ANNUAL REVIEWS Further
Quick links to online content

Ann. Rev. Nutr. 1986. 6:13–40
Copyright © 1986 by Annual Reviews Inc. All rights reserved

Annu. Rev. Nutr. 1986.6:13-40. Downloaded from www.annualreviews.org
by Central College on 01/05/12. For personal use only.

BIOCHEMICAL BASIS FOR THE MANIFESTATIONS OF IRON DEFICIENCY

Peter R. Dallman[1]

Department of Pediatrics, School of Medicine, University of California, San Francisco, California 94143

CONTENTS

INTRODUCTION

The prevention of iron deficiency anemia has attained a high priority among physicians, public health workers, and governmental health agencies. Iron

[1]Supported by NIH Grant # AM 13897.

0199-9885/86/0715-0013$02.00

deficiency tends to be singled out among other nutrient deficiencies because it is common, it is easily identified by laboratory measurements, its laboratory manifestations are readily corrected by iron treatment, and iron is one of the cheapest forms of medication available. However, the correction of laboratory abnormalities is only important if it is accompanied by an improvement in body function and well-being. A program to prevent or treat iron deficiency must ultimately be justified in terms of its physiological consequences. At present, it is easier to define iron deficiency by laboratory measurements on blood than it is to characterize it in terms of its impact on health and well-being (1, 2).

Numerous studies have been aimed at defining the consequences of iron deficiency on work performance, the immune response, and behavior; much of this work is covered in helpful reviews (3–8). These investigations have been stimulated by a better understanding of the biochemical consequences of iron deficiency, not only on the blood and bone marrow, but also on iron compounds in other organs and tissues (3–8). The biochemical abnormalities of iron deficiency also provide the most reliable basis for planning future studies of the manifestations of iron deficiency. It therefore seems useful to review the biochemical basis for the manifestations of iron deficiency, with a particular focus on iron compounds other than hemoglobin.

Annu. Rev. Nutr. 1986.6:13-40. Downloaded from www.annualreviews.org by Central College on 01/05/12. For personal use only.

IRON COMPOUNDS IN THE BODY: DISTRIBUTION AND METABOLIC FUNCTION (3, 4, 9)

The iron-containing compounds in the body are conveniently grouped into two categories (1, 2): those known to serve a metabolic or enzymatic function and those associated with iron storage and transport. The first category of so-called essential or functional iron compounds consists primarily of heme proteins, i.e. proteins with an iron-porphyrin prosthetic group. The function of all heme proteins is related to oxidative metabolism.

Essential Iron Compounds (9–11)

Hemoglobin is the most abundant and easily sampled of the heme proteins and accounts for more than 65% of body iron (total body iron averages about 3.5 g in the adult male). The function of hemoglobin is to transport oxygen via the bloodstream from the lungs to the tissues. Hemoglobin is a tetramer made up of four globin chains, each of which is associated with a heme group that contains one iron atom. The total molecular weight is 66,000. Hemoglobin makes up more than 95% of the protein of the red cell and accounts for well over 10% of the weight of whole blood.

Myoglobin, the red pigment of muscle, transports and stores oxygen for use during muscle contraction. This protein accounts for about 10% of the total body iron. Myoglobin has a molecular weight of 17,800. Its structure is similar

to the monomeric units of hemoglobin; i.e. it is made up of one globin chain attached to one heme group containing a single iron atom. The myoglobin concentration in human muscle is approximately 5 mg per gram of tissue.

Cytochromes are enzymes of electron transport and are located in the mitochondria as well as in other cellular membranes. Cytochromes a, b, and c are present within the cristae of mitochondria in all aerobic cells and are essential for the oxidative production of cellular energy in the form of adenosine triphosphate (ATP).

Cytochrome c, the most easily isolated and best characterized of the cytochromes, is a pink protein with a molecular weight of 13,000. Like myoglobin, it is made up of one globin chain and one heme group containing one atom of iron. Its concentration ranges from 5 to 100 μg per gram of tissue in humans and is somewhat higher in corresponding tissues in the rat. In both species, the highest concentrations are in tissues such as heart muscle that have a high rate of oxygen utilization.

Cytochrome P-450 is located primarily within the microsomal membranes of liver cells but also in the intestinal mucosa. It is involved in oxidative degradation of drugs and endogenous substrates. Cytochrome b_5 is also a component of liver microsomal membranes where it probably provides energy for protein synthesis. Cytochrome b_5 is also present within the cytoplasm of the red blood cell, where it functions as an intermediate in the reduction of methemoglobin. Other heme enzymes are catalase and peroxidase, including the myeloperoxidase of the granulocyte.

Non-heme iron compounds comprise another important group of iron enzymes involved in oxidative metabolism. In these compounds the iron is not in the form of heme. They include the iron-sulfur proteins and metalloflavoproteins, and they account for more iron in the mitochondria than do the cytochromes. Examples are reduced nicotinamide adenine dinucleotide (NADH) dehydrogenase, succinic dehydrogenase, and xanthine oxidase.

Other iron-dependent enzymes, such as tryptophan pyrrolase and phosphoenol-pyruvate carboxykinase, do not contain iron but require iron as a cofactor or activator. Finally, there are some enzymes that contain iron, such as α-glycerophosphate dehydrogenase, in which the form of iron is uncertain.

Iron Storage and Transport Proteins (12, 13)

The major iron storage compounds are *ferritin* and *hemosiderin,* which are located primarily in the liver, reticuloendothelial cells, and erythroid precursors of the bone marrow. The total amount of storage iron can vary over a wide range without apparent impairment of body function. Storage iron may be almost entirely depleted before iron deficiency anemia begins to develop. Conversely, a more than 20-fold increase in iron stores may occur before there is evidence of tissue damage due to iron overload. The protein portion of ferritin, apoferritin,

Annu. Rev. Nutr. 1986.6:13-40. Downloaded from www.annualreviews.org by Central College on 01/05/12. For personal use only.

consists of 24 polypeptide chains, with a total molecular weight of 450,000. These 24 subunits form a spherical cluster around variable amounts of hydrated ferric phosphate contained within a central colloidal core. In hematologically normal adults, ferritin accounts for about half of the storage iron present in the liver, and hemosiderin makes up the other half. Hemosiderin is believed to represent ferritin in various stages of degradation, since hemosiderin will react with antibodies against ferritin. As liver iron stores become abnormally large, hemosiderin makes up an increasingly greater proportion of total iron.

The contribution of the two types of storage iron to total body iron can vary widely from less than 5% to more than 30%. Unless the stores are exhausted, their amount has no discernible influence on any physiologic or biochemical function other than iron absorption.

Transferrin accounts for only about 0.1% of the total body iron. It is protein with a molecular weight of 74,000 and is capable of binding two atoms of ferric iron. Its major role is to transport iron from the reticuloendothelial system and the intestine to the bone marrow for synthesis of hemoglobin in developing red blood cells. The rate at which transferrin delivers iron depends on the relative amount of mono- and diferric transferrin and on the rate of red cell production (14). A high proportion of diferric transferrin and a rapid rate of erythropoiesis both favor increased iron delivery to the cell. Intracellular iron transport involves a less well-defined group of iron compounds of low molecular weight.

Of all the iron compounds that have been discussed, only hemoglobin, serum iron (mostly transferrin-bound), and serum ferritin (which generally reflects the amount of storage iron) are commonly used to assess iron status. These categories account for well over 90% of body iron.

SYNTHESIS AND TURNOVER OF IRON COMPOUNDS (3)

Hemoglobin has a finite life span that, in humans, approximates the roughly 120-day survival of the red cell in the circulation. In the rat, red cell life span is about 60 days. Thus a little less than 1% of the total red cell iron in man and 2% in the rat is released each day from senescent red cells. Because of the large size of the hemoglobin iron pool, this small percentage of iron nevertheless accounts for the major flux of iron within the body. The iron from hemoglobin breakdown can be almost completely re-utilized, as can the amino acids. Heme, on the other hand, is degraded to bilirubin and largely lost via the bile.

In contrast to hemoglobin in the red cell, heme proteins in long-lived cells, such as those of liver and skeletal muscle, do not appear to have a finite life span. Rather, they seem to be subject to random degradation at an exponential rate that is in accord with the rate of turnover of the subcellular structure with

Annu. Rev. Nutr. 1986.6:13-40. Downloaded from www.annualreviews.org by Central College on 01/05/12. For personal use only.

which they are associated. Skeletal muscle cytochrome c, for example, has a half-life of about six days in the rat (15). The implication of the dynamic state of most body iron constituents is that tissue deficits should be largely reparable by treatment with iron. However, the rates at which deficiencies of individual iron compounds are corrected will often differ markedly from the rate at which anemia is reversed (3). Correction is most rapid in rapidly replaced cell populations, such as intestinal mucosa. Deficiencies of muscle myoglobin and iron containing electron transport compounds are corrected more slowly than anemia (3); depleted brain non-heme iron in the rat is replenished at such an extremely slow rate that the abnormality may not be completely reversible (16, 17). In the case of some iron enzymes such as ribonucleotide reductase, a decrease in activity due to iron lack can probably be corrected within less than an hour when increased iron becomes available, without requiring the synthesis of the entire molecule (18).

In an individual with iron deficiency anemia, hemoglobin synthesis can increase several-fold, as indicated by the reticulocyte response and by the increase in hemoglobin concentration almost to the normal value within one month of starting iron treatment. In the rat, the compensatory increase in red cell production during iron treatment is proportionally even greater, and severe anemia can be reversed within one week.

STAGES OF IRON DEFICIENCY (1, 2)

The progression from adequate iron balance to iron deficiency anemia develops in three overlapping stages. The first stage consists of a depletion of storage iron. In man, this stage is characterized by a decrease in the concentration of serum ferritin, which reflects the declining concentration of iron stores in the liver, spleen, and bone marrow. The second stage of iron deficiency consists of a decrease in transport iron and is likely to be transient. This is characterized by an increase in the iron-binding capacity as well as by declining concentration of serum iron. The term "latent iron deficiency" is sometimes used to refer to these first two pre-anemic stages of iron deficiency. The third stage of iron deficiency develops when the supply of transport iron decreases sufficiently to restrict the concentration of hemoglobin and/or other iron compounds that fulfill known physiologic functions. With the few exceptions discussed below, it is safe to assume that there are no harmful consequences of iron deficiency until the third stage, when it is defined in this manner. An alternative definition of the third stage is in terms of a hemoglobin concentration that has decreased sufficiently to fulfill the laboratory definition of anemia (a hemoglobin concentration below the 95% reference range for age and sex) (1). In addition to the effect on hemoglobin, the laboratory characteristics of the third stage include an elevation of erythrocyte protoporphyrin and microcytosis.

Annu. Rev. Nutr. 1986.6:13-40. Downloaded from www.annualreviews.org by Central College on 01/05/12. For personal use only.

TISSUE IRON COMPOUNDS IN RELATION TO PROGRESSION OF ANEMIA

A vast amount of information on iron metabolism and iron deficiency anemia in man has accumulated during the past 50 years (1, 2). Most of this work was based on studies of peripheral blood, bone marrow, liver, and spleen. In contrast, most of our information on the effects of iron deficiency on other tissues and iron compounds other than hemoglobin, ferritin, and hemosiderin is of more recent origin and has been largely derived from studies using the rat as an experimental model (3, 4, 6). These dual sources of information raise questions about the suitability of the rat as a model for iron deficiency in man (19).

Annu. Rev. Nutr. 1986.6:13-40. Downloaded from www.annualreviews.org by Central College on 01/05/12. For personal use only.

The rat has been widely accepted as a model for iron deficiency because it mirrors iron metabolism in man in most respects (20). Experiments in the rat have several advantages: dietary iron can be made the only experimental variable, tissue can be sampled extensively, longitudinal studies are easier, and experimental manipulations can be made that could not be justified in man. Iron kinetics and the steps in the progression of iron deficiency are remarkably similar where comparable studies have been done in both species. Hemoglobin concentration, blood volume as a percentage of total body weight, and the amount of iron per kilogram of body weight are also virtually identical in the rat and in man.

The differences between the two species are primarily a matter of rate of progression and degree of iron deficiency. The rat is less mature at birth, grows rapidly, and has a relatively short life span of three years. During early postnatal development it doubles its body weight in about five days, in contrast to human infants, in whom body weight doubles in four months in early infancy. By three to four weeks of age, the developing rat can be weaned from its mother, and by 40 to 60 days of age it is sexually mature. This rapid rate of growth is naturally associated with a higher peak iron requirement than in man: roughly 6 mg/kg/day (21) in contrast to the high of 1 mg/kg/day in term infants (2). Among the other notable differences in iron metabolism are the differing susceptibilities to iron deficiency in males and females. The female rat has a much lower growth rate compared to the male and is also much less vulnerable to dietary iron deficiency. In contrast, after sexual maturity human females are at much greater risk of iron deficiency (due to menstrual blood loss) than are males. In respect to iron absorption, the rat also differs in that it absorbs dietary heme iron less well than non-heme iron; the opposite relationship is true in man.

Most studies using the rat as an experimental model have provided an iron-deficient diet starting shortly after weaning at 3–4 weeks of age, and then maintained for 2–8 weeks. This regimen takes advantage of the high iron

requirement imposed by the remarkably rapid growth of rats during this period. The rapid rate at which rats grow and develop severe anemia could conceivably exaggerate the degree of tissue iron deficiency in the rat in comparison to man.

Another issue to be considered is that there is usually some degree of growth suppression in rats (as in children) given an iron-deficient diet. In most recent studies, the dietary regimens have not depressed the growth of the iron-deficient rat by more than 10–25% compared to control animals, in contrast to many earlier studies in which the body weight of deficient rats was less than half that of the control group. Studies in which suppression of growth is so extreme raise concerns about secondary effects that might be more directly related to decreased food intake than to iron deficiency.

The concentration of hemoglobin provides the best frame of reference for severity of iron deficiency both in the rat and in man because it is the most easily measured essential iron compound. In the rat, some tissue iron compounds, such as skeletal muscle cytochrome c and myoglobin, become depleted to a similar degree as hemoglobin, not only with severe iron deficiency anemia but also when anemia is relatively mild (21). In terms of the sequence of development of tissue iron deficiency, the cytochrome c concentration of intestinal mucosa is depressed earlier and more profoundly than hemoglobin concentration (22). Presumably this is because the cells lining the intestinal mucosa have a much more rapid turnover than red blood cells, (2 days vs 60 days), which makes it possible for a decreased iron supply to influence the entire cell population more rapidly.

Because the rat's unusually rapid growth makes it particularly vulnerable to iron deficiency, it is pertinent to ask whether similarly impressive changes in tissue iron compounds occur in man. The best data relating to tissue iron compounds in man pertain to biopsies of endothelial tissue and to the neutrophils and lymphocytes sampled from peripheral blood. Humans with relatively mild iron deficiency anemia have a decreased activity of cytochrome oxidase in buccal mucosa (23) and the mucosal cells lining the intestinal villi (24). More quantitative evidence of tissue iron depletion comes from studies of neutrophils and lymphocytes. In neutrophils there is a decreased capacity of the cells to kill ingested bacteria (25–29), presumably because the oxidative burst is iron dependent. Lymphocytes, in most studies, have a decreased response to mitogens (26–28, 30–33), probably because of a decreased activity of the iron-containing enzyme, ribonucleotide reductase, that is required for the production of DNA and subsequent cell division (34). These few examples (discussed in more detail below) provide convincing evidence that tissue iron depletion is a feature of iron deficiency in man and not merely a characteristic of a possibly more vulnerable animal model.

Annu. Rev. Nutr. 1986.6:13-40. Downloaded from www.annualreviews.org by Central College on 01/05/12. For personal use only.

DEFICIENCIES OF IRON COMPOUNDS ASSOCIATED WITH IMPAIRMENTS OF FUNCTION

Hemoglobin, Myoglobin, and Oxygen Transport

Hemoglobin plays the major role in transporting oxygen from the lungs to various tissues. As the concentration of hemoglobin declines, there are several adaptations that minimize the consequences of a decreased oxygen-carrying capacity (35, 36). Initially, the tissues simply extract oxygen from hemoglobin more completely, aided by a decrease in the oxygen affinity of hemoglobin. There is also a redistribution of blood flow that maintains the oxygen supply to the brain and myocardium at the expense of other tissues. As anemia becomes more severe, cardiac output increases, a change that is eventually reflected by cardiac hypertrophy. The effectiveness of these adaptive mechanisms probably accounts for the fact that chronically anemic patients are characteristically symptom-free at rest and that their handicap is only made evident by exercise. In the rat, even with exercise, the decrease in maximal oxygen consumption ($\dot{V}O_2$ max) is relatively modest until anemia becomes severe (37).

The degree to which anemia is responsible for the manifestations of iron deficiency can be distinguished from the role of other tissue iron compounds by experimentally using exchange transfusion to modify the hemoglobin concentration (38). When anemia in iron-deficient rats is corrected, performance in brief exercise tolerance tests is improved, and $\dot{V}O_2$ max returns to almost normal values (39). Recent evidence suggests that impaired ability to maintain body temperature in the cold is also reversed by transfusion (40). In each case, the conclusion is that these manifestations of iron deficiency are more closely linked to anemia than to the effects of iron deficiency on iron compounds other than hemoglobin.

Myoglobin serves an oxygen transport and storage function but one that is restricted to muscle. In the iron-deficient rat, skeletal muscle myoglobin is decreased in concentration (21, 38, 40, 41). Myoglobin deficiency is less marked when rats are subjected to exercise training (42). The important role of work load is supported by the maintenance of closer-to-normal or normal myoglobin concentrations in the myocardium (41–43). Heart hypertrophy in severely anemic rats is presumed to occur in response to an increased work load imposed by anemia (36). The maintenance of a nearly normal cardiac myoglobin concentration probably aids in the increased cardiac output that makes it possible to maintain oxygen delivery to tissues despite anemia.

Cytochromes and Iron-Sulfur Proteins in Relation to Energy Metabolism

Most of the mitochondrial enzymes involved in the oxidative production of ATP contain iron. In cytochromes a, b, and c, iron is in the form of heme. The

Annu. Rev. Nutr. 1986.6:13-40. Downloaded from www.annualreviews.org by Central College on 01/05/12. For personal use only.

NADH- and succinate-dehydrogenases contain iron as part of one or more iron-sulfur complexes. The degree to which the iron enzymes are affected in mitochondria from iron-deficient animals appears to correspond to where they are located in the electron transport chain. The iron-sulfur-containing dehydrogenases that are involved in the first reaction in the electron transport chain are the most severely depleted (41, 44). Cytochrome b and c, which are in the middle of the electron transport chain, are decreased to an intermediate degree and cytochrome a and cytochrome a_3, which comprise the last reaction, are least affected.

The oxidative capacity of the entire electron transport chain, estimated from the rate of oxidation of citric acid cycle intermediates, is as profoundly depressed as the activity of the iron-sulfur-containing dehydrogenases (44). This observation suggests that the deficiency of the iron-sulfur proteins may limit the rate of oxidation in the electron transport chain as a whole.

Although rates of oxidation of citric acid cycle intermediates are decreased in many tissues, the linkage between oxidation and phosphorylation remains normal (38, 44). Thus, the rate of ATP production is decreased in proportion to the diminution in rate of oxidation.

Annu. Rev. Nutr. 1986.6:13-40. Downloaded from www.annualreviews.org by Central College on 01/05/12. For personal use only.

DIFFERENCES IN ORGAN SUSCEPTIBILITY There are large differences in the degree to which the iron-containing electron transport enzymes of the mitochondria are depleted in various organs. The most severely affected tissues are the skeletal muscle and intestinal mucosa (45).

Skeletal muscle Skeletal muscle is severely affected by iron deficiency (3, 38, 39, 41, 44, 45a,b, 46) and, because of the large mass of muscle in the body, the impaired capacity of skeletal muscle for oxidative metabolism probably has a major impact on the energy metabolism of the body as a whole. During the progression of iron deficiency, the depletion of the oxidative enzymes in skeletal muscle occurs more gradually than the development of anemia (22) but can eventually be of comparable or even greater severity (38, 39, 41, 44). Body growth and increase in oxidative capacity of muscle normally progresses at a very rapid rate during the post-weaning period (47) during which rats are usually made iron deficient. Undoubtedly, this rapid growth and differentiation predisposes muscle to the effects of iron deficiency. However, this is unlikely to be the only explanation since tissues such as liver and kidney, which also grow rapidly during this period, are far less affected (43, 48, 49).

Iron deficiency affects all fiber types in skeletal muscle (37, 41, 46). Muscle fiber types are categorized according to their biochemical and contractile characteristics (50). Fast-twitch white fibers are poor in oxidative enzymes. They engage in rapid movements that do not require sustained power and are dependent primarily on glycolysis as an energy source. Red muscle fibers, on

the other hand, are rich in oxidative enzymes, which are their primary source of energy. In the rat, slow-twitch red muscle and fast-twitch red muscle have roughly three and five times the oxidative capacity, respectively, of fast-twitch white muscle.

Experiments in which iron-deficient rats were transfused to raise their hemoglobin concentration led to the conclusion that the major consequence of a decreased muscle capacity for energy metabolism was an impaired capacity for sustained exercise (39, 44). Even though $\dot{V}O_2$ max was virtually corrected by transfusion, the ability to run on a treadmill for a prolonged period remained severely impaired (39). When iron-deficient rats were treated with iron, the improvement in performance of prolonged exercise followed the same time course as correction of decreased muscle oxidative capacity, and was significantly slower than the reversal of anemia (44).

Annu. Rev. Nutr. 1986.6:13-40. Downloaded from www.annualreviews.org by Central College on 01/05/12. For personal use only.

The conclusion that the effects of iron deficiency on muscle particularly impair the capacity for sustained exercise was also supported by experiments with the isolated and perfused hind limb muscles of the rat (45a). This preparation has the advantage of excluding variables unrelated to the direct effects of iron deficiency on muscle function. McLane and coworkers (45a) found that the effect of iron deficiency on contractile force varied with duration of electrical stimulation. During the first two minutes of stimulation there was little difference between iron-deficient and control animals. However, after four minutes, an increasing difference became discernible. After ten minutes of muscle contraction, the iron-deficient animals were only able to develop about 60% of the contractile force of the control animals.

There is an association between the obligatory work load and degree of impairment of oxidative capacity in muscle. Heart muscle, which has an increased work load associated with anemia, and diaphragmatic muscle, which has the continued work load of respiration, retained a much higher oxidative capacity than skeletal muscle compared to control animals (41, 43, 49, 51). Indeed there is little or no effect on cardiac muscle except in one study (52).

A cause-and-effect relationship between work load and oxidative capacity could be inferred in iron-deficient rats that were subjected to an exercise training regimen of four to six weeks (37). The skeletal muscle of trained animals had a far higher oxidative capacity than that of untrained iron-deficient rats. Indeed, the trained, iron-deficient rats achieve an oxidative capacity almost equivalent to that of sedentary animals fed an iron-sufficient diet. Concurrently there was an improvement in their capacity for prolonged exercise. However, neither oxidative capacity nor the ability to run for prolonged periods approached that of iron-sufficient animals that were given a similar exercise regimen. The mechanism of the improvement of the iron-deficient animals with training is uncertain, since training was also associated with a slightly milder anemia than that found in sedentary rats. The results neverthe-

less indicated that exercise training was effective in improving the performance of iron-deficient rats but that they remained handicapped when compared to similarly exercised animals fed a complete diet.

Metabolic adaptations to a decreased capacity for oxidative metabolism in muscle It is reasonable to anticipate some systemic changes in energy metabolism in response to the marked decrease in oxidative capacity of a tissue with so large a mass as skeletal muscle. Recent isotopic studies in the iron-deficient rat indicate that there are substantial alterations in glucose metabolism that allow muscle to utilize more glucose to produce ATP with less dependence on mitochondrial electron transport (52). This is made possible by an increased reconversion of lactate, the end-product of glycolysis, to glucose by the gluconeogenic pathway in the liver. Although hepatic recycling is costly in terms of ATP, this may not constitute a major handicap because the oxidative capacity for production of ATP in the liver is much less impaired by iron deficiency than that of skeletal muscle (41, 43, 49, 54). Glucose recycling in iron-deficient rats occurred at five times the control rate (53). Thus an increased muscle demand for glucose and an increased lactate release from muscle could be managed by the liver, in which mitochondrial oxidative capacity remains relatively high.

Some indication of the effectiveness of the circulatory and metabolic adaptations to iron deficiency can be obtained from studies of exercise performance in rats with iron deficiency of increasing severity (55). $\dot{V}O_2$ max during a brief, hard exercise declined only 16% with a decrease in hemoglobin concentration from 14 to 8 g/dl and fell sharply only below 7 g/dl. These results are in accord with the known effectiveness of compensatory mechanisms that help to maintain oxygen delivery to tissues until iron deficiency becomes severe. The time to exhaustion in a treadmill exercise of submaximal intensity (endurance) showed a different pattern. There was no significant change in performance in iron-deficient rats with a hemoglobin concentration as low as 10 g/dl, which indicates that metabolic reserve or adaptations are relatively effective in rats with mild iron deficiency anemia. However, endurance declined abruptly by 73% between a hemoglobin concentration of 10 and 8 g/dl. This sharp decrease indicates a threshhold of severity of iron deficiency beyond which metabolic adaptations may be adequate in sedentary animals but insufficient to maintain prolonged exercise.

Intestinal mucosa One obvious basis for the susceptibility of the intestinal mucosa to iron deficiency is its rapid cell turnover. Since the mucosal lining is completely replaced every two days in the rat and every three or four days in man, this tissue is particularly vulnerable to day-to-day fluctuations in the supply of iron. However, this rapid cell turnover also has the advantage of

Annu. Rev. Nutr. 1986.6:13-40. Downloaded from www.annualreviews.org by Central College on 01/05/12. For personal use only.

allowing for a rapid recovery following iron treatment. After the administration of iron to iron-deficient rats, mucosal cytochrome c concentration returns to normal values within two or three days (45). Histochemical studies of cytochrome oxidase activity show that young differentiating cells at the base of the villi are the first to restore their normal cytochrome oxidase activity after about 24 hours, following which there is a gradual progression of repair as the cells migrate toward the tip of the villi during the course of their maturation (45, 56).

Intestinal function is impaired in iron deficiency both in man and in the rat. Iron deficiency is often found to be associated with clinically significant intestinal blood loss in infants (57–59), but it is important in interpreting such studies to distinguish between cause and effect. An initial predisposing factor that leads to intestinal blood loss and to the secondary development of iron deficiency is the ingestion of fresh cows' milk (60–62). When the diet is changed from fresh milk to a heat-processed formula, the blood loss can be reversed in most instances (61, 62). Celiac disease also leads to intestinal blood loss and iron deficiency; however, some blood loss persisted for one or more years in patients for whom a gluten-free diet had been recommended (63). The degree of blood loss appeared to be greatest in patients with the greatest villous atrophy and intra-epithelial lymphocytosis and possibly in those with poorer adherence to dietary recommendations.

Dietary iron deficiency appears to be a primary cause of decreased intestinal disaccharide and of lactose malabsorption in children since both were reversed with iron treatment (64). Similar disaccharidase deficiency has been described in iron-deficient rats (65, 66). Absorption of disaccharides is an energy-requiring process, and its impairment may be linked to a decreased capacity for energy metabolism. Dietary iron deficiency was also thought to be primary in other studies that showed malabsorption of sugars (67) or of iron itself (68) because the abnormalities could be corrected by treatment with iron. A similar response to treatment has also been observed in the relatively rare cases of iron deficiency anemia accompanied by hypoproteinemia (69, 70).

The fetus The vulnerability of the fetus to maternal iron deficiency is very different in man and in the rat. In humans, mild iron deficiency during pregnancy has no detectable effect on hemoglobin concentration in the newborn and, at the most, a minor influence on serum ferritin (71–76). On the other hand, severe iron deficiency anemia does result in a decreased hemoglobin concentration in the offspring, but not as great as in the mother (77, 78). In the rat, the effect on the fetus is more profound. In severely iron-deficient, pregnant rats, the rate of fetal resorption is high and the survivors are also severely anemic (79). Biochemical studies of iron-deficient fetuses at 10 days of gestation show less than half of the normal mitochondrial NADH oxidase activity

Annu. Rev. Nutr. 1986.6:13-40. Downloaded from www.annualreviews.org
by Central College on 01/05/12. For personal use only.

(80). The apparent difference in susceptibility of the fetus in the two species may be explained by their relative iron requirements for pregnancy. In the rat, an amount of iron equivalent to about 25% of body iron must be transferred to the uterine contents over a 21-day period. In the human, the comparable requirement is only 15% of body iron, and it is spread out over a much longer period of 280 days. Consequently, the impact of iron deficiency during pregnancy is far more profound in the rat.

Other tissues The mitochondrial oxidative enzymes have been studied in other tissues of the iron-deficient rat: the liver (43, 47, 49, 81), kidney (47, 49), heart muscle (43, 49, 51, 52), and brain (16, 43, 82–84). These enzymes are less severely depleted in liver (as previously mentioned) and in kidney than they are in intestinal mucosa and skeletal muscle. Heart muscle (also discussed) and brain are least affected of all. Indeed brain cytochromes and oxidases are only affected under the most severe conditions of iron deficiency (83) or if the animal is made iron-deficient during early postnatal development when brain growth and development of mitochondrial energy metabolism are most rapid (82, 85).

Possible reasons for differences in organ vulnerability It was mentioned above that rapid growth, cell differentiation, and cell turnover are factors associated with the most profound deficits in oxidative capacity (3). In addition, an altered pattern of work load by organs and cells appears to influence the degree of impairment of oxidative capacity (3). It still remains to be explained how the priorities for production of iron-containing oxidative enzymes in various parts of the body are mediated in iron deficiency. Plasma iron that is bound to transferrin is delivered to all tissues. However, tissues differ substantially in their iron requirement and it is believed that the number of transferrin receptors on the surface of cell membranes can adjust the uptake of iron to the needs of the individual cell (86). The importance of this mechanism is brought out by a recent report of anemia in a patient who developed autoantibodies to the transferrin receptor and in whom incorporation of iron by developing red blood cells was impaired (87).

Altered Metabolism of Biogenic Amines as a Possible Basis for Changes in Behavior

Children with severe iron deficiency were for many years characterized as irritable and uninterested in their surroundings. These manifestations often seemed to be rapidly reversed within a few days of iron treatment. Because such observations were not readily expressed in quantitative terms, it was difficult to distinguish whether they represented medical folklore or astute clinical observation. Scientific interest in the possibility of altered behavior ascribable to iron deficiency increased when it was reported a little more than a decade ago

Annu. Rev. Nutr. 1986.6:13-40. Downloaded from www.annualreviews.org by Central College on 01/05/12. For personal use only.

that iron-deficient rats had a decreased ability to catabolize systemically injected monoamines (88). Monoamine oxidase (MAO) is on the catabolic pathway of the neural mediators, serotonin (5HT), norepinephrine (NE), epinephrine (E), and dopamine (DA) and was thought to be an iron-containing enzyme. A few years later, children with iron deficiency anemia (but not other anemias) were found to have an elevated urinary NE that decreased to normal levels within a week of iron treatment (89). The elevation in urinary NE was considered to be a possible basis for the behavioral changes believed to characterize iron-deficient patients. The above-mentioned two papers stimulated numerous studies of the effects of iron deficiency on either behavior or on the metabolism of biogenic amines (catecholamines and serotonin). Because the studies of behavior are the subject of several recent reviews (8, 90, 91), the emphasis in this report is on the possible roles of iron in the metabolism of the biogenic amines.

Iron is involved at several steps in the synthesis and degradation of the biogenic amines. Although, iron deficiency appears to affect systemic catecholamine metabolism, the changes in the brain seem less profound. The notable exceptions, to be described in more detail, involve not synthesis or catabolism, but an impairment in the binding of DA to the D_2 receptor of the caudate nucleus (92) and of 5HT to brain synaptic vesicles (93).

BINDING OF BIOGENIC AMINES TO RECEPTOR SITES Dopamine plays an important role as the major neurotransmitter of the extrapyramidal system of the brain. Youdim and associates (92) investigated the effect of iron deficiency on DA binding sites in the caudate nucleus, an area of the extrapyramidal system that is iron-rich and in which DA receptors are concentrated. There are two types of DA receptors, one is linked to adenylcyclase (the D_1 receptor) and another is unrelated to activation of cyclic nucleotides (the D_2 receptor). In iron-deficient rats, D_2 binding sites, estimated by ^{3}H-spiroperidol binding, were decreased to about half of the control number. When the rats were then given an iron-rich diet for two weeks, the anemia as well as the abnormality in binding were corrected. However, decreased binding did not appear to be caused by the anemia per se, because phenylhydrazine-induced hemolytic anemia did not result in any change in ^{3}H-spiroperidol binding.

In contrast to the marked effect of iron deficiency on the D_2 binding site, there was no evidence of altered binding on the D_1 receptor: neither the basal activity of caudate nucleus DA-sensitive adenylcyclase nor its response to increasing concentrations of DA were affected by iron deficiency.

Analogous results for serotonin (5HT) were reported by Kaladhar & Rao (93). 5HT is also an important neurotransmitter whose function is linked to slow-wave sleep. Binding of 5HT by synaptic vesicles prepared from whole brain was about one-third lower in the iron-deficient compared to control rats;

Annu. Rev. Nutr. 1986.6:13-40. Downloaded from www.annualreviews.org by Central College on 01/05/12. For personal use only.

this abnormality was corrected by one month of iron treatment. A plausible basis for the decreased binding of 5HT is the earlier observation that ferrous iron increases the rate of 5HT uptake by synaptic vesicles (94).

A relationship has been postulated between the decreased number of D_2 receptors for DA and an elevated pain threshold after administration of β-endorphin, morphine, or haloperidol (a blocker of DA receptors) to iron-deficient rats. Yehuda & Youdim (95) reported an increase in pain threshold in iron-deficient rats compared to control animals. In addition, there was a reversal of the normal circadian difference in pain threshold. In control rats, evidence of discomfort on a surface with a temperature of 58°C occurred more slowly when rats were tested during the 12-hour light period (when they are normally least active) than during the dark (their normal period of wakefulness and activity). This pattern was initially also observed in rats on the iron-deficient regimen. However, after 3 to 4 weeks of the dietary regimen, the iron-deficient rats had developed a marked delay in their pain response during the dark but not during the light period. This reversal in the light-dark response was even more evident after treatment with β-endorphin. Not only was the pain response markedly delayed over control values, but the delay was more prolonged during the dark than during the light period. Morphine and haloperidol produced a similarly greater delay in iron-deficient compared to control rats. After 25 days of iron treatment, all of these abnormalities were reversed.

It seems that the reversal of the circadian activity pattern may only occur under certain conditions of age and duration of diet. The altered circadian pattern of pain threshold in iron-deficient rats (96) seems in accord with the reversed pattern of eating and drinking and/or activity observed in another laboratory (97). However, other investigators have shown maintenance of the normal circadian pattern of nocturnal activity (98–100). This is a critical point to establish in future experiments in order to distinguish behavioral alterations that take place without an altered activity pattern from those that represent solely a change in diurnal pattern.

SYNTHESIS AND CATABOLISM OF BIOGENIC AMINES Two iron-dependent hydroxylases are on the direct synthetic pathways of the biogenic amines. Tyrosine hydroxylase catalyzes the hydroxylation of tyrosine to L-DOPA (3,4-dihydroxyphenylalanine), a precursor of the catecholamines NE and E. Tryptophan hydroxylase catalyzes the production of serotonin from tryptophan. Tryptophan hydroxylase activity in the brain of iron-deficient rats was either normal or only slightly decreased, even with very severe iron deficiency (84). Tyrosine hydroxylase could be inferred to retain normal activity from indirect evidence (84). In accord with these findings is the observation that brain concentrations of NE and DA were normal (84). The brain content of

Annu. Rev. Nutr. 1986.6:13-40. Downloaded from www.annualreviews.org by Central College on 01/05/12. For personal use only.

serotonin was found to be only slightly decreased by one group (84) but slightly increased by another (83).

The turnover and degradation of biogenic amines in brain also remained close to normal despite the fact that one or more of the catabolic steps is iron dependent (84). Aldehyde oxidase (or dehydrogenase) is an iron-dependent reaction. Mackler and associates (83) found the enzyme to be decreased in activity, and they interpreted this to be in accord with their finding of slightly increased brain concentrations of 5HT. However, these results were in disagreement with those of Youdim and coworkers (84), who found aldehyde dehydrogenase to be normal in activity and 5HT unchanged in concentration (84). Both groups agree that monamine oxidase (MAO) activity in brain is not altered by iron deficiency. MAO is now known not to contain iron. Nevertheless, MAO was found to be decreased in activity in the liver (though not by Mackler et al), heart, and adrenal of rats (84) and in the platelets of humans with iron deficiency (101). Intravenously injected synthetic monoamines were also catabolized at a less than normal rate in iron-deficient rats (88). From these findings, it has been inferred that iron may be involved in MAO synthesis even though it is not part of the MAO molecule (101).

Rats that become iron deficient within the first few weeks of postnatal development have a lower concentration of brain non-heme iron than do control animals (16, 84, 102). This deficit is unusual in that it cannot be reversed, even after other manifestations of iron deficiency have been corrected by iron treatment. The physiological implications of this finding remain uncertain since the relatively few other biochemical manifestations of iron deficiency in brain that have been described are reversed with iron administration (83, 92, 93).

Evidence for systemic alterations in catecholamine metabolism, aside from decreased MAO activity in the rat (88), is based primarily on increased blood and urine concentrations of NE in the rat (101, 103) and in man (89, 104). Since there is relatively little indication of substantial changes in synthesis and degradation of biogenic amines in brain, it seems more promising to seek systemic explanations for elevated blood and urine NE. Dillmann and coworkers determined the rate of disappearance of labelled NE from the blood after intravenous injection (105). Hemoglobin was first adjusted to an identical concentration of about 10 g/dl in the deficient and control groups by exchange transfusion. NE disappeared at a more rapid rate in the iron-deficient than in control rats. However the difference in disappearance rate was not interpreted as being great enough to account for the marked elevation in blood NE. It was proposed instead that the major factor was an increase in rate of production of NE in relation to impaired thermogenesis in the iron-deficient rat.

Iron-deficient rats (105–107) and humans (108) have been found to have an impaired capacity to maintain their body temperature in a cold environment. Two major systems are involved in maintaining body temperature, NE and

Annu. Rev. Nutr. 1986.6:13-40. Downloaded from www.annualreviews.org by Central College on 01/05/12. For personal use only.

thyroid hormone. Dillmann and coworkers (106) found that iron deficiency was associated with an impaired conversion of T4, the inactive precursor, to T3, the active thyroid hormone. They hypothesized that NE production was elevated in iron deficiency as a compensatory mechanism for an impaired thyroid hormone response.

More recent results from the same laboratory may require a modification of this hypothesis. Impaired thermogenesis in response to cold exposure was reversed when iron-deficient rats were transfused to correct their anemia (107). Similarly, the responses in T3 and thyroid stimulating hormone (TSH) to cold exposure could be modified by simply adjusting the hematocrit in iron-deficient or control rats. In either group, T3 and TSH responses were generally normal when the hematocrit was above 25% and were subnormal when the hematocrit was below 25%. Thus, the abnormalities in thermogenesis, T3, and TSH appeared to be a nonspecific consequence of anemia rather than a result of iron deficiency. Furthermore, the impaired TSH response provides evidence that not only iron deficiency, but anemia per se, can result in impaired pituitary and possibly central nervous system function. These conclusions require a reexamination of the earlier reports that NE elevation was characteristic of iron deficiency and not merely a consequence of anemia (105). If NE elevation in iron deficiency is indeed independent of anemia whereas the defect thermogenesis is anemia related, then it becomes difficult to attribute the NE elevation to a compensatory overproduction for maintenance of body temperature. Perhaps, after all, a decrease in NE catabolism is a more plausible explanation for blood and urine NE elevation.

The linkages between altered behavior and biogenic amine metabolism in iron deficiency remain tenuous. The review of Lozoff & Brittenham (8) emphasizes the useful distinction between behavioral changes that are primarily cognitive and those that are noncognitive. Cognitive functions include attention span, learning ability, and performance on IQ tests whereas spontaneous activity, apathy, responsiveness, alertness, apprehensiveness, and irritability are noncognitive characteristics (even though they can influence cognition). When considering possible biochemical bases for altered behavior it is also useful to make a distinction between primary alteration in brain metabolism and systemic changes, e.g. anemia and blood NE levels, that might influence behavior in a less direct manner.

Most of the studies of behavior in iron-deficient infants have used a single test, the Bailey Scale, that has both cognitive and noncognitive components (109–113). Despite the careful design of the studies, many of the reports have features that do not allow an unambiguous conclusion about the effects of iron deficiency on behavior. There is also disagreement whether (109, 111, 113) or not (108, 115) the behavioral abnormalities are reversed by treatment at the same rapid rate reported for the reversal of elevated blood and urine NE values.

Annu. Rev. Nutr. 1986.6:13-40. Downloaded from www.annualreviews.org
by Central College on 01/05/12. For personal use only.

None of the studies have followed the subjects for more than a month. Studies of older children, using IQ tests (114–116) and other measures of cognitive function (117), all indicated impaired performance associated with iron deficiency. Improvement with iron treatment was observed (115–117), but the possibility of long-term impairment was suggested by one report (114).

Lozoff & Brittenham (8) made cautious conclusions in their review of human and rat data. Studies in the iron-deficient rat did not demonstrate deficits in cognitive performance but did show differences in noncognitive factors such as responsiveness to environmental stimuli. Analysis of the human studies led to somewhat analogous conclusions. Although there were indicators of impaired cognitive function, there remains the possibility that the changes observed during testing could be due to noncognitive factors such as short attention span, fearfulness, and increased body tension and a striking increase in the maintenance of body contact between the iron-deficient infant and its mother.

Annu. Rev. Nutr. 1986.6:13-40. Downloaded from www.annualreviews.org by Central College on 01/05/12. For personal use only.

The Immune Response: Bacterial Killing by Neutrophils and Activation of T Lymphocytes

Two abnormalities in the response to infection have been relatively well documented in iron-deficient patients and in rats. One of these is the decreased capacity of neutrophils to kill ingested bacteria (25–29, 118) and the other is the impairment in T-cell proliferation in response to stimulation by mitogens (26, 27, 30–33) or by antigens.

NEUTROPHIL FUNCTION Neutrophils provide a major defense of the body against invasion by bacteria, as clearly demonstrated by the fact that profound deficiencies in neutrophil number or function are associated with a high rate of mortality from bacterial infections. Most studies of neutrophil function and enzyme activity in iron deficiency have used blood samples from humans. Although the results of human studies should be most conclusive, interpretation of the results was often complicated by the presence of additional nutritional deficiencies or concurrent or recent infections. Furthermore, the constraints imposed by small blood samples fostered the use of semiquantitative histologic tests of the oxidative function, such as reduction of the dye nitroblue tetrazolium (NBT), and the histochemical estimation of myeloperoxidase activity on blood smears. For these reasons, both animal and human data contribute to the evaluation of the immune response in iron deficiency.

There is general agreement that neutrophil number and ability to ingest bacteria by phagocytosis are normal or close to normal in iron-deficient patients (27, 32, 119) and rats (118). The major defect in iron deficiency is the capacity for killing certain types of bacteria once they have been ingested (25–29, 118). Bacterial killing involves a sharp increase in oxygen consumption that is known as the "respiratory burst" (120). The respiratory burst results

from the activation of NADPH oxidase (presumably an iron-sulfur enzyme), which in turn produces O_2^- and H_2O_2. The heme protein, cytochrome b, is associated with the respiratory burst but its precise role has not been defined. H_2O_2 and O_2^- are then used as the starting materials for the production of oxidized halogens and hydroxyl radicals that are most effective in bacterial killing. The production of the oxidized halogens from H_2O_2 is catalyzed by the iron enzyme, myeloperoxidase which is located in the azurophilic granule of the neutrophil (121). The production of hydroxyl radical from O_2^- is catalyzed by iron that is donated by lactoferrin, (122) an iron binding protein that is present in high concentration in the specific granule of the neutrophil (121). Consequently, there are at least two or three iron-dependent steps involved in bacterial killing.

In studies of iron-deficient patients, the results of the NBT dye test were decreased in some (25, 27) but normal in other (26, 29, 123) reports. Myeloperoxidase, estimated histochemically, was decreased in two studies (118, 124) but normal in two others (29, 123). These discordant results involving the presumed biochemical mechanisms are puzzling when there is virtual unanimity about the end result, bacterial killing. The confounding influences of additional nutrient deficiencies and the known stimulation of NBT reduction by infection could explain the disparity among various studies.

Two recent studies of iron-deficient rats provide additional information on the basis for impaired bacterial killing by neutrophils. Mackler and coworkers (125) measured the rate of oxygen consumption before and after activation in neutrophils that were isolated from iron-deficient and control rats. The baseline rates of respiration were similar in the two groups of animals but the maximum rate of oxygen consumption after activation in the iron-deficient group was less than half of the control value. The cytochrome b concentration of neutrophils was unaffected by iron deficiency. These results suggested that iron deficiency impaired the NADPH oxidase reaction and that the impairment was not attributable to cytochrome b. In the same study, myeloperoxidase was measured enzymatically. Its activity in the iron deficiency group was less than one third that of the control value. These results carry greater weight than the less quantitative histochemical data from other studies. Moore & Humbert (118) also studied neutrophil function in iron-deficient rats. NBT reduction and myeloperoxidase measured histochemically were decreased in iron deficiency.

An important factor in the ability of neutrophils to kill bacteria is whether the bacteria themselves contain catalase (118). Catalase-positive bacteria, such as *Staphylococcus aureus* and *Escherichia coli,* can break down H_2O_2 that the neutrophil has generated and consequently be more resistant to killing. These bacteria are not readily killed by neutrophils from iron-deficient rats and humans. With catalase-negative bacteria such as *Streptococcus pneumoniae,* H_2O_2 can be maintained at a high enough level to enhance bacterial killing by

Annu. Rev. Nutr. 1986.6:13-40. Downloaded from www.annualreviews.org by Central College on 01/05/12. For personal use only.

oxidized halogens and hydroxyl radical. Killing of this organism was unimpaired in neutrophils from iron-deficient rats.

ACTIVATION OF T LYMPHOCYTES A continuous supply of iron is required for the activity of mammalian ribonucleotide reductase (18), an obligatory step in the production of DNA (34). In fact, ribonucleotide reductase purified from calf thymus has an activity half-life of only 10 minutes when the supply of iron and oxygen are removed (18). For this reason iron appears to have an important regulatory role in DNA production.

The nearly normal growth of rats made iron deficient after weaning indicates sufficient availability of iron for DNA production under some circumstances. However, there may be a decreased capacity for maintaining an extremely rapid rate of DNA production. When rats are made iron deficient during the immediate postnatal period of lactation, there is a slower weight gain and a marked decrease in the concentration of DNA in thymus and spleen compared to normally nourished animals (128). Impaired DNA synthesis is also evident after partial hepatectomy in the iron-deficient rat (129) and in the decreased capacity of the T lymphocyte to respond to mitogens in iron-deficient children (as already discussed). It has also been shown more directly that deferoxamine, a potent chelator of iron, inhibits DNA production by human T and B lymphocytes and that this inhibition can be reversed by restoration of iron (130).

Studies of cell-mediated immunity in man yield some discordant data, as summarized in two recent reviews (5, 7) and in the article by Keusch & Farthing in this volume (131). As in the case of neutrophil function, there are the potential confounding influences of other nutritional deficiencies and concurrent or antecedent infections in the patient populations studied. Indeed, since infection results in many of the same laboratory abnormalities as iron deficiency (1, 2), it is possible that the anemia of infection was occasionally misdiagnosed as iron deficiency.

Resistance to intracellular invasion by bacteria, fungi, and viruses is mediated largely by thymus-dependent lymphocytes or T cells. T-cell function is most commonly estimated from the number of circulating T cells, the delayed hypersensitivity skin test response to antigens, and the in vitro lymphocyte response to mitogens such as phytohemagglutinin (PHA). Circulating T cells were decreased in three studies of patients (27, 28, 32). Skin reactivity to a variety of antigens was decreased in some but not all patients (26, 27, 31) and returned toward normal after iron treatment (26, 31). The in vitro response of lymphocytes from iron-deficient patients to PHA was decreased in most (26, 27, 32) but not all (132) studies. The group that reported negative results subsequently showed a T-cell-dependent impairment of delayed cutaneous hypersensitivity (133) as well as decreased splenic lymphocyte response to mitogens (134) in iron-deficient mice.

Annu. Rev. Nutr. 1986.6:13-40. Downloaded from www.annualreviews.org
by Central College on 01/05/12. For personal use only.

Humoral immunity has generally been considered normal in iron-deficient patients on the basis of IgG, IgA, and IgM concentrations (26, 27) and antibody production in response to T-cell-dependent and -independent antigens (27). However, studies in iron-deficient rats show a clear-cut deficit in antibody production to Salmonella and tetanus toxoid (135). Furthermore when nursing rats are made iron-deficient through maternal iron deficiency during pregnancy and lactation, there is a long-term deficit in antibody production that is not corrected by an iron-rich diet (136). The lack of accord between the human and rat studies of humoral immunity in iron deficiency makes this an area requiring further study.

Despite the many studies on the effects of iron deficiency on the immune response, the extent of the impairment remains unclear. As discussed in recent reviews (5, 7), serious flaws in experimental design make it difficult to draw any conclusions from two commonly cited studies that come to opposite conclusions regarding prevalence of infections in iron-deficient infants (126, 127). This is still an area that requires clarification. Differences in morbidity may be difficult to detect in industrialized countries where iron deficiency can be very mild (137), environmental sanitation is satisfactory, and antibiotic treatment readily available. Impaired resistance to infection might be easier to detect among populations in developing countries where none of the above conditions apply and where morbidity from infection is high. However, in those settings it is difficult to exclude the role of other nutrient deficiencies. Even if there were a clear-cut association between iron deficiency and infection, it might be difficult to determine cause and effect. Not only may iron deficiency lead to infection but infection can lead to iron deficiency by impairing iron absorption (138, 139).

An additional complicating factor is the protective role of the iron-binding proteins, transferrin and lactoferrin, in the defense against infection (139a). These proteins act by withholding iron from invading organisms that require it for their proliferation. This protective property can be lost as the iron-binding proteins become increasingly iron saturated with iron excess but could be modestly enhanced with decreased iron saturation in iron deficiency. However, the latter effect is likely to be counterbalanced by impairments of cellular and humoral immunity that have already been discussed. It will be a challenge to disentangle this in man.

Cytochrome P-450 and the Defense of the Body Against Foreign Compounds

A large number of drugs, environmental pollutants, and other nonnutritive chemicals are readily absorbed but poorly eliminated from the body. Their elimination frequently involves oxidative transformation, primarily in the liver and intestine, to water-soluble products that are more readily excreted (140,

Annu. Rev. Nutr. 1986.6:13-40. Downloaded from www.annualreviews.org by Central College on 01/05/12. For personal use only.

141). This oxidative transformation is mediated by a group of cytochrome-P-450-dependent mixed-function oxidations. In contrast to the oxidative production of cellular energy, which is localized in mitochondria, the mixed-function oxidations take place in the endoplasmic reticulum.

Among the iron-containing compounds involved in mixed-function oxidations are the group of heme proteins termed cytochrome P-450 (on the basis of their spectral absorption maximum) and cytochrome b_5. The response of these compounds to iron deficiency and its consequences for drug metabolism have been studied only in the rat. In the liver, where the mixed-function oxidation system is present in high concentration and has been best characterized, there was little or no effect of iron deficiency on the concentration of cytochrome P-450 (142–144) and no change in cytochrome b_5 (143, 144). Even the stimulation of cytochrome P-450 by barbiturate treatment was normal in iron deficiency (143). Rats with severe iron deficiency anemia, had the same fourfold increase in cytochrome P-450 in response to three days of phenobarbital as control animals. This normal response to phenobarbital treatment implies an unusually high priority for the limited amounts of iron from the deficient diet and/or the catabolism of hemoglobin and other iron proteins. In fact, the activity of several drug-metabolizing enzymes and the clearance of aniline and aminopyrine from the plasma were actually increased in iron deficiency (144).

In striking contrast to the situation in the liver is the marked vulnerability of intestinal cytochrome P-450 to iron deficiency (145, 146). An interesting feature of the production of intestinal cytochrome P-450 is its dependence on the day-to-day dietary supply of iron (and selenium), not on iron from systemic sources. As a consequence, the iron-replete rat with abundant storage iron nevertheless shows a decrease in intestinal cytochrome P-450 concentration within a day of starting an iron-deficient diet. Intestinal cytochrome b_5 and NADPH-cytochrome P-450 reductase were not affected. When intestinal cytochrome P-450 is acutely depleted by 1 to 3 days of a low-iron diet, drug oxidation by everted sacs of duodenum is impaired in acutely iron-deprived rats.

An important implication of the findings in respect to intestinal cytochrome P-450 is that day-to-day fluctuations in the diet can influence metabolic processes even when there is no systemic evidence of iron deficiency. This phenomenon is therefore an exception to the general rule that tissue abnormalities do not occur in iron deficiency until iron stores are depleted and hemoglobin production begins to be compromised. The applicability of the rat data to man remain to be demonstrated (147). Nevertheless, it seems quite possible that the response to drugs and other chemicals might vary considerably as a function of the day-to-day adequacy of a dietary nutrient such as iron.

Annu. Rev. Nutr. 1986.6:13-40. Downloaded from www.annualreviews.org by Central College on 01/05/12. For personal use only.

CONCLUDING REMARKS

In the past decade, there has been an increasing awareness of the role of non-hemoglobin iron compounds in the pathogenesis of iron deficiency. Indeed, the literature on this topic has become too large to encompass in a single review. The topics included for discussion in this review were selected because they are characterized by reasonable links between biochemical abnormalities and physiologic manifestations. In certain instances, including the immune response, there are many discordant data. In attempting to integrate and reconcile these studies, one runs the risk of oversimplification. Most of the topics that have been discussed bring to mind additional questions to be asked and studies to be performed. The rapid pace of progress in determining the subtle manifestations of iron deficiency is encouraging because it should provide a necessary perspective for planning measures to prevent, detect, and treat this common disorder.

Annu. Rev. Nutr. 1986.6:13-40. Downloaded from www.annualreviews.org by Central College on 01/05/12. For personal use only.

Literature Cited

1. Bothwell, T. H., Charlton, R. W., Cook, J. D., Finch, C. A. 1979. *Iron Metabolism in Man.* Oxford: Blackwell. 576 pp.
2. Dallman, P. R., Siimes, M. A., Stekel, A. 1980. Iron deficiency in infancy and childhood. *Am. J. Clin. Nutr.* 33:86–118
3. Dallman, P. R. 1974. Tissue effects of iron deficiency. In *Iron in Biochemistry and Medicine,* ed. A. Jacobs, M. Worwood, pp. 437–76. London: Academic
4. Beutler, E., Fairbanks, V. F. 1980. The effect of iron deficiency. See Ref. 10, pp. 394–425
5. Strauss, R. G. 1978. Iron deficiency, infections, and immune function: a reassessment. *Am. J. Clin. Nutr.* 31:660–66
6. Dallman, P. R. 1982. Manifestations of iron deficiency. *Semin. Hematol.* 19:19–30
7. Beisel, W. R. 1982. Single nutrients and immunity. *Am. J. Clin. Nutr.* 35:417–68
8. Lozoff, B., Brittenham, G. M. 1985. Behavioral aspects of iron deficiency. *Prog. Hematol.* In press
9. Worwood, M. 1977. The clinical biochemistry of iron. *Semin. Hematol.* 14: 3–30
10. Jacobs, A., Worwood, M., eds. 1980. *Iron in Biochemistry and Medicine.* London: Academic. 706 pp.
11. Saltman, P., Hegenauer, J., eds. 1982. *The Biochemistry and Physiology of Iron.* New York: Elsevier Science. 836 pp.
12. Hershko, C. 1977. Storage iron regulation. *Prog. Hematol.* 10:105–48
13. Deiss, A. 1983. Iron metabolism in reticuloendothelial cells. *Semin. Hematol.* 20:81–90
14. Huebers, H. A., Finch, C. A. 1984. Transferrin: physiologic behavior and clinical implications. *Blood* 64:763–67
15. Booth, F. W., Holloszy, J. O. 1977. Cytochrome c turnover in rat skeletal muscles. *J. Biol. Chem.* 252:416–19
16. Dallman, P. R., Siimes, M. A., Manies, E. C. 1975. Brain iron: persistent deficiency following short-term iron deprivation in the young rat. *Br. J. Haematol.* 31:209–15
17. Dallman, P. R., Spirito, R. A. 1977. Brain iron in the rat: extremely slow turnover in normal rats may explain long-lasting effects of early iron deficiency. *J. Nutr.* 107:1075–81
18. Thelander, L., Gräslund, A., Thelander, M. 1983. Continual presence of oxygen and iron required for mammalian ribonucleotide reduction: possible regulation mechanism. *Biochem. Biophys. Res. Commun.* 110:859–65
19. Crnic, L. S. 1984. Animal models of early malnutrition: A comment on bias, dependability, and human importance. In *Malnutrition and Behavior: Critical Assessment of Key Issues,* ed. J. Brozek, pp. 460–68. Lausanne: Nestle Found.

Annu. Rev. Nutr. 1986.6:13-40. Downloaded from www.annualreviews.org by Central College on 01/05/12. For personal use only.

20. Morgan, E. H. 1980. Comparative iron metabolism. See Ref. 10, pp. 641–87
21. Siimes, M. A., Refino, C., Dallman, P. R. 1980. Manifestations of iron deficiency at various levels of dietary iron intake. *Am. J. Clin. Nutr.* 33:570–74
22. Dallman, P. R., Refino, C. A., Yland, M. J. 1982. Sequence of development of iron deficiency in the rat. *Am. J. Clin. Nutr.* 35:671–77
23. Jacobs, A. 1961. Iron-containing enzymes in the buccal epithelium. *Lancet* 2:1331–33
24. Dagg, J. H., Jackson, J. M., Curry, B., Goldberg, A. 1966. Cytochrome oxidase in latent iron deficiency (sideropenia). *Br. J. Haematol.* 12:331–33
25. Chandra, R. K. 1973. Reduced bactericidal capacity of polymorphs in iron deficiency. *Arch. Dis. Child.* 48:864–66
26. Macdougall, L. G., Anderson, R., McNab, G. M., Katz, J. 1975. The immune response in iron-deficient children: Impaired cellular defense mechanisms with altered humoral components. *J. Pediatr.* 86:833–43
27. Chandra, R. K. 1975. Impaired immunocompetence associated with iron deficiency. *J. Pediatr.* 86:899–902
28. Srikantia, S. G., Prasad, J. S., Bhaskaram, C., Krishnamachari, K. A.V. R. 1976. Anemia and immune response. *Lancet* 1:1307–9
29. Yetgin, S., Altay, C., Ciliv, G., Laleli, Y. 1979. Myeloperoxidase activity and bactericidal function of PMN in iron deficiency. *Acta Haematol.* 61:10–14
30. Vyas, D., Chandra, R. K. 1984. Functional implications of iron deficiency. In *Iron Deficiency in Infancy and Childhood,* ed. A. Stekel, pp. 45–69. New York: Raven
31. Joynson, D. H. M., Jacobs, A., Walker, D. M., Dolby, A. E. 1972. Defect of cell-mediated immunity in patients with iron-deficiency anaemia. *Lancet* 2:1058–59
32. Bhaskaram, C., Reddy, V. 1975. Cell-mediated immunity in iron and vitamin-deficient children. *Br. Med. J.* 3:522
33. Fletcher, J., Mather, J., Lewis, M. J., Whiting, G. 1975. Mouth lesions in iron-deficient anemia: Relationship to *Candida albicans* in saliva and to impairment of lymphocyte transformation. *J. Infect. Dis.* 131:44–50
34. Reichard, P., Ehrenberg, A. 1983. Ribonucleotide reductase—a radical enzyme. *Science* 221:514–19
35. Woodson, R. D., Wills, R. E., Lenfant, C. 1978. Effect of acute and established anemia on O_2 transport at rest, submaximal and maximal work. *J. Appl. Physiol.* 44:36–43
36. Varat, M. A., Adolph, R. J., Fowles, N. O. 1972. Cardiovascular effects of anemia. *Am. Heart J.* 83:415–26
37. Perkkiö, M. V., Jansson, L. T., Henderson, S., Refino, C., Brooks, G. A., Dallman, P. R. 1985. Work performance in the iron deficient rat: improved endurance with exercise training. *J. Appl. Physiol.* 249:E306–11
38. Finch, C. A., Miller, L. R., Inamdar, A. R., Person, R., Seiler, K., Mackler, B. 1976. Iron deficiency in the rat: physiological and biochemical studies of muscle dysfunction. *J. Clin. Invest.* 58:447–53
39. Davies, K. J. A., Donovan, C. M., Refino, C. J., Brooks, G. A., Packer, L., Dallman, P. R. 1984. Distinguishing effects of anemia and muscle iron deficiency on exercise bioenergetics in the rat. *Am. J. Physiol.* 246:E535–43
40. Beard, J., Green, W., Miller, L., Finch, C. A. 1984. Effect of iron-deficiency anemia on hormone levels and thermoregulation during cold exposure. *Am. J. Physiol.* 247:R114–19
41. McKay, R. H., Higuchi, D. A., Winder, W. W., Fell, R. D., Brown, E. B. 1983. Tissue effects of iron deficiency in the rat. *Biochim. Biophys. Acta* 757:352–58
42. McDonald, R., Hegenauer, J., Sucec, A., Saltman, P. 1984. Effects of iron deficiency and exercise on myoglobin. *Eur. J. Appl. Physiol.* 52:414–19
43. Dallman, P. R., Schwartz, H. C. 1965. Distribution of cytochrome and myoglobin in rats with dietary iron deficiency. *Pediatrics* 35:677–86
44. Davies, K. J. A., Maguire, J. J., Brooks, G. A., Dallman, P. R., Packer, L. 1982. Muscle mitochondrial bioenergetics, oxygen supply, and work capacity during dietary iron deficiency and repletion. *Am. J. Physiol.* 242:E418–27
45. Dallman, P. R., Schwartz, H. C. 1965. Myoglobin and cytochrome c response during repair of iron deficiency in the rat. *J. Clin. Invest.* 44:1631–38

45a. McLane, J. A., Fell, R. D., McKay, R. H., Winder, W. W., Brown, E. B., Holloszy, J. O. 1981. Physiological and biochemical effects of iron deficiency on rat skeletal muscle. *Am. J. Physiol.* 241:C47–54

45b. Ackrell, B. A. C., Maguire, J. J., Dallman, P. R., Kearney, E. B. 1984. Effect of iron deficiency on succinate and NADH-ubiquinone oxidoreductases in skeletal muscle mitochondria. *J. Biol. Chem.* 259:10053–59

Annu. Rev. Nutr. 1986.6:13-40. Downloaded from www.annualreviews.org by Central College on 01/05/12. For personal use only.

46. Mackler, B., Grace, R., Finch, C. A. 1984. Iron deficiency in the rat: effects on oxidative metabolism in distinct types of skeletal muscle. *Pediatr. Res.* 18:499–500
47. Dallman, P. R., Schwartz, H. C. 1964. Cytochrome c concentration during rat and guinea pig development. *Pediatrics* 33:106–10
48. Beutler, E. 1957. Iron enzymes in iron deficiency. I cytochrome c. *Am. J. Med. Sci.* 234:517–27
49. Salmon, H. A. 1962. The cytochrome content of the heart, kidney, liver, and skeletal muscle of iron-deficient rats. *J. Physiol.* 164:17–30
50. Brooks, G. A., Fahey, T. H. 1984. *Exercise Physiology: Human Bioenergetics and Its Applications*. New York: Wiley
51. Goodman, J. R., Warshaw, J. B., Dallman, P. R. 1970. Cardiac hypertrophy in iron and copper deficiency: The quantitative contribution of mitochondrial enlargement. *Pediatr. Res.* 4:244–56
52. Blayney, L., Bailey-Wood, R., Jacobs, A., Henderson, A., Muir, J. 1976. The effects of iron deficiency on the respiratory function and cytochrome content of rat heart mitochondria. *Circ. Res.* 39: 744–48
53. Henderson, S. A., Dallman, P. R., Brooks, G. A. 1986. Glucose turnover and oxidation are increased in iron deficiency. *Am. J. Physiol.* In press
54. Ohira, Y., Hegenauer, J., Strause, L., Chen, S., Saltman, P., Beinert, H. 1982. Mitochondrial NADH dehydrogenase in iron-deficient and iron-repleted rat muscle: an EPR and work performance study. *Br. J. Haematol.* 52:623–30
55. Perkkiö, M. V., Jansson, L. T., Brooks, G. A., Refino, C. A., Dallman, P. R. 1984. Work performance in iron deficiency of increasing severity. *J. Appl. Physiol.* 58:1477–80
56. Dallman, P. R., Sunshine, P., Leonard, Y. 1967. Intestinal cytochrome response with repair of iron deficiency. *Pediatrics* 39:863–71
57. Rasch, C. A., Cotton, E. K., Harris, J. W., Griggs, R. C. 1960. Blood loss as a contributing factor in the etiology of iron-lack anemia in infancy. *Am. J. Dis. Child.* 100:627
58. Hoag, M. S., Wallerstein, R. O., Pollycove, M. 1961. Occult blood loss in iron deficiency anemia of infancy. *Pediatrics* 27:199–203
59. Elian, E., Bar-Shani, S., Liberman, A., Matoth, Y. 1966. Intestinal blood loss: A factor in calculation of body iron in late infancy. *J. Pediatr.* 69:215–19
60. Fomon, S. J., Ziegler, E. E., Nelson, S. E., Edwards, B. B. 1981. Cow milk feeding in infancy: gastrointestinal blood loss and iron nutritional status. *Pediatrics* 98:540-45
61. Wilson, J. F., Heiner, D. C., Lahey, M. E. 1964. Milk-induced gastrointestinal bleeding in infants with hypochromic microcytic anemia. *J. Am. Med. Assoc.* 189:568–72
62. Woodruff, C. W., Clark, J. L. 1972. The role of fresh cows milk in iron deficiency. I. Albumin turnover in infants with iron deficiency anemia. *Am. J. Dis. Child.* 124:18–23
63. Kosnai, I., Kuitonen, P., Siimes, M. A. 1979. Iron deficiency in children with celiac disease on treatment with gluten-free diet. Role of intestinal blood loss. *Arch. Dis. Child.* 54:375–78
64. Lanzkowsky, P., Karayalcin, G., Miller, F., Lane, B. P. 1981. Disaccharidase values in iron-deficient patients. *J. Pediatr.* 99:605–8
65. Sriratanaban, A., Thayer, W. R. 1971. Small intestinal disaccharidase activities in experimental iron and protein deficiency. *Am. J. Clin. Nutr.* 24:411–15
66. Buts, J.-P., Delacroix, D. L., Dekeyser, N., Paquet, S., Horsmans, Y., et al. 1984. Role of dietary iron in maturation of rat small intestine at weaning. *Am. J. Physiol.* 246:G725–31
67. Naiman, J. L., Oski, F. A., Diamond, L. K., Vawter, G. F., Schwachman, H. 1964. The gastrointestinal effects of iron deficiency anemia. *Pediatrics* 33:83–99
68. Kimber, C., Weintraub, L. R. 1968. Malabsorption of iron secondary to iron deficiency. *N. Engl. J. Med.* 279:453–59
69. Schubert, W. K., Lahey, M. E. 1959. Copper and protein depletion complicating hypoferric anemia of infancy. *Pediatrics* 24:710–33
70. Lundström, U., Perkkiö, M., Savilahti, E., Siimes, M. 1983. Iron deficiency anaemia with hypoproteinaemia. *Arch. Dis. Child.* 58:438-41
71. Sturgeon, P. 1959. Studies of iron requirements in infants. III. Influence of supplemental iron during normal pregnancy on mother and infant. B. The infant. *Br. J. Haematol.* 5:45–55
72. Shott, R. J., Andrews, B. F. 1972. Iron status of a medical high-risk population at delivery. *Am. J. Dis. Child.* 124:369–71
73. Jansson, L., Holmberg, L., Ekman, R. 1979. Variation of serum ferritin in low birth weight infants with maternal ferritin, birth weight and gestational age. *Acta Haematol.* 62:273–77
74. MacPhail, A. P., Charlton, R. W., Both-

Annu. Rev. Nutr. 1986.6:13-40. Downloaded from www.annualreviews.org by Central College on 01/05/12. For personal use only.

well, T. H., Torrance, J. D. 1980. The relationship between maternal and infant iron status. *Scand. J. Haematol.* 25:141–50
75. Blot, I., Rey, A., Kaltwasser, J. P., Francoual, J., Papiernik, E., Tchernia, G. 1982. Folate and iron deficiencies in mothers and their newborn children. *Blut* 44:297–303
76. Wallenburg, H. C. S., van Eijk, H. G. 1984. Effect of oral iron supplementation during pregnancy on maternal and fetal iron status. *J. Perinat. Med.* 12:7–12
77. Sisson, T. R. C., Lund, C. J. 1958. The influence of maternal iron deficiency on the newborn. *Am. J. Clin. Nutr.* 6:376–84
78. Singla, P. N., Chand, S., Khanna, S., Agarwal, K. N. 1978. Effect of maternal anaemia on the placenta and the newborn infant. *Acta Paediatr. Scand.* 67:645–48
79. Finch, C. A., Huebers, H. A., Miller, L. R., Josephson, B. M., Shepard, T. H., Mackler, B. 1983. Fetal iron balance in the rat. *Am. J. Clin. Nutr.* 37:910–17
80. Mackler, B., Grace, R., Person, R., Shepard, T. H., Finch, C. A. 1983. Iron deficiency in the rat. Biochemical studies of fetal metabolism. *Teratology* 28:103–7
81. Bailey-Wood, R., Blayney, L. M., Muir, J. R., Jacobs, A. 1975. The effects of iron deficiency on rat liver enzymes. *Br. J. Exp. Pathol.* 56:193–98
82. Dallman, P. R. 1969. Iron restriction in the nursing rat: Early effects upon tissue heme proteins, hemoglobin, and liver iron. *J. Nutr.* 97:475–80
83. Mackler, B., Person, R., Miller, L. R., Inamdar, A. R., Finch, C. A. 1978. Iron deficiency in the rat: biochemical studies of brain metabolism. *Pediatr. Res.* 12:217–20
84. Youdim, M. B. H., Green, A. R., Bloomfield, M. R., Mitchell, B. D., Heal, D. J., Grahame-Smith, D. G. 1980. The effects of iron deficiency on brain biogenic monoamine biochemistry and function in rats. *Neuropharmacology* 19:259–67
85. Land, J. M., Booth, R. F. G., Berger, R., Clark, J. B. 1977. Development of mitochondrial energy metabolism in rat brain. *Biochem. J.* 164:339–48
86. Huebers, H. A., Finch, C. A. 1984. Transferrin: physiologic behavior and clinical implications. *Blood* 64:763–67
87. Larrick, J. W., Hyman, E. S. 1984. Acquired iron-deficiency anemia caused by an antibody against the transferrin receptor. *N. Engl. J. Med.* 311:214–18
88. Symes, A. L., Missala, K., Sourkes, T. L. 1971. Iron and riboflavin-dependent metabolism of a monoamine in the rat in vivo. *Science* 174:153–55
89. Voorhess, M. L., Stuart, M. J., Stockman, J. A., Oski, F. A. 1975. Iron deficiency anemia and increased urinary norepinephrine excretion. *J. Pediatr.* 86:542–47
90. Pollitt, E., Leibel, R. L., eds. 1982. *Iron Deficiency: Brain Biochemistry and Behavior*, p. 214. New York: Raven
91. Pollitt, E. 1985. Effects of iron deficiency with and without anemia on mental development among infants and preschool children. In *Nutritional Anthropology*, ed. F. E. Johnston. New York: Liss. In press
92. Ashkenazi, R., Ben-Shachar, D., Youdim, M. B. H. 1982. Nutritional iron and dopamine binding sites in the rat brain. *Pharmacol. Biochem. Behav.* 17(Suppl. 1):43–47
93. Kaladhar, M., Rao, B. S. N. 1982. Effects of iron deficiency on serotonin uptake in vitro by rat brain synaptic vesicles. *J. Neurochem.* 38:1576–81
94. Tamir, H., Klein, A., Rapport, M. M. 1976. Serotonin binding protein: enhancement of binding by Fe^{2+} and inhibition of binding by drugs. *J. Neurochem.* 26:871–78
95. Yehuda, S., Youdim, M. B. H. 1984. The increased opiate action of β-endorphin in iron deficient rats: the possible involvement of dopamine. *Eur. J. Pharmacol.* 104:245–51
96. Youdim, M. B. H., Yehuda, S., Ben-Uriah, Y. 1981. Iron deficiency–induced circadian rhythm reversal of dopaminergic-mediated behaviors and thermoregulation in rats. *Eur. J. Pharmacol.* 74:295–301
97. Glover, J., Jacobs, A. 1972. Activity pattern of iron-deficient rats. *Br. Med. J.* 2:627–28
98. Edgerton, V. R., Bryant, S. L., Gillespie, C. A., Gardner, G. W. 1972. Iron deficiency anemia and physical performance and activity of rats. *J. Nutr.* 102:381–400
99. Martin J. C., Martin, D. C., Dillmann, E., Day, H. E., Sigman, G. 1980. Effects of ambient temperature upon diurnal activity in nutritionally iron-deficient rats. *Bull. Psychon. Soc.* 15:18–20
100. Dallman, P. R., Refino, C. A., Dallman, M. F. 1984. The pituitary-adrenal response to stress in the iron-deficient rat. *J. Nutr.* 114:1747–53
101. Youdim, M. B. H., Grahame-Smith, D. G., Woods, H. F. 1975. Some properties

Annu. Rev. Nutr. 1986.6:13-40. Downloaded from www.annualreviews.org by Central College on 01/05/12. For personal use only.

of human platelet monamine oxidase in iron-deficiency anemia. *Clin. Sci. Mol. Med.* 50:479–85

102. Weinberg, J., Levine, S., Dallman, P. R. 1979. Long-term consequences of early iron deficiency in the rat. *Pharmacol. Biochem. Behav.* 11:631–38
103. Groeneveld, D., Smeets, H. G. W., Kabra, P., Dallman, P. R. 1985. Urinary catecholamines in iron-deficient rats at rest and following surgical stress. *Am. J. Clin. Nutr.* 42:263–69
104. Wagner, A., Fortier, N., Giroux, A., Lukes, J., Snyder, L. M. 1978. Catecholamines in adult iron deficient patients. *Experimentia* 35:102–3
105. Dillmann, E., Johnson, D. G., Martin, J., Mackler, B., Finch, C. A. 1979. Catecholamine elevation in iron deficiency. *Am. J. Physiol.* 237:R297–300
106. Dillmann, E., Gale, C., Green, W., Johnson, D. G., Mackler, B., Finch, C. A. 1980. Hypothermia in iron deficiency due to altered triiodothyronine metabolism. *Am. J. Physiol.* 239:R377–81
107. Beard, J., Green, W., Miller, L., Finch, C. 1984. Effect of iron-deficiency anemia on hormone levels and thermoregulation during cold exposure. *Am. J. Physiol.* 247:R114–19
108. Martinez-Torres, C., Cubeddu, L., Dillmann, E., Brengelmann, G. L., Leets, I., et al. 1984. Effect of exposure to low temperature on normal and iron-deficient subjects. *Am. J. Physiol.* 246:R380–83
109. Oski, F. A., Honig, A. S. 1978. The effects of therapy on the developmental scores of iron-deficient infants. *J. Pediatr.* 92:21–25
110. Lozoff, B., Brittenham, G. M., Viteri, F. E., Wolf, A. W., Urrutia, J. J. 1982. The effects of short-term oral iron therapy on developmental deficits in iron-deficient anemic infants. *J. Pediatr.* 100:351–57
111. Walter, T., Kovalskys, J., Stekel, A. 1983. Effect of mild iron deficiency on infant mental development scores. *J. Pediatr.* 102:519–22
112. Lozoff, B., Brittenham, G. M., Viteri, F. E., Wolf, A. W., Urrutia, J. J. 1982. Developmental deficits in iron-deficient infants: Effects of age and severity of iron lack. *J. Pediatr.* 101:948–52
113. Oski, F. A., Honig, A. S., Helu, B., Howanitz, P. 1983. Effect of iron therapy on behavior performance in nonanemic, iron-deficient infants. *Pediatrics* 71: 877–80
114. Palti, H., Pevsner, B., Adler, B. 1983. Does anemia in infancy affect achievement on developmental and intelligence tests? *Hum. Biol.* 55:189–94
115. Seshadri, S., Hirode, K., Naik, P., Malhotra, S. 1982. Behavioural responses to young anaemic Indian children to iron-folic acid supplements. *Br. J. Nutr.* 48:233–40
116. Pollitt, E., Soemantri, A. G., Yunis, F., Scrimshaw, N. S. 1985. Cognitive effects of iron-deficiency anaemia. *Lancet* 1:158
117. Pollitt, E., Viteri, F., Saco-Pollitt, C., Leibel, R. L. 1982. Behavioral effects of iron deficiency anemia in children. See Ref. 90, pp. 195–208
118. Moore, L. L., Humbert, J. R. 1984. Neutrophil bactericidal dysfunction towards oxidant radical-sensitive microorganisms during experimental iron deficiency. *Pediatr. Res.* 18:684–89
119. Kulapongs, P., Vithayasai, V., Suskind, R., Olson, R. E. 1974. Cell-mediated immunity and phagocytosis and killing function in children with severe iron-deficiency anaemia. *Lancet* 2:689–91
120. Babior, B. M. 1983. The respiratory burst of phagocytes. *J. Clin. Invest.* 73:599–601
121. Cramer, E., Pryzwansky, K. B., Villeval, J.-L., Testa, U., Breton-Gorius, J. 1985. Ultrastructural localization of lactoferrin and myeloperoxidase in human neutrophils by immunogold. *Blood* 65: 423–32
122. Ambruso, D. R., Johnson, R. B. Jr. 1981. Lactoferrin enhances hydroxyl radical production by human neutrophils, neutrophil particulate fractions, and an enzymatic generating system. *J. Clin. Invest.* 67:352–60
123. Higgs, J. M., Wells, R. S. 1972. Chronic mucocutaneous candidiasis: associated abnormalities on iron metabolism. *Br. J. Dermatol.* 86(Suppl. 8):88–102
124. Prasad, J. S. 1979. Leucocyte function in iron-deficiency anemia. *Am. J. Clin. Nutr.* 32:550–52
125. Mackler, B., Person, R., Ochs, H., Finch, C. A. 1984. Iron deficiency in the rat: effects on neutrophil activation and metabolism. *Pediatr. Res.* 18:549–51
126. Andelman, M. B., Sered, B. R. 1966. Utilization of dietary iron by term infants: A study of 1,048 infants from slow socioeconomic population. *Am. J. Dis. Child.* 111:45–55
127. Burman, D. 1972. Hemoglobin levels in normal infants aged 3–24 months and the effect of iron. *Arch. Dis. Child.* 47:261–71
128. Kochanowski, B. A., Sherman, A. R. 1985. Cellular growth in iron deficient

rats: effect of pre- and postweaning iron repletion. *J. Nutr.* 115:279–87
129. Siimes, M. A., Dallman, P. R. 1974. Iron deficiency: impaired liver growth and DNA synthesis in the rat. *Br. J. Haematol.* 28:453–62
130. Lederman, H. M., Cohen, A., Lee, J. W. W., Freedman, M. H., Gelfand, E. W. 1984. Deferoxamine: a reversible S-phase inhibitor of human lymphocyte proliferation. *Blood* 64:748–53
131. Keusch, G. T., Farthing, M. J. G. 1986. Nutrition and infection. *Ann. Rev. Nutr.* 6:131–54
132. Kulapongs, P., Vithayasai, V., Suskind, R., Olson, R. E. 1974. Cell-mediated immunity and phagocytosis and killing function in children with severe iron-deficiency anaemia. *Lancet* 2:689–91
133. Kuvibidila, S. R., Baliga, B. S., Suskind, R. M. 1981. Effects of iron deficiency anemia on delayed cutaneous hypersensitivity in mice. *Am. J. Clin. Nutr.* 34:2635–40
134. Kuvibidila, S., Nauss, K. M., Baliga, B. S., Suskind, R. M. 1983. Impairment of blastogenic response of splenic lymphocytes from iron-deficient mice: in vivo repletion. *Am. J. Clin. Nutr.* 37:15–25
135. Nalder, B. N., Mahoney, A. W., Ramakrishnan, R., Hendricks, D. G. 1971. Sensitivity of the immunological response to the nutritional status of rats. *J. Nutr.* 102:535–42
136. Kochanowski, B. A., Sherman, A. R. 1985. Decreased antibody formation in iron-deficient rat pups—effect of iron repletion. *Am. J. Clin. Nutr.* 41:278–84
137. Dallman, P. R., Yip, R., Johnson, C. 1984. Prevalence and causes of anemia in the United States, 1976–1980. *Am. J. Clin. Nutr.* 39:437–45
138. Beresford, C. H., Neale, R. J., Brooks, O. G. 1971. Absorption and pyrexia. *Lancet* 1:568–72
139. Bender-Götze, C., Ludwig, U., Schafer, K. H., Heinrich, H. C., Oppitz, K. H. 1976. Cytochemische knochenmarksbefunde und diagnostische $^{59}Fe^{2+}$-absorption wahrend des akuten und chronischen infektes im kindesalter. *Monatsschr. Kinderheilkd.* 124:305–7
139a. Weinberg, E. D. 1984. Iron withholding: a defense against infection and neoplasia. *Physiol. Rev.* 64:65–102
140. Remmer, H. 1970. Role of the liver in drug metabolism. *Am. J. Med.* 49:617–29
141. Gram, T. E., ed. 1980. *Extrahepatic Metabolism of Drugs and Other Foreign Compounds*. New York: SP Medical and Scientific. 601 pp.
142. Catz, C. S., Juchau, M. R., Yaffe, S. J. 1970. Effects of iron, riboflavin and iodine deficiencies on hepatic drug-metabolizing enzymes systems. *J. Pharmacol. Exp. Ther.* 174:197–205
143. Dallman, P. R., Goodman, J. R. 1971. The effects of iron deficiency on the hepatocytes: A biochemical and ultrastructural study. *J. Cell. Biol.* 48:79–90
144. Becking, G. C. 1972. Influence of dietary iron levels on hepatic drug metabolism in vivo and in vitro in the rat. *Biochem. Pharmacol.* 21:1585–93
145. Hoensch, H., Woo, C. H., Raffin, S. B., Schmid, R. 1976. Oxidative metabolism of foreign compounds in rat small intestine: cellular localization and dependence on dietary iron. *Gastroenterology* 70:1063–70
146. Pascoe, G. A., Correia, M. A. 1985. Structural and functional assembly of rat intestinal cytochrome P-450 isoenzymes. Effects of dietary iron and selenium. *Biochem. Pharmacol.* 34:599–608
147. O'Malley, K., Stevenson, I. H. 1972. Iron deficiency anemia and drug metabolism. *J. Pharm. Pharmacol.* 25:339–40

Annu. Rev. Nutr. 1986.6:13-40. Downloaded from www.annualreviews.org by Central College on 01/05/12. For personal use only.