A REVIEW OF THE SIGNIFICANCE OF DIETARY IRON ON IRON STORAGE PHENOMENA

NOVEMBER 1972

PREPARED FOR

DIVISION OF NUTRITION
BUREAU OF FOODS
FOOD AND DRUG ADMINISTRATION
WASHINGTON, D.C. 20204

CONTRACT NO. FDA 71-294
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by

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FOREWORD

The Life Sciences Research Office (LSRO), Federation of American Societies for Experimental Biology (FASEB), provides scientific assessments of topics in the biomedical sciences. These reports are based upon comprehensive literature reviews and the perceptive observations of knowledgeable scientists engaged in work in the field. Although LSRO reports are recognized by FASEB as contributions to societal needs and most LSRO consultants are members of FASEB constituent societies, the reports do not necessarily reflect the views of the members of its six constituent societies. However, the report has been reviewed for policy matters by the LSRO Advisory Committee, which includes representatives of each constituent society.

This technical report was prepared for the Division of Nutrition, Bureau of Foods, Food and Drug Administration, by the Staff of the Life Sciences Research Office, FASEB, in accordance with the provisions of FDA contract number 71-294.

The report reflects the varied opinions of the participants in an ad hoc review group that met at Beaumont House, FASEB, November 30 - December 1, 1971, and the other consultants. The first draft of the report, prepared by the LSRO Staff, was reviewed by these experts. Because there were major differences of scientific opinion regarding the conclusions of the report, a second draft was prepared and submitted to the consultants. It proved to be impossible to reconcile the divergent views into a single consensus; therefore, the final report reflects all viewpoints expressed by the consultants.

The listing of the consultants' names in Section XI must not be construed as indicating that they are authors of the report or that they endorse the study conclusions.

C. Jelleff Carr, Ph.D.
Director
Life Sciences Research Office

- 3 -
SUMMARY

This report provides a review and assessment of the possible hazard that might result from increased iron enrichment of wheat flour and flour-containing dietary items. It identifies the iron compounds of the body associated with iron storage phenomena and emphasizes the importance of the intestinal mucosa in regulating iron absorption. Particular attention is focused upon the several forms of iron storage disorders including hemosiderosis, hemochromatosis, and other diseases characterized by accumulation of excess body iron.

The report reviews the evolution of animal models of iron storage disorders and suggests that adequate models of human iron storage phenomena remain to be developed. The significance of heme and non-heme iron in different food categories is considered in relation to regulation of iron absorption through the intestinal mucosa of both normal individuals and those afflicted with iron storage disorders.

From estimates of food intake and iron content of different foods, the increase in average daily iron intake was calculated for a representative group of males and one of females. The proposed increased iron enrichment would raise the average daily intake of the group of males from the present 17.9 to about 21.5 mg/day. The average daily iron intake of the females would be increased from 11.3 to 13.4 mg/day. These averages do not describe the amount of iron that would be ingested by individuals at either end of the curve of normal distribution. Data are not available concerning the amount of iron ingested by these segments of the population.

It is concluded that the proposed increase in the iron content of enriched flour and flour-containing dietary items will have little or no effect on the accumulation of iron by normal males, and that the extra iron per se will not precipitate hemochromatosis or other hereditary iron storage disorders, although it may accelerate the course of these diseases. The consultants were not unanimous in their opinion as to the significance of the proposed increase in the non-heme iron content of the diet on the accumulation of iron in the latent stages of these disorders. It was agreed that additional research would be required to resolve conclusively the effect of the proposed level of dietary iron on iron accumulation.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foreword</td>
<td>3</td>
</tr>
<tr>
<td>Summary</td>
<td>5</td>
</tr>
<tr>
<td>I. Background</td>
<td>9</td>
</tr>
<tr>
<td>II. Statement of the Problem</td>
<td>11</td>
</tr>
<tr>
<td>III. Scope of the Study</td>
<td>13</td>
</tr>
<tr>
<td>IV. Iron Metabolism</td>
<td>15</td>
</tr>
<tr>
<td>A. Iron Compounds in the Body</td>
<td>15</td>
</tr>
<tr>
<td>B. Physiological Regulation of Iron Balance</td>
<td>20</td>
</tr>
<tr>
<td>V. Iron Overload and Iron Storage Disorders</td>
<td>23</td>
</tr>
<tr>
<td>A. Methods for Measuring Iron Stores</td>
<td>23</td>
</tr>
<tr>
<td>B. Hemosiderosis</td>
<td>25</td>
</tr>
<tr>
<td>1. General Aspects</td>
<td>25</td>
</tr>
<tr>
<td>2. Bantu Siderosis</td>
<td>25</td>
</tr>
<tr>
<td>C. Hemochromatosis</td>
<td>28</td>
</tr>
<tr>
<td>D. Other Iron Storage Disorders</td>
<td>33</td>
</tr>
<tr>
<td>E. Animal Models</td>
<td>34</td>
</tr>
<tr>
<td>VI. Iron Intake</td>
<td>39</td>
</tr>
<tr>
<td>A. Food Iron and Its Absorption</td>
<td>39</td>
</tr>
<tr>
<td>1. Absorption from Single Foods</td>
<td>40</td>
</tr>
<tr>
<td>2. Absorption from Mixtures of Foods</td>
<td>40</td>
</tr>
</tbody>
</table>
I. BACKGROUND

The Food and Drug Administration (FDA) has proposed that the iron content of enriched wheat flour be increased from the present 13.0-16.5 mg to 40 mg per pound; and that of enriched bread, buns and rolls be increased from the present 8.0-12.5 mg to 25 mg per pound (Federal Register, December 3, 1971, 36 F.R. 23074). These increases in the iron content of enriched flour-containing dietary items have been proposed to prevent or reduce the prevalence of iron-deficiency in large segments of the population of the United States. On the other hand, the question has been raised as to possible untoward effects of the additional iron enrichment on certain individuals.

It has been suggested by some that the increased iron intake may pose some hazard for those adults who do not require additional dietary iron. Concern has been expressed about the possibility of increasing the severity of iron overload in patients with diseases involving deranged iron metabolism. In addition, there has been speculation that the increase in dietary iron may accelerate the deposition of iron in persons with latent iron storage disorders. Therefore, FDA requested that LSRO review current scientific knowledge concerning dietary iron in iron overload syndromes and related iron storage phenomena in man and experimental animals.

Historically, in the United States, flour enrichment with iron and certain vitamins to improve nutrient intake has been practiced since 1942. This supplementation of the diet via cereal products was accepted as a logical way to make available these nutrients to all segments of the population. More recently, the proposed increase in iron enrichment is contemplated in response to improved knowledge of the prevalence of iron deficiency in the United States and to the recommendations of groups such as the Food and Nutrition Board, National Academy of Sciences, National Research Council (NAS-NRC); the American Medical Association (AMA) Council on Foods and Nutrition; and several panels of the 1969 White House Conference on Food, Nutrition and Health.
The specific objective of this review has been to provide the FDA with additional information necessary for evaluation of any possible hazards of iron overload in the U. S. population that could conceivably result from the proposed increase in the level of iron in cereal products. In this respect, the report will contribute to the assessment of the significance of current and potential sources of dietary iron in the American diet.
II. STATEMENT OF THE PROBLEM

Increasing the dietary iron intake may influence the absorption, tissue iron accumulation, and the pathogenesis of iron overload syndromes. However, to assess this effect adequately, one must clearly define the incidence and severity of human iron storage disorders in the United States population. Therefore, it is necessary to examine critically the evidence for diet-related iron overload and the factors known to control the rates of absorption and storage, especially over long periods of time. Although the subject of iron utilization in the human body has been investigated by numerous workers, many of the details of knowledge concerning absorption, transport, utilization, storage, and excretion are unknown.

The problems of determining the relative importance of the various forms of dietary iron, the significance of siderosis without clinically overt disease, and the hazards of increasing dietary iron to the potential hemochromatotic are discussed. The report also includes the identification of those subjects, usually adults, who may be at risk, and the factors of liver disease or other states involved in enhanced iron deposition.
III. SCOPE OF THE STUDY

Preliminary planning meetings with outstanding authorities assisted in outlining the objectives and scope of this report. The scientific experts in the field were to identify gaps in existing knowledge and suggest experimental studies to generate the required information. An attempt has been made to indicate these opportunities for exploitation. Finally, this review was to assess the available information on iron metabolism and any relationship to dietary iron, and to draw some tentative conclusions on the effects of increased enrichment on iron overload.

The study includes a brief discussion of iron metabolism in normal man and in iron storage diseases, the different sources of dietary iron and the influence of other food items on iron absorption. Such factors as the influence of alcohol on the intestinal absorption of iron and its subsequent distribution and storage were considered relevant. The significance of non-food iron and other dietary constituents have been considered within the scope of the study because they influence the total amount of ingested iron. The study includes a literature survey to document opportunities to develop satisfactory experimental animal models for the study of iron absorption, storage and iron overload injury.

The diagnostic, preventive and therapeutic measures currently employed in iron overload disorders have been discussed. Throughout this study, attention has been directed to the important desiderata of procedures and tests to permit the early detection of those individuals who, for unknown reasons, absorb iron beyond their needs and add iron to their tissue stores.

The primary concern of this study has been to examine the possibility of untoward effects of the proposed addition to the level of iron in the national diet. Under the terms of the FDA contract, the nutritional value of increased iron in the diet was not a part of the study.
IV. IRON METABOLISM

A. IRON COMPOUNDS IN THE BODY

Because this report is of interest to individuals in fields other than iron metabolism, it is appropriate to review briefly the structure and function of biologic iron compounds and the interrelationships among the various compounds in the human body. For more comprehensive review of these subjects, one or more of the several recent reviews should be consulted (Bothwell and Finch, 1962; Moore, 1968; Conrad, 1970; Finch et al., 1970).

Although iron, quantitatively, is a minor constituent of the animal body, it is involved in the regulation of some most important metabolic reactions. In performing each of these functions the iron is combined with a distinctive protein of high molecular weight, forming the active center of the prosthetic group, the functional part of the molecule. According to Bothwell and Finch (1962) and Finch (1969) the total body iron for the normal adult male is 3 to 4 g distributed as follows: hemoglobin 65-70 per cent; myoglobin 3-5 per cent; ferritin and hemosiderin 25-30 per cent. The remaining amount of body iron is contained in the transferrin and the various iron-containing enzymes outlined in Table 1. In the female, during her reproductive years, the total body iron is closer to 2.5 g because of a smaller blood volume and its lesser content of hemoglobin. In addition, iron stores in the normal female are lower because of menstruation and parturition.

The hemoglobin of the red blood cell is the transporter of molecular oxygen to the tissues, while the iron-containing enzymes function at the cellular level in a variety of metabolic, oxygen-requiring energy cycles. There are three iron-containing compounds of the body that function, not in the transport and utilization of oxygen, but in the transport and storage of iron itself: transferrin, ferritin and hemosiderin. Transferrin is the iron-binding protein of the blood that functions in iron transport. Ferritin and hemosiderin are the two forms of storage iron. Ferritin is soluble and more labile than hemosiderin. Both are found mainly in the liver, spleen and bone.
### TABLE 1

**APPROXIMATE COMPOSITION OF IRON-CONTAINING COMPOUNDS IN THE ADULT MALE**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Prosthetic Groups per Molecule</th>
<th>Iron in Grams</th>
<th>Percentage of Total Iron</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron porphyrin (heme) compounds</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>4 hemes</td>
<td>650-750</td>
<td>2.1-2.5</td>
</tr>
<tr>
<td>Myoglobin</td>
<td>1 heme</td>
<td>40</td>
<td>0.13</td>
</tr>
<tr>
<td>(i) Mitochondrial cytochromes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c</td>
<td>1 heme</td>
<td>0.8</td>
<td>0.004</td>
</tr>
<tr>
<td>a₃, a, c₁, b</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(ii) Microsomal cytochromes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b₅</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(iii) Catalase</td>
<td>-</td>
<td>5.0</td>
<td>0.004</td>
</tr>
<tr>
<td>(iv) Peroxidase</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Nonheme compounds</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flavin-Fe enzymes</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Iron chelate enzyme aconitase</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Transport protein - transferrin</td>
<td>2 Fe</td>
<td>10.0</td>
<td>0.004</td>
</tr>
<tr>
<td>Storage compounds</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferritin</td>
<td>4(FeOOH)n</td>
<td>-</td>
<td>0.8-1.5</td>
</tr>
<tr>
<td>Hemosiderin</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total body iron</td>
<td></td>
<td>3-4</td>
<td>100</td>
</tr>
</tbody>
</table>

Modified from Bothwell and Finch, 1962.
marrow which, under normal circumstances, are sites of iron storage.

From the clinical viewpoint and in the context of this report, the most important of the iron-containing compounds are the two found in blood, hemoglobin and transferrin, and the two storage compounds, ferritin and hemosiderin. Although the functions of myoglobin and the iron-containing enzymes are of unquestioned importance in the daily metabolism of the body, they do not appear to have a significant role in the phases of iron metabolism associated with iron overload phenomena.

The iron content of normal hemoglobin is 0.34 per cent. The hemoglobin content of the blood of normal adult males is approximately 15.0 g per 100 ml. Therefore, 100 ml whole blood contains 50 mg of iron. These figures emphasize the importance of blood loss or, conversely, blood transfusion on the iron balance of the body. The donation of one unit (475 ml) of blood reduces the body iron of the donor by 237 mg and increases that of the recipient by the same amount. In the presence of normal iron stores this amount can be spared without difficulty by the donor but it may take several months to replenish the stores.

The average life span of the normal red blood cell has been determined to be approximately 120 days (Bothwell and Finch, 1962). This implies that all of the corpuscles present in the blood at any one time will become senescent and be replaced during the succeeding 120 days. Using the values for hemoglobin in Table 1, this indicates a daily synthesis of 5.4 to 6.2 g hemoglobin and a daily turnover of 17 to 21 mg of iron. These values apply only to the normal. In the presence of any of the various hemoglobinopathies or other causes of hemolysis, the life span of the red cells is shortened resulting in a greater demand on the erythropoietic marrow for hemoglobin production. Because the body conserves the iron from senescent red cells and returns it promptly to the marrow for the production of new hemoglobin, such conditions increase the turn-over rate of iron but do not impose a demand for additional iron. Indeed, in some hemolytic conditions the intestinal mucosa responds by absorbing more iron than is needed, leading to an increase in the iron stores which may be excessive in some cases.
Transferrin is an iron-binding glycoprotein that occurs not only in the blood plasma but also in the extravascular fluids of the body. It may be characterized as the "iron broker," accepting and carrying iron to tissues from storage sites. The transferrin molecule is a true carrier because the protein (apotransferrin) is neither consumed nor degraded when the bound iron is released.

According to Harrison (1969), human serum transferrin has a molecular weight of 86,000-93,000; each molecule can bind two atoms of ferric iron. At physiological pH it has a high affinity for iron but it may complex less firmly with other metals, such as copper. Although there is some evidence that the iron atom at one of the transferrin sites is more readily exchanged than that at the other (Harrison, 1969), in vivo evidence is consistent with a homogeneous behavior of transferrin-bound iron (Finch et al., 1970). Only 0.1 per cent of the total body iron is bound to transferrin; i.e., at any one time, about 4.0 mg of iron is bound in the plasma. It is estimated that the total transferrin iron turnover is 30 to 40 mg per day, the bulk of which is delivered to the erythropoietic bone marrow (Harrison, 1969). The transferrin iron is selectively taken up by immature RBC actively synthesizing hemoglobin.

The apotransferrin content of normal human plasma (or serum) is between 0.24 and 0.28 g per 100 ml. Under certain disease conditions its concentration may be increased, under others, decreased. However, its concentration is more easily and more usefully given in terms of the amount of iron which is bound to it. Under normal conditions the transferrin is only about one-third saturated, representing serum iron (SI) values ranging from 70 to 150 μg iron per 100 ml plasma. When completely saturated with iron, values of 200 to 300 μg may be obtained and such figures are referred to as total iron binding capacity (TIBC).

SI and TIBC are important determinations in assessing the iron metabolism of the body. In iron deficiency with lower than normal transferrin saturation, SI is low and TIBC is increased. In the presence of excess body stores of iron, the per cent transferrin saturation is higher. Crosby (1968) has emphasized the homeostasis of transferrin. Thus, if physiological adjustments can be made, the transferrin soon returns to the normal range of saturation. If either iron deficiency or iron overload is pushed to an extreme, the transferrin becomes either almost devoid of iron or almost completely
saturated. A more complete discussion of the functions of transferrin is given by Bothwell and Finch (1962) and Harrison (1969).

Ferritin and hemosiderin are the two compounds in which iron is stored in the body. Neither participates actively in metabolic processes, their function being to hold a relatively large amount of iron in the form that is non-toxic to the cell in which it occurs. Harrison (1969) has reviewed the metabolism of ferritin and hemosiderin in some detail.

Ferritin is a well-characterized iron-protein complex which has been isolated in crystalline form from the liver or spleen of many animal species. Its synthesis may be stimulated by administration of excess iron (Granick, 1942; Fineberg and Greenberg, 1955), a characteristic which is thought by some to be important in regulating the absorption of iron by the mucosal cells of the intestine (Crosby, 1968). The iron of ferritin occurs as a central core or "micelle" and is surrounded by a shell of protein. Different ferritin preparations contain variable amounts of iron but the average is around 20 per cent of the dry weight (Granick, 1942). It is estimated that between 4000 to 5000 iron atoms are held in each molecule of ferritin (Harrison, 1969). The ability of the cells of the body to synthesize the iron-free protein, apoferritin, in response to the presence of uncommitted iron is of obvious importance in the iron storage role of ferritin.

Hemosiderin, or siderin, is related to ferritin both chemically and in metabolic function. In general, hemosiderin represents aggregated ferritin molecules together with other cell constituents (Harrison, 1969). Preparations of hemosiderin are not homogeneous but vary in iron, phosphorus and protein content. Compared with ferritin, hemosiderin is insoluble and a much less active and less mobile source of iron; however, both will exchange their iron. The greater the iron stores, the more visible are the hemosiderin granules in histologic preparations, e.g. reticuloendothelial cells of the liver and bone marrow. If body stores become depleted the hemosiderin essentially disappears.
B. PHYSIOLOGICAL REGULATION OF IRON BALANCE

Total body iron balance is maintained by the interaction of absorption through the small intestine, recycling of iron from senescent RBC, and limited excretion (Crosby, 1969; Conrad, 1970). From a nutritional viewpoint, iron is an unusual nutrient; once absorbed into the body, most iron is conserved and there is limited excretion. Absorption probably plays the most active role in regulation of iron balance (Conrad, 1970).

The total daily exchange of iron between the healthy adult male and his environment is estimated to be 0.9 to 1.25 mg (Green et al., 1968; Heinrich, 1970). A small amount of iron is eliminated through loss of desquamated cells of the skin, gastrointestinal and urinary tracts, and perhaps through the bile. There is a small, but measurable, normal loss of RBC into the gastrointestinal tract. These losses are offset by absorption in the small intestine. Most authorities agree that, in the normal adult male, the amount of iron that must be replaced by absorption from the gut is approximately 1.0 mg per day.

An important part of the control of iron balance of the body resides in the cells of the intestinal mucosa which are thought to regulate the amount of iron that is absorbed. As discussed in Section VI (see p. 39), the amount and form of the iron in the daily diet also have an influence on the amount of iron absorbed. Nevertheless, a control or feedback mechanism is thought to be present in the mucosal cells that control the avidity with which iron is absorbed and transferred to the blood. The size of the body iron stores is recognized as perhaps the most important factor in influencing the feedback mechanism which in turn regulates iron absorption from the gut.

Because of the central role which transferrin plays in the interchange of iron among the several body pools, Crosby (1969) has suggested that transferrin saturation or turnover may be involved in regulation of iron balance. Because transferrin is critical to transport of iron from the intestine or stores to the erythropoietic tissues in the bone, it is logical to conclude that it is involved in regulation of mucosal absorption and transfer. This concept is not universally accepted; for example, Harrison (1969) concluded that
neither percentage saturation of transferrin nor iron level in stores influenced iron absorption.

The various methods used in the measurement of the intestinal absorption of iron in man have been evaluated and the more precise procedures, based on the use of radioiron isotopes, for assessing the iron status of human subjects have been reviewed by Heinrich (1970).
V. IRON OVERLOAD AND IRON STORAGE DISORDERS

While the intestinal mucosa functions in regulating iron absorption, other factors can influence the amount that will be absorbed. These include the amount of iron and the form in which it exists in the foodstuffs that comprise the normal diet. The presence of components such as phytates and phosphates which tend to form insoluble iron compounds may decrease absorption. Other dietary items such as alcohol and ascorbic acid are known to increase iron absorption.

It appears that complete control of iron absorption does not reside in the intestinal mucosal cells, although under normal conditions and in healthy individuals this control is adequate. However, in certain disease states or under unusual dietary conditions, the absorptive function of the mucosa may be deranged or overwhelmed, leading to increased absorption and ultimately to excessive body stores of iron.

Hemosiderosis (or, more simply, siderosis) is a term used by some investigators to denote a focal or general increase in tissue iron stores without obvious associated tissue damage. The term hemochromatosis is used by some investigators to denote a condition of widespread iron deposits including organs not normally involved in iron storage, and evidence of disease or malfunction, particularly of the liver. The term iron overload has come into use as a designation covering all forms and degrees of excess iron content of the body. Some authorities consider the term imprecise, because the upper limit, or significance, of excess iron stores is unknown. Other authorities do not agree that the term is imprecise, or that the significance of iron stores is unknown.

A. METHODS FOR MEASURING IRON STORES

The most frequently used clinical method of assessing iron stores in patients is histological examination for stainable iron in specimens of bone marrow or liver obtained by needle biopsy. The
grading of the amount of iron seen in histological sections by experienced personnel agrees well with the chemical determinations that have been made on the same organs (Gale et al., 1963).

Another procedure is the parenteral injection of one of the strong iron chelating agents such as diethylene-triamine-pentaacetic acid (DTPA) or desferrioxamine (Desferal®). These compounds form complexes with the iron from the various pools of the body which are then excreted in the urine and also in the feces by way of the bile. Although the iron removed from the body in this manner is relatively small, the amount is considered to be proportional to that of the body stores. Some workers (Barry et al., 1969, 1970a, 1970b) claim that the desferrioxamine test gives a reasonably accurate estimate of the size of the iron stores in patients with hemochromatosis. However, in other forms of liver disease the results may not reflect reliably the amounts of stored iron (Barry et al., 1970a, 1970b).

The measurement of SI and TIBC are useful screening tests to suggest the presence of either low or increased iron stores, especially since these determinations have become automated laboratory procedures. In idiopathic hemochromatosis the serum iron values are generally high but also the serum transferrin may be between 90 and 100 per cent saturated (Bothwell and Finch, 1962). Because other factors influence these serum values they cannot be accepted as diagnostic of iron overload without other confirmation (MacDonald, 1970; Bothwell and Finch, 1962).

The most accurate, but inconvenient, method for measuring body iron stores is repeated phlebotomy at regular intervals until mild iron deficiency anemia persists. The amount of iron removed is easily calculated on the basis of the hemoglobin level of the blood and its known iron content. Phlebotomy removes both forms of storage iron, ferritin and hemosiderin, from all of the storage sites (Balcerzak, 1969). Phlebotomy also affords a method of evaluating maximal increases in iron absorption after the mild iron deficiency anemia is established.

Conrad (1968) has classified the known iron storage disorders (Table 2). The most important of these disorders that involve some degree of iron overload are discussed on the following pages.
B. HEMOSIDEROSIS

1. General Aspects

The precise definition and the proper usage of terminology employed is always difficult. It is particularly so in dealing with the various forms of iron overload, especially in differentiating between hemosiderosis and hemochromatosis. Some authors state or imply that the former is a stage in the development of the latter and that further addition of iron to the stores is likely to hasten the transformation (MacDonald, 1964; Balcerzak, 1969). The term hemosiderosis is applied here to other conditions of increased iron stores confined mainly to the cells of the reticuloendothelial system and without obvious malfunction or disease which can be attributed to the presence of the iron.

Bantu siderosis is discussed in the following section as an unusual and well-studied form of hemosiderosis, of particular interest because of the source of the iron and the amount and the form in which it is ingested. Note is taken of the fact that some of these individuals develop a condition similar to that of hemochromatosis.

In the discussion of hemochromatosis, the assumption is made that it is a separate entity, distinguished from hemosiderosis by well recognized clinical signs and symptoms, even though the total amount of iron in the body may not be different in the two conditions.

2. Bantu Siderosis

The most outstanding example of iron overload associated with excess iron ingestion is that widely distributed throughout the Bantu in several southern African countries. The high body stores in this population were first identified in 1929 and since then have been studied systematically and quantitatively.

The general occurrence of excess iron stores in the Bantu is the result of the high iron content of their diet and the

---

¹Some consultants disagree with this conclusion.
TABLE 2

CLASSIFICATION OF THE IRON-STORAGE DISORDERS

1. Idiopathic generalized massive iron overloading (hemochromatosis)

2. Secondary iron overloading diseases (hemosiderosis)
   
   A. Generalized and massive siderosis
   
      1. Bantu siderosis
      2. Transfusional siderosis
      3. Microcytic hypochromic anemia with defective hemoglobin synthesis
         
         a. Thalassemias
         b. Pyridoxine-responsive anemia
         c. Sex-linked hypochromic anemia

   B. Relative and limited siderosis
   
      1. Pernicious anemia
      2. Hemolytic disorders
      3. Aplastic anemia
      4. Pancreatic insufficiency

   C. Focal siderosis
   
      1. Renal siderosis
      2. Pulmonary siderosis
      3. Laennec's cirrhosis

Modified from Conrad, 1968.
extremely high iron content of the alcoholic beverage which the Bantu consume. Much of the ingested iron comes from iron utensils in which their food is cooked and in which the acidic beer is brewed and stored. Adults, especially males, consume large quantities of the fermented alcoholic beverage. The average concentration of iron in the beverage has been determined to be about 4 mg per 100 ml, most of which is in a soluble form (Walker and Arvidsson, 1953; Bothwell et al., 1964). Estimates from these and other studies suggest that the average iron intake of adult Bantu males may be 100 mg per day (Walker and Arvidsson, 1953; Bothwell et al., 1964). In heavy drinkers, the iron intake may be considerably higher. Charlton and Bothwell (1966) stated, "The fact that alcoholic drinks provide the major source of iron in the Bantu diet is of further importance, since it implies that those subjects with the most severe degrees of siderosis are also those who have consumed the largest amount of alcohol."

The Bantu siderosis first manifests itself in early adulthood and increases in severity with age, reaching its greatest degree between the ages of 40 and 60. It occurs in both sexes but is more prevalent and of greater severity in males. Siderosis is reported to be extremely rare in Caucasian and Indian populations of South Africa who consume diets different from that of the Bantu (Mayet and Bothwell, 1964).

The tissue distribution of iron in the siderotic Bantu is remarkably predictable (Charlton and Bothwell, 1966). In the early stages hemosiderin is seen in the liver parenchymal and Kupffer cells. With increasing iron concentration, deposits become more prominent in portal tract phagocytes. Characteristically, the iron concentrations in the portal tracts are greater than those in other parts of the liver. Other evidence of reticuloendothelial cell involvement includes observations of abnormal iron deposits in the spleen and bone marrow. Concentrations in the spleen increase with minor degrees of siderosis and tend to be higher than those that occur in the liver in more severe siderosis. It has been calculated from isotopic and chemical analyses that as much as 10 g iron may be stored in the reticuloendothelial cells of the bone marrow in Bantu subjects with severe degrees of siderosis.

This pattern of hepatic and reticuloendothelial system involvement is characteristic of iron overload in the majority of the Bantu with the condition. Significant involvement of parenchymal
tissues in organs other than the liver is very uncommon, even in the presence of marked siderosis. Thus, the pattern of both organ and the cellular distribution of iron in Bantu siderosis appears to be different from that found in idiopathic hemochromatosis.

A different pattern of iron distribution has been observed in siderotic Bantu suffering from portal cirrhosis. In such subjects hemosiderin is also seen in the parenchymal cells of a number of organs that do not normally store iron. The amount of iron in organs such as the pancreas, adrenal, thyroid, pituitary, and heart is several times greater than in those of Bantu subjects with equally severe siderosis of liver and spleen but with no cirrhosis. Whatever the reason for this difference in iron distribution, the pathological features in those subjects with portal cirrhosis resemble closely those described in idiopathic hemochromatosis.

Clinical features in these individuals with cirrhosis also resemble in part those of patients with hemochromatosis. Approximately 20 per cent of the autopsied Bantu subjects with cirrhosis and with iron in organs other than the liver suffered from diabetes prior to death. Further, between 5 and 10 per cent of living Bantu diabetics have been found by liver biopsy to have heavy siderosis with portal cirrhosis. Other clinical features regarded as characteristic of hemochromatosis, such as cardiac involvement, are uncommon in the Bantu condition.

Charlton and Bothwell (1966) concluded that while the clinical and pathological features of idiopathic hemochromatosis are markedly different from those exhibited by the great majority of siderotic Bantu, a few with portal cirrhosis have a condition resembling hemochromatosis. However, Charlton and Bothwell (1966) also concluded that the incidence of hemochromatosis per se in the adult Bantu was difficult to estimate.

C. HEMOCHROMATOSIS

Although there is controversy as to its etiology, hemochromatosis is regarded by most clinicians as a disease entity. Some authorities define hemochromatosis as a disease characterized by
excessive iron absorption (probably related to an inborn error of metabolism) pigmentation of the skin and hemosiderin deposits in the liver, pancreas and other organs; often associated with cirrhosis. However, other investigators define hemochromatosis as an inborn disease characterized by excessive iron absorption. The word is also used to describe the organ injury that results from the deposition of the excess iron. According to this latter definition, hemochromatosis is a disorder of iron absorption leading to excessive stores of body parenchymal iron with evidence of disease, especially cirrhosis.

Historically, the clinical diagnosis was based mainly on the tetrad of liver disease, diabetes mellitus, skin pigmentation and cardiac insufficiency. More recently it has been established that the pathological diagnosis requires, in addition to cirrhosis, extensive deposits of iron in the liver parenchymal cells, connective tissue, and bile duct epithelium (Charlton and Bothwell, 1966). There is pancreatic fibrosis with parenchymal iron deposits, and iron deposits in other organs such as the skin, heart, and endocrine glands. Iron does not seem to be found in excessive amounts in the bone marrow. The concentration of iron in most of these organs is on the order of five to ten times the normal levels. The total body iron stores are excessive as demonstrated by the quantity of iron removed by phlebotomy. The amounts range from 10 to 36 g of iron calculated from the repeated bleeding over many months or years (Williams et al., 1969).

Clinical manifestations of the disease appear most often between the ages of 40 and 60 years, although some diagnosed cases have been in their twenties. The disease affects males more often than females, in a ratio of about ten to one, and females exhibit it at a somewhat later age (Charlton and Bothwell, 1966).

Reviews of current concepts of the etiology and pathogenesis of hemochromatosis usually start with reference to Sheldon's monograph in 1935 in which he reviewed 365 previously reported cases of hemochromatosis. Of these, Sheldon accepted 311 as valid examples of hemochromatosis. He had suggested previously that hemochromatosis might be an inborn error of metabolism and this concept seemed to fit the overall picture that emerged from this larger study. He was impressed by the long, slow accumulation of iron in the tissues without evidence of excessive intake and noted that a small number of cases were reported in which the disease was found in more than one member of a family.
Since 1935, a great deal of information on iron metabolism has been amassed. The limited capacity of the body to excrete iron and the function of the intestinal mucosa in regulating iron absorption were elucidated. The stimulating effects of blood loss on iron absorption and the effects of high iron stores in limiting absorption were shown. The difference in iron requirements and in the amount of the iron stores between males and menstruating and pregnant females was recognized. These findings agreed well with the concept that idiopathic hemochromatosis was the result of a metabolic error, or some manifestation of a derangement in gut mucosal function that permitted a greater than normal absorption of iron. To acquire the degree of excess body iron stores characteristic of hemochromatosis after middle age, approximately 2 to 4 mg of extra iron per day is required to be absorbed and retained.

Similar concepts of the nature of idiopathic hemochromatosis emerged from a review of 787 cases by Finch and Finch in 1955. The signs and the symptoms that characterize the disease, and the prevalence with which they are seen were described with greater precision. Of greater importance perhaps was the concept that idiopathic hemochromatosis represented an "iron storage disease" and that the tissue damage was the result of the excessive iron deposits. The concept recognized that the degree of iron overload in any individual may also be influenced by factors other than the severity of the gastrointestinal mucosal defect, such as the amount of iron in the diet, non-food sources of iron, and age and sex. It was suggested that agents such as alcohol which are associated with cirrhosis might hasten the appearance of clinical manifestations of hemochromatosis.

This concept of hemochromatosis is accepted by the majority of research workers in this field. Attention is focused almost entirely on iron and its unusual distribution in the tissues. It is believed that the amount and the tissue distribution of iron can account for the changes that characterize the diseased condition. The nature of the defect and the metabolic reactions that become deranged are still unknown; however, therapy is possible.

In addition to supportive measures for control of cirrhosis, diabetes and cardiac complications, the only definitive therapy is repeated phlebotomy until the excess iron stores have been removed. Recent reports (Weintraub et al., 1966a; Conrad, 1968) recommend that phlebotomy be applied vigorously to reduce the iron stores as
rapidly as possible by the removal of 2 or 3 units of blood (500 ml each) per week at the beginning. The rate and duration of the pro-
gram of phlebotomy is continued until evidence is obtained that the
iron stores are depleted, following which a maintenance program
of phlebotomy is instituted. In a majority of patients subjected to
such therapy there is definite improvement in their clinical condition,
both subjectively and objectively (Bothwell and Finch, 1962; Charlton
and Bothwell, 1966; Weintraub et al., 1966a), including an improve-
ment in the prognosis of hemochromatosis (Powell, 1970; Williams
et al., 1969).

The concept of an inborn, or genetically transmitted, error
of metabolism as the cause of the excess absorption of iron from
normal diets is generally held by most authorities. There seems
little doubt that a familial occurrence of the disease, or what has
been regarded as latent stages of the disease, is evident. However,
some investigators have concluded the evidence for an inborn error
of metabolism is unsatisfactory (MacDonald, 1964, 1970). While
clinical reports of a familial incidence of hemochromatosis and iron
overload are numerous, the adequacy of these observations to
sustain the concept of heritability of the disease has been questioned
(MacDonald, 1964). Until the transmission through several genera-
tions has been established on unequivocal genetic evidence, this point
will remain unresolved.²

MacDonald (1964, 1970) and his associates have challenged
some of the accepted tenets in their publications over more than a
decade. They question whether hemochromatosis should be regarded
as a single metabolic entity or two separate conditions, iron overload
and cirrhosis, that occur together. They have suggested that the
iron overload could be the result of excessive dietary iron from sources
not recognized as iron contributors, such as alcoholic beverages (wines
in particular), iron cooking utensils and containers, and the use of
iron medications. The portal cirrhosis was considered to be caused
by excessive use of alcohol, malnutrition, viral hepatitis, vascular
congestion, or biliary obstruction. The cirrhosis of hemochromatosis
is not different from the cirrhosis associated with these conditions
and it had been established that abnormal iron absorption frequently
occurs with cirrhosis and with chronic pancreatic disease. There-
fore, MacDonald (1964, 1970) did not believe it was necessary to post-
ulate an inherited metabolic defect to explain the tissue iron levels seen in
hemochromatosis. Further, many attempts to produce tissue damage in

²For footnote 2 see page 38.
experimental animals by excess dietary or parenteral iron had failed unless nutritionally deficient diets were used. These investigators concluded that iron alone was not responsible for the organ changes observed in hemochromatosis but that some other factor, perhaps nutritional, in addition to excessive iron was needed. A similar concept has been put forward by Block (1969) and by Necheles et al., (1969).

In concluding the discussion of hemochromatosis it may be instructive to review briefly the special features of the disease as seen in younger patients. Charlton and Bothwell (1966) have reviewed six cases from their own experience. It was established that none of the six drank alcohol in any form and their diets contained only normal amounts of iron. Iron cooking utensils were not used in any of their families, and no iron-containing tonics or medications were taken. None of the patients had had blood transfusions, jaundice, or any relevant illnesses or symptoms. Because the full hemochromatotic syndrome appeared in the second and third decades of life, Charlton and Bothwell concluded that the accumulation of iron must have been unusually rapid and could be accounted for only by excessive absorption from a normal diet.

The clinical picture was remarkably uniform: excellent hepatic function (in contrast with older patients); five of the six patients showed obvious hypogonadism; three were diabetic; all of them exhibited clinical and electrocardiographic evidence of cardiomyopathy. Five of the patients died, all from cardiac failure. The diagnosis of hemochromatosis was confirmed at autopsy. Iron concentrations of more than 2.8 per cent of the dry weight of the liver were found. The surviving patient was treated by repeated phlebotomy, more than 20 g of iron being removed at the time of the report, which resulted in significant improvement in the cardiac signs and symptoms. The specific involvement of the endocrine system and the heart appears to be characteristic of hemochromatosis in the younger patient.

The incidence in the U.S. population of "iron storage diseases" or persons with recognizable excessive iron deposition in specific body tissues is not known. The publication of Finch and Finch (1955) is the only documented estimate of the incidence of hemochromatosis. They calculate that idiopathic hemochromatosis is recognized once in about 20,000 hospital admissions and once in about 7,000 hospital
deaths. On the basis of these data, they suggest that in this country there are about 20,000 people with hemochromatosis of which only a small fraction are in the symptomatic stage. Other workers have estimated that hemochromatosis may be diagnosed in about one case in 10,000 autopsies (Darby, 1972). Recently published comments on these estimates of incidence provide no further data (Butterworth, 1972; Crosby, 1971).

D. OTHER IRON STORAGE DISORDERS

The preceding discussion has dealt with two forms of iron overload: hemosiderosis as found in the Bantu, and idiopathic hemochromatosis. Although there are similarities in these two conditions, i.e., excess amounts of iron in the body, resulting in each case from excess absorption from the gut, there are also differences. The most important difference is the location of the iron in the tissues and the harm which it causes. In Bantu siderosis the bulk of the iron is confined to the reticuloendothelial system, while in hemochromatosis most of the iron is found in the parenchymal cells of the liver, pancreas, endocrines and in the muscles of the heart. Bothwell and Finch (1962), admitting the difficulty of fully understanding the reasons for this difference in location of the iron, have discussed possible mechanisms by which it might occur.

Certain anemias are also characterized by excess absorption of iron from the gut. These are the anemias associated with excessive but ineffective red cell production or with blocks in the incorporation of iron into hemoglobin. It has been shown that the behavior of the mucosa is in some way conditioned by the rate of erythropoiesis and bone marrow activity with the result that iron overload may develop. Because many of the patients with these anemias may have received iron therapy by mouth or repeated blood transfusions, it is difficult to assess the effects of the level of iron in the diet on the amount and location of their iron stores. While there is no direct evidence, the prevailing opinion is that dietary iron might possibly be a complicating factor in thalassemia major, pyridoxine-responsive anemia, and sex-linked hypochromic anemia.
In the management of thalassemia major the physician is faced with the dilemma of having to transfuse patients regularly to maintain hemoglobin at levels that will support health and provide for acceptable growth and development, while always being aware of the increasing degree of iron overload. The parenteral use of iron-chelating agents, such as desferrioxamine and DTPA, to mitigate in some degree the iron overload has been reported (Beard et al., 1969; Piomelli et al., 1969; Wolff and Luke, 1969; Wolman and Ortolani, 1969).

Pyridoxine-responsive anemia and sex-linked hypochromic anemia are diseases which usually become manifest in adult life and both are associated with generalized iron overload. The underlying defect is ineffective utilization of iron for hemoglobin synthesis with accompanying elevation of SI. Some patients may have had inappropriate iron medication but, in the main, the tissue iron was derived from increased absorption of dietary iron. Phlebotomy may be tolerated in certain cases and this means of controlling iron stores has been recommended (Weintraub et al., 1966b).

In sickle cell anemia, iron absorption is unlikely to be increased and iron stores are usually within normal limits (Lehmann and Huntsman, 1968; MacDonald, 1964). In the discussion of sickle cell anemia in textbooks of medicine, the possibility of excess iron stores is not considered to be a consequence of the disease (Wintrobe, 1967; Mann and Lessof, 1970; Beeson and McDermott, 1971). In the management of this disease transfusions and iron therapy are avoided if possible.

E. ANIMAL MODELS

Many investigators have attempted to produce iron overload in experimental animals to study the phenomena of excessive storage. A wide variety of animal species, various forms of iron preparations administered for long periods of time, and experimentally induced dietary deficiencies have been studied without marked success. These studies have been reviewed by MacDonald (1964). Unfortunately, no satisfactory animal model has been found that appears to simulate hemochromatosis in man.
Dogs administered iron saccharate intravenously or whole blood transfusions that produced SI elevations greater than normal failed to produce liver cirrhosis, pancreatic fibrosis or evidence of fibrous tissue reaction over a 4 to 7 year observation period (Brown et al., 1957). Lisboa (1971) reported the production of hepatic cirrhosis with massive siderosis in 5 of 6 dogs after 35 to 47 months of repeated administrations of an intravenous iron-dextran or an intramuscular iron-sorbitol preparation. The total dose of iron given was enormous compared with the quantity of dietary iron consumed by hemochromatotics. While the results are inconclusive, they do illustrate a form of experimental hepatic cirrhosis apparently caused by iron overload. Brown et al. (1957) concluded from their studies that the reticuloendothelial cells were able to remove effectively the iron load under their experimental conditions and thus prevent parenchymal localization. This explanation received support from the subsequent work of MacDonald et al., in 1968 when they found that substances blocking the reticuloendothelial cells would enhance parenchymal iron storage in rats administered a lipotrope deficient diet containing 6 per cent ferric ammonium citrate.

From their studies Brown et al. (1957) postulated that, "The role of iron in causing the tissue damage of hemochromatosis may have been overemphasized." It is generally agreed that the oral administration of large amounts of iron to normal animals on a normal diet does not produce excessive iron stores or cirrhosis in the liver.

Whipple and his colleagues in their classical experiments on dogs postulated in 1943 the existence of a "mucosal block" to the absorption of radioactive dietary iron after plasma iron had reached normal values following experimental anemia (Hahn et al., 1943). These experiments supported the concept of a physiological saturation level of iron that controlled acceptance or refusal of ingested iron by the gastrointestinal mucosal epithelium. Clearly the normal animal will not show evidence of damage associated with parenchymal iron storage from excess dietary sources unless some additional influence contributes to the disorder. Numerous investigators have tried agents that cause liver cirrhosis, e.g., carbon tetrachloride (Witzleben and Chaffey, 1962), dietary deficiencies such as choline and other nutrients (MacDonald and Pechet, 1965), or pancreatic duct ligation to cause exocrine cell destruction (Kinney et al., 1950; Kinney et al., 1955) as experimental techniques to encourage iron storage. These experiments have not been successful in producing an acceptable animal model of iron storage disease.
MacDonald (1964) discussed in considerable detail the studies on oral administration of iron to animals on deficient diets. Dietary iron when fed in large doses led to the deposition of iron in the liver and other tissues if the animals were fed a high fat diet deficient in choline and other nutrients (MacDonald and Pechet, 1965). A deficiency of folic acid appeared to produce effects similar to choline deficiency; however, methyltetrahydrofolate acid did supply methyl groups needed for choline synthesis, suggesting that the two dietary factors may play similar roles. When choline was added to the diet, protection against iron storage followed and there was no evidence that folic acid deficiency per se was still a factor (MacDonald et al., 1965). The authors concluded that there was a difference in the distribution of tissue iron when excess iron was given to normal rats as compared with animals receiving a lipotrope deficient diet or a diet low in folic acid. These findings are cited as support for the hypothesis that hemochromatosis is a disorder of excess iron storage related to some metabolic defect.

Subsequent experiments by these investigators showed that $^{59}$Fe administration to rats fed a lipotrope deficient diet increased iron absorption and storage in the liver, spleen and bone marrow as measured by whole body counting (MacDonald et al., 1968). Rapid iron absorption often exceeded the iron binding capacity of the plasma transferrin. After 2 or more months of feeding the rats, iron deposits were identified in the parenchymal cells of the pancreas and heart. Histologically these changes were most prominent in the pancreas and heart tissue. In these tissues the cells adjacent to blood vessels and ducts had the highest stainable iron.

Thorium dioxide was used to produce reticuloendothelial cell blockage, followed by carbon black clearance studies, to assess the influence of this system on the production of parenchymal cell deposition of iron (MacDonald et al., 1968). In these rats it was reasoned the liver, pancreas and heart tissues of lipotrope deficient animals would tend to deposit more iron because the reticuloendothelial cells were blocked. In the experimentally produced reticuloendothelial cell blocked animals, iron deposits occurred in the parenchymal cells of the pancreas and heart. When the reticuloendothelial cells and the liver are unable to remove iron from the vascular system efficiently, it appears that in these animals iron is deposited in parenchymal cells of the pancreas and heart. From these studies, the authors concluded that a disorder of the reticuloendothelial system together with excessive dietary iron were the two factors responsible for the "experimental hemochromatosis."
Chronic tissue hypoxia in mice associated with high oral and parenteral iron dosage has been reported to lead to a form of myocardial hemosiderosis (Necheles et al., 1969). Cell injury produced by keeping mice in an atmosphere of 8 to 10 per cent oxygen was considered responsible for the iron loading in the liver, spleen, and heart tissue after 12 weeks. Although blood hemoglobin levels were essentially normal, the combination of hypoxia and high iron dosage led to an increase in the myocardial iron content although neither factor alone produced the effect. Relatively little stainable iron was present in the myocardium of the non-iron-loaded mouse, and iron loading alone or hypoxia alone caused only a slight increase in myocardial iron content of tissue sections. However, the increased iron was mostly within the reticuloendothelial system in the animals under hypoxia and receiving high iron dosage. Occasional heart muscle cells were observed to have iron deposits as fine perinuclear granules. It was suggested by the authors that a longer period of hypoxia might have led to progressively more iron transferred from the reticuloendothelial cells to myocardial cells. In this case the experimental conditions might have elicited a histologic picture resembling hemochromatosis or transfusion-induced hemosiderosis.

Ascorbic acid deficiency in guinea pigs has been found to cause changes in the body distribution of storage iron (Lipschitz et al., 1971). Total non-heme iron concentrations were lower in the livers and considerably higher in the spleens of scorbutic guinea pigs than in control animals. Other workers have found similar high iron concentrations in the spleens of scorbutic guinea pigs. The in vivo metabolic iron distribution and storage were explained on the basis of a diminished release of iron from the reticuloendothelial cells in the scorbutic animals. The close interrelationship of ascorbic acid and iron metabolism has been recognized for many years. However, there is a need to explore further the significance of ascorbic acid in iron storage phenomena in experimental animals.

In summary, numerous basic questions on iron absorption and subsequent metabolism are best approached by animal studies. However, the role of excess iron in causing liver and other tissue damage can be examined prospectively only in animal systems that adequately mimic human iron storage disorders. Additional research will be required to develop satisfactory animal models that will elucidate the physiological and biochemical events controlling iron absorption, distribution and storage.
One consultant suggested the following paragraph be added to this discussion of the genetic basis of hemochromatosis:

VI. IRON INTAKE

A. FOOD IRON AND ITS ABSORPTION

Discussion of the physical and physiological factors affecting the availability of iron from the gut is outside the scope of this report and has been reviewed recently (Conrad, 1970; MacDonald, 1970). The important concern of this report is the amount and the availability of the iron in the various foods which make up the American diet. A brief summary is of value in identifying those food categories that are important contributors of iron to the diet and as background to assess the effect of the proposed increase in the iron enrichment of wheat flour and related products.

There is great variability in the reported values of the iron content of individual foods, particularly those of plant origin. Food composition tables represent the best average value that may be calculated from the divergent results reported for each food (Watt and Merrill, 1963). The iron content of a mixed diet, calculated from tabulated values for each item, are often at variance with the chemical analysis of the same mixed diet (Moore, 1968).

Equal or greater variability is found in the results obtained when the amount of iron absorbed from food in the digestive tract is measured. Older methods using human subjects based on the difference between amount of iron ingested and the amount found in the feces were subject to large errors (Heinrich, 1970). More recent methods using radioiron isotopes are more elegant and more precise but the results still show individual variability (Layrisse and Martinez-Torres, 1971). In reviewing studies on intestinal absorption of iron, it must be kept in mind that normal individuals are not good test subjects because of the small amounts of iron they absorb each day. Usually individuals who are iron deficient in some degree and who absorb more iron are used to add to the precision of the test. Also, relatively small test doses of iron, either as an iron salt or in food, are usually given after an overnight fast and without other food for several hours to avoid interference with absorption. These procedures improve the sensitivity of the test, have merit in
comparing one food with another, and in ranking them for absorbability of their iron content. But questions may be raised as to the significance of the results when assessing the availability of iron from the same food eaten in larger quantities or as part of a mixed diet.

1. **Absorption from Single Foods**

However, much valuable information has been accumulated during recent years on the average absorbability of iron from single foods representative of different food categories. Use has been made of radioiron isotopes in measuring iron absorption and certain foods have been produced with the intrinsic iron labelled by supplying radioactive iron to the plant during growth or by injecting it into animals. The results of such experiments have been summarized by Layrisse and Martinez-Torres (1971) and are illustrated in Figure 1. The greater availability for human subjects of the iron in individual foods of animal origin as compared with those of plant origin is clearly demonstrated. Undoubtedly, the difference is due to the fact that most of the iron in animal tissues and organs is in the form of heme compounds, such as hemoglobin and myoglobin, where it is protected from other chelators which might inhibit its absorption, and from which it is more easily absorbed by the cells of the intestinal mucosa. Also, the amino acids resulting from the digestion of meat or fish have a favorable effect on the absorption of iron. Iron contained in vegetables, cereals, and legumes is exposed during digestion to several chelating agents, some of which may favor absorption but others bind the iron so firmly as to inhibit its absorption. It may be noted that in these experiments the iron in wheat products is absorbed on the average to the extent of about 5 per cent.

2. **Absorption from Mixtures of Foods**

The absorption of iron from one food is greatly influenced by the simultaneous presence in the digestive tract of one or more other foods. By means of two different radioiron isotopes ($^{59}$Fe and $^{56}$Fe) Layrisse and his associates have assessed the interaction between two different foods when they are ingested together (Layrisse et al., 1968; Martinez-Torres and Layrisse, 1971). They measured in the same subject the iron absorption from black beans and from corn when each was fed alone or, in turn, with veal muscle
FIGURE 1

IRON ABSORPTION FROM VARIOUS PLANT AND ANIMAL FOODS*

<table>
<thead>
<tr>
<th>Food of vegetable origin</th>
<th>Food of animal origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice</td>
<td>Ferritin</td>
</tr>
<tr>
<td>Spinach</td>
<td>Veal liver</td>
</tr>
<tr>
<td>Black beans</td>
<td>Fish muscle</td>
</tr>
<tr>
<td>Corn</td>
<td>Hemo-globin</td>
</tr>
<tr>
<td>Lettuce</td>
<td>Veal muscle</td>
</tr>
<tr>
<td>Wheat</td>
<td></td>
</tr>
<tr>
<td>Soybean</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dose of food Fe</th>
<th>2 mg</th>
<th>2 mg</th>
<th>3-4 mg</th>
<th>2-4 mg</th>
<th>1-17 mg</th>
<th>2-4 mg</th>
<th>3-4 mg</th>
<th>3 mg</th>
<th>3 mg</th>
<th>1-2 mg</th>
<th>3-4 mg</th>
<th>3-4 mg</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>N° cases</td>
<td>11</td>
<td>9</td>
<td>137</td>
<td>73</td>
<td>13</td>
<td>42</td>
<td>38</td>
<td>17</td>
<td>11</td>
<td>34</td>
<td>39</td>
<td>96</td>
<td>520</td>
</tr>
</tbody>
</table>

Data were derived from a collaborative study of the Departments of Botany and Medicine, University of Washington at Seattle, and Department of Pathophysiology, Instituto Venezolano de Investigaciones Científicas at Caracas, Venezuela. The horizontal line represents the geometrical mean and the cross-hatched area shows the limits of one standard error.

*From Layrisse and Martinez-Torres, 1971, with permission.
or with fish. The presence of the animal products almost doubled the absorption of iron from either plant product. In the combination of veal with beans or corn, the increase in the iron absorption from the latter was accomplished at the expense of about a 20 per cent reduction in the absorption of the veal iron. The combination of fish with plant products did not seem to inhibit the iron absorbed from the former. Such results emphasize the difficulties of interpreting quantitatively the effect of food combinations on the total iron intake of the individual subjects. It must be emphasized that these experiments were conducted with small doses of iron (3-4 mg total).

Martinez-Torres and Layrisse (1970) have reported that the amino acids in the number and proportions found in fish, when added to black beans, increase the iron absorption from the latter approximately threefold. The most effective of the amino acids was cysteine.

Until recently no method was available for assessing the multiple factors that influence iron absorption from a diet containing more than two components. It is of interest to note that the Food and Nutrition Board, NAS-NRC, in compiling the table of Recommended Dietary Allowances (RDA) has assumed a 10 per cent absorption of the total iron present in the average American diet and all RDA values for iron are calculated on this basis (1968). There is no adequate experimental evidence for this assumption. However, three publications have appeared very recently describing procedures which offer much promise in assessing the iron absorption from a diet of mixed foodstuffs (Cook et al., 1972; Layrisse and Martinez-Torres, 1972; Björn-Rasmussen et al., 1972).

The methods are based on the concept that all food iron may be regarded as consisting of two iron pools: heme iron and non-heme iron. It has been shown that when a small amount of a radioactive iron salt (extrinsic tag) is mixed with vegetable foods, intrinsically labeled with a different iron isotope, the two labels are absorbed in a constant proportion by subjects ingesting the foods (Cook et al., 1972; Björn-Rasmussen et al., 1972). Similarly, when a small amount of $^{59}$Fe-labeled hemoglobin (extrinsic tag) is consumed with $^{55}$Fe-labeled iron in meat, the two labels are absorbed in a constant proportion, close to unity (Layrisse and Martinez-Torres, 1972). In both cases, the relationship of absorbed extrinsic iron to absorbed intrinsic iron remains constant over a range of 0.001 mg to 0.5 mg of the former and 2 mg to 4 mg of the latter.
These findings provide a simple and inexpensive procedure for measuring the amount of iron absorbed from all combinations of foods. By using a labeled iron salt or a differently labeled hemoglobin, or both, it is possible to estimate the absorption of iron from plant foods, from meat products, or from mixtures of the two.

B. THE IRON CONTRIBUTION OF VARIOUS FOOD CATEGORIES

The U.S. Department of Agriculture publication, Food Intake and Nutritive Value of Diets of Men, Women and Children in the United States, Spring 1965 (USDA, 1969), provides the best available calculations of the relative contribution of each of twelve food categories to the total daily intake of iron. From the several age-sex groupings under which these data have been reported, those of ages 20-34 for males and for females have been selected to illustrate the amount and source of iron in the present diets of these individuals (Table 3). The reasons for selecting the 20-34 age group of males is that their average daily iron intake of 17.9 mg is presently the highest average value among all the age groups within the U.S. population. The differences among the age and sex groups with respect to percentage contribution to the diet by the various food categories are minor in all age groups over 9 years old.

The average value for daily iron intake does not describe adequately the population of values it represents. In addition, individual variation is poorly defined. Even the most recent extensive survey of dietary intakes does not include adequate data on the range of daily iron intakes for adult males under 60 years of age (U.S. Department of Health, Education, and Welfare, 1972).

The importance of the meat group of foods as a contributor of iron to the diet is immediately obvious (Table 3). The meat group, together with the grain products group, contribute between 65 and 70 per cent of the total daily iron intake. The average contributions by eggs (6.7 per cent) and "other vegetables, fruit" (6.6 per cent) are significant; other food categories individually contribute less, although in total they contribute about 17 per cent.
TABLE 3

CONTRIBUTION OF DIFFERENT FOODS IN THE U.S. DIET TO IRON INTAKE OF NORMAL ADULTS*

<table>
<thead>
<tr>
<th>Food Categories</th>
<th>Contribution to Iron Intakes (%)</th>
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<tr>
<td></td>
<td>Males (20 - 34 yrs)</td>
<td>Females (20 - 34 yrs)</td>
<td></td>
</tr>
<tr>
<td>Meat, poultry, fish</td>
<td>46.3</td>
<td>43.1</td>
<td></td>
</tr>
<tr>
<td>Grain products</td>
<td>23.3</td>
<td>24.9</td>
<td></td>
</tr>
<tr>
<td>Eggs</td>
<td>6.7</td>
<td>5.2</td>
<td></td>
</tr>
<tr>
<td>Other vegetables, fruit</td>
<td>6.6</td>
<td>7.4</td>
<td></td>
</tr>
<tr>
<td>Legumes, nuts</td>
<td>4.5</td>
<td>4.1</td>
<td></td>
</tr>
<tr>
<td>Potatoes</td>
<td>3.7</td>
<td>3.4</td>
<td></td>
</tr>
<tr>
<td>Beverages other than milk and</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>fruit juice</td>
<td>2.5</td>
<td>3.8</td>
<td></td>
</tr>
<tr>
<td>Sugars, sweets</td>
<td>2.1</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>Tomatoes, citrus fruit</td>
<td>1.5</td>
<td>2.1</td>
<td></td>
</tr>
<tr>
<td>Dark green, deep yellow vegetables</td>
<td>1.0</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>Milk, milk products</td>
<td>1.0</td>
<td>1.7</td>
<td></td>
</tr>
<tr>
<td>Fats, oils</td>
<td>0.7</td>
<td>0.6</td>
<td></td>
</tr>
</tbody>
</table>

Total Daily Iron Intake (mg) 17.9 11.3

*Taken from figures in Tables 1a and 16, USDA, 1969.
Because the proposed FDA regulation (Federal Register, December 3, 1971, 36 F.R. 23074) to increase the level of iron in enriched baked wheat flour products (bread, buns, rolls) will affect only the grain products category, the average daily increase in iron intake can be calculated. Using the quantities consumed daily of bread, rolls and biscuits as reported in Table 6A (USDA, 1969) by the same two age-sex groups shown in Table 3, the iron intake calculations are as follows:

a) Quantity of bread, rolls, biscuits consumed daily:
   Males (20-34 years old) 120 g
   Females (20-34 years old) 71 g

b) Proposed level of iron enrichment of bread 25.0 mg/lb
   Current level of iron enrichment of bread 11.3 mg/lb*
   Difference or net increase 13.7 mg/lb

c) For males:
   120 g would supply an increased intake of
   \[ \left( \frac{120g}{454g} \times 13.7 \text{ mg/lb} \right) \]
   For females:
   71 g would supply an increased intake of
   \[ \left( \frac{71g}{454g} \times 13.7 \text{ mg/lb} \right) \]

   d) Thus the average daily intake at the proposed level of enrichment would be:
   For males \( 17.9 + 3.6 \) 21.5 mg/day
   For females \( 11.3 + 2.1 \) 13.4 mg/day

*This figure was used in calculating iron contribution of the "grain products" category in the reference cited (USDA, 1969).
Some consultants noted that in the absence of information concerning the ranges of bread and flour ingestion, it is not possible to predict how great an amount of excess dietary iron would be imposed upon those who eat large quantities of bread and flour. They suggested that the risk to this segment of the population should be a matter of particular concern. Other consultants did not agree that consumption of large quantities of bread and flour would constitute a hazard to normal individuals.

Under the category, "grain products," (Table 6A, USDA, 1969) there is an additional food item, "other baked goods." These are consumed in smaller amounts than the bread, rolls and biscuits. Baked goods, either purchased or baked at home, may be made with unenriched flour. Their contribution to the iron intake was calculated on the basis of unenriched flour use. Should the proposed FDA regulation become effective, it is possible that some baked goods would be made with enriched flour and this represents an additional but indeterminate, small increment that might have to be added to the above calculated total iron intakes.
C. OTHER SOURCES OF IRON

In addition to the iron in foods, that in water, beverages, contamination of dietary items before consumption, and iron medications are sources of iron. However, the iron from these sources is in diverse chemical forms and its availability is extremely variable. It is not possible to quantitate the amount of iron that may be ingested and absorbed from these several sources.

The iron that accompanies food as a contaminant may or may not be absorbed. For example, in Ethiopia the iron-rich soil contaminates the cereal grain, tef, during threshing and represents an extreme example of high dietary iron intake; as much as 500 mg/day. Hofvander (1968) concluded that this iron is mainly in the form of oxides and hydroxides and presumably is poorly absorbed. It is generally assumed that foods in the average U.S. diet are relatively free of soil and other extraneous matter that might contain iron. Even if present, the iron probably would be in forms that are poorly absorbed.

Iron in water is usually present as ferric hydroxide and it is doubtful that it contributes significant amounts of the element to total iron intake (MacDonald, 1964). It is probable that measurable amounts of extraneous iron may be added inadvertently during cooking or preparing food, e.g. by use of iron cookware. In a study of iron requirements of pregnant Bantu, Becker et al., (1970) referred to their diet as "fortuitously fortified." In the average U.S. diet, the importance of such fortuitous fortification is obscure. Most investigators have found that the longer a food is cooked or stored in iron utensils and the higher the acidity, the more iron released into the food (Burroughs and Chan, 1972). Although iron cookware and utensils may be a source of iron in the U.S. diet, the relative importance is considered minimal.

Alcoholic beverages, especially wines, are important sources of iron for individuals who consume large amounts of these beverages. The consequences of ingestion of alcohol-iron have been reviewed by MacDonald (1964). The long term significance of the iron in such beverages on non-alcoholic individuals is unknown.

Iron medications may be prescribed in relatively large doses in the treatment of anemias and iron "tonics" are
frequently taken daily without medical supervision for long periods of time. Numerous products containing relatively large amounts of ferrous or ferric salts or organic iron compounds are available to the general public without restriction. Many of these products contain in excess of 50 mg of iron per average dose. In addition these preparations are often consumed three times a day for periods of months or years. The consumption of iron-containing medications and dietary supplements is not known precisely; however, their use appears to be widespread.

In the ad hoc review group discussions, it was suggested that the proposed increase in iron enrichment may be insignificant in relation to the intake from medications and dietary supplements by certain individuals. The majority of the consultants agreed that this was possible, but definitive data do not exist. A minority of the consultants disagreed with this suggestion.

In summary, there are sources of iron beyond those considered as dietary food iron. In the case of iron medications, the amounts ingested may be significant for some individuals. However, in general, the contribution of other sources of iron to total daily iron intake of most individuals is probably minor when compared to the amounts of iron from the daily diet.
VII. ASSESSMENT OF POSSIBLE HAZARDS ASSOCIATED WITH INCREASED IRON ENRICHMENT

The primary requirement of this study has been to identify and assess any possible hazard that might result from the proposed increase in the iron enrichment of wheat flour and flour-containing dietary items. In the preceding pages we have tried to estimate the effect of this increased enrichment on the average total daily iron intake of a representative group of males and one of females in the U.S. population. We are quite aware that the average value alone for daily iron intake does not describe adequately the population of values it represents. However, data on the range of daily intakes making up the average were not available.

It is recognized that the present general diet of normal adult males contains more than enough iron to meet their nutritional needs, and does not appear to constitute any hazard. In the example given in Table 3, the total average daily intake of the males is approximately 180 per cent of their estimated requirements based on an RDA of 10 mg per day. The proposed increase in iron enrichment (see p 45) will raise the average daily intake of normal males to approximately 215 per cent of the present RDA. Concern has been expressed that such an increased intake might have untoward effects on a relatively small, but unknown, number of ostensibly healthy individuals who are unaware of abnormalities in their iron metabolism. These are persons who may develop, at some later date, the clinical problems of hemochromatosis or other iron overloading disorders.

During the conduct of this study, it became evident that there was no unanimity of scientific opinion concerning the possible hazards associated with the proposed increased iron enrichment. It was impossible to reconcile these strong differences into a single consensus. Therefore, the following section of the report presents the various points of view of all the scientific consultants and comments by the LSRO staff.
A. **OPINIONS OF THE CONSULTANTS**

1. **Majority Opinion**

   The majority of the ad hoc group members agreed that the proposed increase in iron enrichment will not jeopardize the health of U.S. males who have normal iron metabolism. They noted that adult males have always consumed more dietary iron than required to meet their nutritional needs. It is difficult to conceive of any combination of foods, in amounts consumed to meet caloric requirements, that would not provide more iron than the adult male requires. It is believed by some that in the past, because of greater physical activity and consumption of greater quantities of food, the average daily intake of iron exceeded that presently proposed (Finch and Monsen, 1972).

   If it is accepted that hemochromatosis is an inborn error of metabolism, then it may be concluded that the proposed increase in the iron content of the general diet will not affect the number of individuals who have the inborn error. In addition, the majority of the consultants agreed that the incidence of hemochromatosis will not be increased. The consultants were aware of the published opinions

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3 Some consultants restricted this conclusion to those normal adult males whose dietary iron intakes approximates the calculated average values on page 45.

4 One consultant was of the opinion that the evidence for an inborn error of metabolism is insufficient.

5 One consultant proposed that the following sentences be substituted for the first two sentences of this paragraph:

"If it is accepted that hemochromatosis is an inborn error of metabolism such as to promote an increase in absorption of dietary iron, then it may be concluded that the proposed increase in the iron content of the general diet will not affect the number of individuals who have that inborn error. Since it is not known whether all individuals with this error ultimately develop clinical hemochromatosis, it is impossible to be certain that increasing the dietary iron levels will or will not increase the frequency of clinical hemochromatosis."
that the rate of accumulation of iron in the latent hemochromatotic may be accelerated and presumably this would result in an increase in the severity of the clinical manifestations (Finch and Monsen, 1972; Crosby, 1971). This opinion is plausible but there is no substantial evidence to prove or disprove it. Thus, it would appear that the proposed increase in the iron content of enriched flour and related products should have little or no untoward effects on iron accumulation of individuals with latent iron storage disorders.

However, the consultants acknowledged that all these opinions were based on informed judgment rather than direct experimental evidence and that additional research would be required to resolve conclusively the significance of the amount of dietary iron on iron accumulation. 6

The majority of the consultants indicated that it would be desirable to perform such research along with implementation of any regulation increasing the iron enrichment of flour and related cereal products. They stated that if and when the proposed regulation is implemented, continuous careful surveillance of representative segments of the U.S. population will be needed not only to evaluate efficacy in the prevention of iron deficiency, but also to provide information as to whether or not there is any effect on the incidence and severity of iron storage disorders.

2. Minority Opinion

A minority of the consultants were of the opinion that the proposed increase in the iron content of enriched flour and related products would have no untoward effects on iron accumulation of normal males whose intake of dietary iron approximates the average shown for males on page 45. Certain hematologists in this group expressed the belief that any increase in dietary iron over a period of time will result in increased rate of accumulation in those with the inborn error of metabolism.

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6One consultant suggested that the following sentences should be added to this paragraph:
"The additional research should include the development of predictability of iron overload diseases, with a view of providing a warning to those susceptible individuals to avoid foods which contain significant amounts of added iron. The majority of consultants were agreed that this state of affairs should not stand as a deterrent to the promulgation of regulations relating to the increase of iron content of cereal products."
If it is accepted that hemochromatosis is an inborn error of metabolism, then it may be concluded that the proposed increase in iron content of the general diet will not affect the number of individuals who have the inborn error. These consultants concluded that the incidence of hemochromatosis will not be increased, and they noted that Crosby (1971) and Finch and Monsen (1972) have expressed the opinion that the rate of accumulation of iron in the latent hemochromatotic may be accelerated and the damage resulting from the accumulation made more severe. This is plausible but there is no substantial evidence to prove or disprove the hypothesis.

However, the consultants acknowledge that these opinions are not based on experimental evidence and that additional research would be required to resolve conclusively the significance of the amount of dietary iron on iron accumulation.

These consultants proposed that such research should be performed before implementing the regulation increasing the iron enrichment of flour and related cereal products. When implementation is begun, continuous careful surveillance of hemoglobin values and serum iron concentrations will be needed not only to evaluate efficacy in the prevention of iron deficiency but also to provide evidence concerning the incidence of detectable hemochromatosis and other iron storage diseases.

B. STAFF COMMENTS

When hemochromatosis is first diagnosed, the amount and distribution of iron in the tissues is believed to be the result of one or more decades of excess absorption. The cause of the mucosal defect, which occurred at some indefinite time in the past, is unknown. However, there is no evidence that the amount of iron in the diet at that time was the inciting factor. Hemochromatosis has been defined as "an inborn error of metabolism" and its familial or genetic occurrence suggests that something other than dietary iron led to the derangement in the intestinal mucosa allowing continued excessive iron absorption. Therefore, it may be concluded that the proposed increase in the iron content of the general diet will not affect the number of individuals who will develop hemochromatosis.
Although we agree that the incidence of hemochromatosis will not be increased, Crosby (1971) has expressed the opinion that the rate of accumulation of iron in the latent hemochromatotic may be accelerated and the damage resulting from the accumulation made more severe. Finch and Monsen (1972) have also considered this possibility, but concluded that iron overload is a rare disorder which will occur regardless of manipulation of dietary iron intake. There is no experimental evidence that the rate of absorption in individuals with latent iron storage disorders would be influenced significantly by the proposed increase in the iron content of the cereal portion of the diet. The literature on hemochromatosis discloses surprisingly little concern for the levels of iron in the various diets consumed by victims of the disease or any effect which the different levels might have had on the severity of the symptoms when the disease was diagnosed. The usual statement is that the excess iron was absorbed from "a normal diet".

Crosby et al., (1963) studied the rate of iron accumulation in three patients with hemochromatosis and one with hereditary hypochromic iron-loading anemia. Each of these patients was subjected to at least two series of phlebotomies. In each case blood removal was continued until the patient was distinctly anemic as the result of iron deficiency. It was found that the rate of iron accumulation, as measured by replenishment of the hemoglobin mass, was distinctly increased during the periods of iron deficiency. Prior to phlebotomy, these patients accumulated iron at a rate of 1.5 to 5 mg per day; during the periods of added iron deficiency, the rate increased to 3 to 10 mg per day. This suggested to the investigators that response to iron deficiency depended upon some mechanism other than that which is at fault in hemochromatosis. Another possible interpretation may be that the mucosa retains a degree of control over the amount of iron that is absorbed and accumulated even in the hemochromatotic state.

Although the diet that these patients consumed was described only as a freely chosen "standard American diet," it may be argued that, because the diet supported the absorption of 10 mg of iron per day during the months of iron deficiency, the diet contained an excess of iron during the many months between phlebotomies when only 1.5 to 5 mg were accumulated. These findings suggest that variations in dietary iron intake such as occurs in consuming a "normal diet" or a "standard American diet" may have negligible influence on the amount of iron absorbed by the intestinal mucosa even in the
deranged conditions characteristic of iron-storing disorders. It seems reasonable to speculate that the effect of the proposed increase in the iron content of flour and flour-containing dietary items would have a similarly negligible effect.
IX. PROPOSED ADDITIONAL RESEARCH

Another part of this overall LSRO study dealt with the feasibility of developing protocols of clinical research that would detect and assess any possible hazard that might result from the proposed increase in the iron enrichment of cereal products (Waddell et al., 1972). Two clinical research protocols were developed that gave special consideration to two segments of the population: (a) those afflicted with various diseases in which some degree of iron overload is characteristic; and (b) healthy adult males who do not need additional iron in their diet.

The experiment outlined in the first protocol (a) was designed to permit the measurement of any difference in iron absorption over a ten day period from two diets identical except for the two breads with different levels of iron enrichment. The experiment was to compare the amount of iron absorbed from each of the two breads: one containing the present level of iron enrichment (10 mg/lb) labeled with $^{59}$Fe, and the other containing the proposed higher level of iron (25 mg/lb) and labeled with $^{55}$Fe. The breads were to be fed on alternate days for ten days as part of standard meals which would otherwise be identical. Fourteen days later (day 24 of the experiment) the $^{59}$Fe and $^{55}$Fe activities of the circulating hemoglobin were to be estimated to determine the amount of iron absorbed from each bread. The subjects for the experiment were to include normal individuals and patients with various diagnosed iron-storing disorders.

Although differences in the amount of iron absorbed over a ten day period from the two breads with different levels of enrichment cannot be translated into amounts of body stores which might accumulate with time, such an experiment would be a first step in the evaluation of any untoward effects of the proposed increased enrichment. It would be advantageous if subjects with latent as well as diagnosed iron storage disorders could be included in such an investigation. It is extremely difficult to detect the individual with asymptomatic hemochromatosis.  

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7One consultant suggested that the following sentence be added and the remainder of the paragraph deleted: "This can only be done by screening techniques applied to asymptomatic individuals whereby those with an elevated serum iron and increased transferrin saturation could be detected."
However, it has been suggested that health service testing laboratories are able to detect by biochemical analyses those individuals who have elevated serum iron and increased percentage transferrin saturation, conditions that are associated with excess iron stores.

The objective of the experiment outlined in the second protocol (b) was to acquire information on the response of three groups of healthy adult males over a long period of time to three different levels of iron intake provided in bread or other flour-containing food items. All of the subjects were to start with iron stores at a level standardized by phlebotomy; this procedure was to be repeated at the time any subject was removed from the experiment to measure any increase in iron stores. It was recognized that because of the large number of subjects and the long duration of the experiment, the study would present great difficulties and would be very costly. Because the estimated extra iron intake from the enriched breads ranged from 5 to 30 mg per day over prolonged periods, this information would provide a fuller understanding of the response of healthy males to the three levels of iron in a mixed diet. In spite of the enormous difficulties of conducting this experiment, the protocol exemplifies the type of extensive investigation that would be required to obtain a definitive answer to the question of the effects of increased iron enrichment in the general diet.
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XI. GLOSSARY OF TERMS

Bioavailability .......... The amount or proportion of iron in the diet absorbed from the gastrointestinal tract that is utilized by the body in life processes or stored.

Chelation ............... A process forming a chelate. A chelate is a type of coordination compound with a central atom (most frequently a metal) joined to two or more other atoms of other molecules or ions (called ligands) forming heterocyclic rings, with the central atom as a part of each ring. The term is derived from claw to signify the two or more bonds holding the central atom (metal) to the ligand. Metal chelates of biological systems include the iron-binding porphyrin of hemoglobin and the magnesium-binding of chlorophyll. Ethylenediaminetetraacetic acid (EDTA) is a common chelating agent.

Erythropoietin .......... A hormone secreted by the kidneys that stimulates the bone marrow to make red blood cells in erythropoiesis.

Ferritin ................. An iron-protein complex that can store up to 5,000 atoms of iron in each molecule. Formed by the union of ferric iron with apoferritin in the intestinal mucosa.

Fibrosis ................. Growth of fibrous tissues (e.g. scar tissues) usually as a reparative or reactive process.

Hemosiderin ............. See siderin.

Hemochromatosis ...... See Section V (C), page 28.
Hypochromic anemia .... The red blood cells contain less hemoglobin than normal in this form of anemia.

Idiopathic disease ........ A disease of unknown origin.

Intrinsic iron .......... Iron originally or naturally present in foods. Iron not naturally present is extraneous, or adventitious iron.

Kupffer cells ........... Specialized reticuloendothelial liver cells that degrade hemoglobin.

Lipotrope ............... A dietary factor that aids the digestion and metabolism of fats.

Microcytic anemia ...... An anemia in which the average size of the erythrocytes is smaller than normal. Includes syndromes of nutritional hypochromic anemia and chlorosis.

Parenchymal cells ...... Distinctive cells of a tissue or organ contained in and supported by the connective tissue framework or stroma.

Parenteral .............. Injected into the body, not ingested.

Phagocytes .............. Scavenger cells that engulf fragments of body wastes.

Phlebotomy .............. Surgical blood-letting. Venesection means the same.

Portal cirrhosis ........ Fibrosis of liver tissues around portal system veins that carry blood from the gut to the liver.

Reduced iron ............ A pure form of elemental iron obtained by a chemical process in the form of a grayish black amorphous powder. Used as a dietary supplement.
Reticuloendothelium ..... Cells of the reticuloendothelial system. Collectively the cells in different organs chiefly concerned with phagocytosis. Cells specialized for storage found mainly in the liver, spleen and bone marrow.

Siderin ............... Siderin (hemosiderin) is a granular, amorphous complex in which iron is more concentrated than in ferritin.

Siderosis .............. The presence of siderin mainly in reticuloendothelial cells usually implies that iron stores are excessive.

Transferrin ............ The blood plasma protein that accepts iron from the gut (at 2 atoms iron per molecule transferrin) and transports it to other tissues.

Venesection ............ Same as phlebotomy.
A. PROPOSED IMPROVEMENT OF NUTRIENT LEVELS OF ENRICHED FOODS.

(Reprinted from Federal Register of December 3, 1971; 36 F.R. 30412)

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Food and Drug Administration
[21 CFR Parts 15, 17]

WHEAT FLOUR AND RELATED PRODUCTS AND PROCESSED PRODUCTS
Proposed Improvement of Nutrient Levels of Enriched Foods

A notice of proposed rule making was published in the Federal Register of April 1, 1970 (35 F.R. 5412), based on a petition jointly filed by the American Bakers Association, 1750 Pennsylvania Avenue NW, Washington, D.C. 20006, and the Millers’ National Federation, National Press Building, 520 14th Street NW, Washington, D.C. 20004, proposing that (1) iron be required at a level of not less than 50 milligrams and not more than 80 milligrams per pound of enriched flour (21 CFR 15.10) and enriched self-rising flour (21 CFR 15.60) and (2) that iron be required at a level of not less than 20 milligrams and not more than 38 milligrams per pound of enriched bread and enriched rolls or buns (21 CFR 17.2).

In the same publication, the Commissioner of Food and Drugs, on his own initiative, proposed that the standard for enriched bread and enriched rolls or buns also be amended by inserting a statement that iron and calcium may be added only in forms which are harmless and assimilable. The standards for enriched flour and enriched self-rising flour already bear such a statement.

Concerning the quantity of iron proposed, comments both in favor of and in opposition to the proposal were received. Because of the issues raised by these comments and on the basis of other relevant information the Commissioner concludes that an alternative proposal should be published.

The major public health significance of widespread iron deficiency anemia was recognized by the 1969 White House Conference on Food, Nutrition, and Health. The Conference recommendations urged that the nutrient levels of calcium, thiamine, riboflavin, niacin, and iron in enriched foods be increased, with particular emphasis on increasing iron enrichment ("White House Conference on Food, Nutrition, and Health, Final Report" for sale by the Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20405; price $3). In a statement of November 1969 entitled "Recommendations for Increased Iron Levels in the American Diet" the Food and Nutrition Board, National Academy of Sciences-National Research Council, endorsed increasing the iron enrichment levels in wheat flour and bread (copies of this statement available without charge from the Food and Nutrition Board, National Academy of Sciences-National Research Council, 2101 Constitution Avenue NW, Washington, D.C. 20410). The Council on Foods and Nutrition, American Medical Association, has also endorsed increasing the iron levels to 40 milligrams per pound for enriched flour and 25 milligrams per pound for enriched bread, buns, and rolls. The Ten-State Nutrition Survey conducted by the Department of Health, Education, and Welfare has clearly substantiated the widespread, high prevalence rates of iron deficiency anemia ("Ten-State Nutrition Survey in the United States, 1966-1967, Preliminary Report to the Congress, April 1971") prepared by the Center for Disease Control, U.S. Department of Health, Education, and Welfare, Public Health Service, Health Services and Mental Health Administration, Atlanta, Ga. 30339). There is also evidence to indicate that the average iron intake in the United States has declined in recent decades due to decreased caloric intakes, less contamination of foods with extraneous sources of iron, and the virtual disappearance of iron cooking vessels.

Grain products are almost universally consumed and continue to be the most suitable vehicle for iron enrichment of the American diet. These products currently provide approximately 28 percent of the average total caloric intake, even though their per capita consumption dropped substantially during the period 1940-69. Also, approximately two-thirds of the flour currently consumed in the United States is enriched.

In view of the above, the Commissioner concludes that an increase in the amount of iron in the diet should be made available. The Commissioner also concludes that the amounts of the nutrients, calcium, thiamine, riboflavin, and niacin presently provided for in the standards should be changed to make it easier to prepare enriched bread and significantly fortified nonstandardized bakery products from enriched flour alone. The Commissioner proposes that the amounts of nutrients in enriched flour be so adjusted that bakers, relying on the enrichment provided in enriched flour, will be able in most instances to produce enriched bread meeting the requirements of the enriched bread standard.

The Commissioner also proposes that the amounts of nutrients added to enriched self-rising flour and enriched farina be changed so that they are substantially the same as those added to enriched flour. This measure will help to ensure an improved nutritional quality of the diet when home-prepared products made from enriched self-rising flour or enriched farina are consumed in place of enriched bread.

The present standards for flour and bread provide a range for the quantities of most added nutrients with both maximum and minimum levels specified. In order to insure uniformity and maximum benefit to the consumer, the Commissioner now proposes that the present ranges for flour and bread be deleted and that single level requirements, with provisions for reasonable variance within the limits of good manufacturing practice, be substituted. With certain exceptions, as pointed out below, the single level requirements proposed in this notice are close to the maximum levels in the ranges presently provided for in the existing standards.

The proposed level of iron for enriched flour (§ 15.10 (which affects § 15.60 by cross reference) and § 15.60) is 40 milligrams per pound. This level is 33.8 milligrams more than the maximum level now permitted and 20 milligrams less than the maximum level proposed in the notice of April 1, 1970 (35 F.R. 8412). With respect to enriched bread (§ 17.2), the proposed 25 milligrams per pound is 12.5 milligrams more than the maximum level now permitted and 13 milligrams less than the maximum level proposed in the April 1, 1970, notice. In order to insure uniformity, the amount of iron proposed for enriched farina is also 40 milligrams per pound of finished food, as compared with a minimum of 13 milligrams and no maximum in the present standard.

The proposed level for calcium in enriched flour and in enriched farina is 900 milligrams per pound of finished food, except that when more calcium is needed for technical purposes in enriched self-rising flour the quantity may exceed 900 milligrams per pound but the excess shall be no greater than that necessary to accomplish the intended effect. The ranges provided for in the existing standard are 600-235 milligrams for enriched flour, 560-1,560 milligrams for enriched...
an enriched flour, and 500 milligrams sodium or less, maximum, for enriched farina. The proposed level for calcium in enriched bread is 400 milligrams per pound of finished food, as compared with a range of 300–900 milligrams in the present standard.

The need for vitamin D in human nutrition and the importance of maintaining a daily intake sufficient to protect infants and growing children from developing rickets is well established. The use of whole milk, evaporated milk, infant formula products, processed breakfast cereals, and dietary supplement preparations that contain added vitamin D to provide 400 U.S.P. units daily in normal usage is ample for this purpose. The addition of vitamin D to other foods may serve only to increase to excessive levels the intake of vitamin D by infants, children, and pregnant women. The Commissioner concludes that avoiding excessive intakes of vitamin D is in the public interest, and accordingly, in the amendment proposed below, provisions for the addition of vitamin D have been deleted from the standards for enriched flour, enriched cereal, enriched farina, and enriched bread and enriched rolls or buns.

Therefore, based on available information, the Commissioner proposes that:

1. Section 15.10 be amended by revising paragraphs (a), (b), and (c), as follows:

§ 15.10 Enriched flour; identity; label statement of optional ingredients.

(a) It contains in each pound 2.9 milligrams of thiamine, 1.8 milligrams of riboflavin, 24 milligrams of niacin, and 40 milligrams of iron (Fe).

(b) It may contain added calcium in such quantity that the total calcium (Ca) content is 980 milligrams per pound of enriched flour. Enriched flour may be acidified with monocalcium phosphate within the limits prescribed by § 15.70 for phosphated flour but, if insufficient additional calcium is present to meet the requirement for calcium as an optional nutrient, no claim may be made on the label for calcium as a nutrient.

(c) The requirements of paragraphs (a) and (b) of this section will be deemed to have been met if reasonable overages of the vitamins and minerals, within limits of good manufacturing practice, are present to insure that the required levels of the vitamins and minerals are maintained throughout the expected shelf life of the food under customary conditions of distribution.

Due to a cross reference, amendment of § 15.10 would have the effect of simplifying, and providing for enriched bromaet flour (§ 15.20).

2. Section 15.60 be amended by revising paragraphs (a) and (b) and by revising the closing text of the section, as follows:

§ 15.60 Enriched self-rising flour; identity; label statement of optional ingredients.

(a) It contains in each pound 2.9 milligrams of thiamine, 1.8 milligrams of riboflavin, 24 milligrams of niacin, 50 milligrams of iron (Fe), and 960 milligrams of calcium (Ca). A calcium compound is added for ornamental purposes to give self-rising characteristics to the flour; the amount of calcium (Ca) per pound of flour may exceed 980 milligrams provided that the excess is no greater than that necessary to accomplish the intended effect.

(b) The requirements of paragraph (a) of this section will be deemed to have been met if reasonable overages of the vitamins and minerals, within limits of good manufacturing practice, are present to insure that the required levels of the vitamins and minerals are maintained throughout the expected shelf life of the food under customary conditions of distribution.

Iron and calcium may be added only in forms which are harmless and assimilable. The substances referred to in paragraph (a) of this section may be added in a form of calcium which does not impair the enriched self-rising flour; such carrier is used only in the quantity necessary to effect an intimate and uniform admixture of such substances with the flour.

3. Section 15.140 be amended by revising paragraphs (1), (2), and (3) and the closing text in paragraph (a), as follows:

§ 15.140 Enriched farina; identity; label statement of optional ingredients.

(a) * * *

(1) It contains in each pound 2.9 milligrams of thiamine, 1.8 milligrams of riboflavin, 24 milligrams of niacin, and 40 milligrams of iron (Fe).

(2) It may contain added calcium in such quantity that the total calcium (Ca) content is 980 milligrams per pound of finished enriched farina.

(3) The requirements of subparagraphs (1) and (2) of this paragraph will be deemed to have been met if reasonable overages of the vitamins and minerals, within limits of good manufacturing practice, are present to insure that the required levels of the vitamins and minerals are maintained throughout the expected shelf life of the food under customary conditions of distribution.

Iron and calcium may be added only in forms which are harmless and assimilable. The substances referred to in subparagraphs (1) and (2) of this paragraph may be added in a form of calcium which does not impair the enriched farina; such carrier is used only in the quantity necessary to effect an intimate and uniform admixture of such substances with the farina.

4. Section 17.2 be amended by revising paragraph (a) (1), (2), and (3) and the closing text in paragraph (a), as follows:

Pursuant to provisions of the Federal Food, Drug, and Cosmetic Act (secs. 401, 701, 52 Stat. 1046, 1055, as amended 70 Stat. 943, 944, 92 Stat. 909, 910, 21 U.S.C. 341, 342, 371) and in accordance with authority delegated to the Commissioner of Food and Drugs (21 CFR 2.120), interested persons are invited to submit their views in writing (preferably in quintuplicate) regarding this proposal within 60 days after its date of publication in the Federal Register. Such views and comments should be addressed to the Hearing Clerk, Department of Health, Education, and Welfare, Room 6–48, 5600 Fishers Lane, Rockville, Md. 20852, and may be accompanied by a memorandum or brief in support thereof. Received comments may be seen in the above office during work hours, Monday through Friday.

Dated: November 22, 1971.

SAM D. FINE, Associate Commissioner for Compliance.

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Food and Drug Administration

[21 CFR Parts 15, 17]

WHEAT FLOUR AND RELATED PRODUCTS AND BAKED PRODUCTS

Proposed Improvement of Nutrient Levels of Enriched Foods; Extension of Time for Filing Comments

The notice published in the Federal Register of December 3, 1971 (36 F.R. 23074), proposing to improve the nutrient levels of enriched foods, provided 60 days for the filing of comments.

The Commissioner of Food and Drugs has received many requests to extend such time and, good reason therefor appearing, the time for filing comments is hereby extended to May 1, 1972.

This action is taken pursuant to provisions of the Federal Food, Drug, and Cosmetic Act (secs. 401, 701, 52 Stat. 1046, 1065 as amended by 70 Stat. 519 and 72 Stat. 948; 21 U.S.C. 341, 371) and under authority delegated to the Commissioner of Food and Drugs (21 CFR 2.129).


SAM D. FINE,
Associate Commissioner
for Compliance.

[FR Doc.72-3714 Filed 2-11-72; 8:48 am]
XIII. SCIENTIFIC CONSULTANTS
ON
A REVIEW OF THE SIGNIFICANCE OF DIETARY IRON ON IRON STORAGE PHENOMENA

A. ATTENDEES, AD HOC STUDY GROUP MEETING
   November 30 and December 1, 1971

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* Deceased, August 1972.