Chapter 1

History of iron overload disorders

In 1704 in Berlin, Heinrich Diesbach and Johann Konrad Dippel attempted to manufacture a synthetic red pigment. By accident, Dippel mixed potash, animal oil derived from blood, and iron sulfate. Thereafter, he discovered that he had produced an insoluble, light-fast, dark blue pigment. This color was first used extensively to dye the uniforms of the Prussian army, and became known as "Prussian blue." Almost 150 years later, physicians and scientists recognized the feasibility of visualizing iron in tissue using a similar staining sequence. After more than 250 years, it became practical to quantify iron in blood and tissue, permitting case finding and screening for hemochromatosis and iron overload. Maneuvers to treat iron overload began in the same era. In the interval 1994–1996, the genetic bases of four different iron disorders (X-linked sideroblastic anemia, aceruloplasminemia, hereditary hyperferritinemia-cataract syndrome, and *HFE* hemochromatosis) were elucidated. The pace of basic science, clinical, and sociological revelations pertinent to hemochromatosis and iron overload disorders continues to accelerate. This chapter provides an abbreviated chronology of these discoveries.

Iron in tissue

In 1847, Rudolph Virchow reported the occurrence of golden brown granular pigment at sites of hemorrhage and congestion in tissue examined by microscopy. The pigment was soluble in sulfuric acid, yielded a red ash on ignition, and produced a positive Prussian blue reaction.[1] In 1867, Max Perls formulated the first practical acidified ferrocyanide reaction for histologic analysis of iron, and applied the staining reaction to a variety of tissues.[2] In 1962, Scheuer and colleagues reported a method of grading iron stained using Perls' technique in hepatic biopsy specimens in patients with hemochromatosis and their relatives, and described the characteristic gradient of iron distribution in hepatocytes in hemochromatosis.[3] The method is widely used today.

Iron overload disorders

Armand Trousseau described the syndrome of hepatic cirrhosis, pancreatic fibrosis, and cutaneous hyperpigmentation in 1865, but he did not recognize the involvement of iron in its pathogenesis (Table 1.1).[4] Troisier's account of *diabète bronzé et cirrhose pigmentaire* in 1871 confirmed and extended that of Trousseau, and described in detail the iron-reactive pigment in various tissues.[5] Troisier's syndromic triad became the *sine qua non* of hemochromatosis diagnosis for decades. In 1889, von Recklinghausen reported the use of the methods of Virchow and Perls to identify excess iron in tissues obtained at autopsy of persons who had *hämochromatose*.[6] Following the theories of Virchow, von Recklinghausen believed that the iron-containing pigment was derived from blood (due to hemorrhage or hemolysis), rather than from the primary deposition of iron. The syndrome of "juvenile hemochromatosis" was first recognized and described by French authors in the early 1930s who called it *le syndrome endocrine-hepato-cardiaque*.[7,8] Early cases were summarized by Royer de Vericourt in his 1935 thesis (Table 1.1).[9]

The first substantive characterization of iron overload in non-European peoples was published in 1929 by Scottish medical student A.S. Strachan.[10] He was appointed as the first Professor of Pathology in 1926 at the newly created Medical School of the University of the Witwatersrand in Johannesburg.[11] His 1929 thesis was based on a necropsy study of 876 individuals from several parts of southern and central Africa who died in Johannesburg from 1925 to 1928. Strachan concluded that

> haemochromatosis is a not uncommon disease in the South African native; the chief factor in its production

Table 1.1. An abbreviated chronology of major heritable primary iron overload disorders[a]

Disorder	Date, author of first clinical report	Gene (protein)	Date, author of first genetic definition	Reference(s)
HFE hemochromatosis	1871, Troisier	*HFE* (HFE)	1996, Feder	5,43
Early age-of-onset ("juvenile") hemochromatosis	1932, Bezançon	*TFR2* (transferrin receptor-2)	2000, Camaschella	65
		HAMP (hepcidin)	2003, Merryweather-Clarke; Roetto	66,67
		HJV (hemojuvelin)	2004, Papanikolaou	68
		SLC40A1 (ferroportin)[c]	2005, Sham	69
X-linked sideroblastic anemia	1945, Cooley	*ALAS2* (δ-aminolevulinate synthase)	1994, Cotter	70,71
Atransferrinemia	1961, Heilmeyer	*TF* (transferrin)	2000, Beutler	72,73
Aceruloplasminemia	1987, Miyajima	*CP* (ceruloplasmin)	1995, Harris; Yoshida	74–76
Hereditary hyperferritinemia-cataract syndrome[b]	1995, Beaumont; Girelli	*FTL* (ferritin light chain)	1995, Beaumont; Girelli	77–79
Autosomal dominant iron overload with predominance of macrophage iron deposition	1999, Pietrangelo	*SLC40A1* (ferroportin)[d]	2001, Montosi	80,81
Autosomal dominant iron overload	2001, Kato	*FTH1* (ferritin heavy chain)	2001, Kato	82
DMT1 hemochromatosis	2004, Priwitzerova	*DMT1* (divalent metal transporter-1)	2005, Mims	83,84
Anemia and iron overload due to glutaredoxin-5 deficiency	2007, Camaschella	*GLRX5* (glutaredoxin-5)	2007, Camaschella	85

Notes: [a]The clinical descriptions of the disorder and the genetic definitions were not necessarily made from the same subjects or kinships. There may be a heritable component of African iron overload, although a putative African iron overload gene[86] remains unidentified.
[b]Hereditary hyperferritinemia-cataract syndrome is not associated with iron overload, but is included here because it mimics autosomal iron overload in many kinships.
[c]"Gain-of function" *SLC40A1* mutations are typically associated with this phenotype.
[d]"Loss-of function" *SLC40A1* mutations are typically associated with this phenotype.

appears to be the diet. The development of the complete picture of bronzed diabetes depends on the degree of deposition of the pigment and the rate of its deposition.[10,11]

Possible causes of iron overload

In 1935, the English gerontologist Joseph H. Sheldon[12] (Fig. 1.1) summarized 311 carefully selected "haemochromatosis" cases from the literature.[13] He concluded that the absorption of iron (and possibly that of other metals) is increased in hemochromatosis, and suggested that the disorder is an inborn error of metabolism that primarily affects men. Sheldon rejected hypotheses that diabetes, infections, intoxication, alcoholism, and other conditions cause hemochromatosis, and he viewed Strachan's findings with skepticism.[11] In the 1960s, Richard A. MacDonald opined that iron overload and tissue and organ injury in whites with hemochromatosis are typical consequences of alcoholism and other nutritional factors, and not the result of an inborn error that enhances intestinal absorption of iron.[14] MacDonald

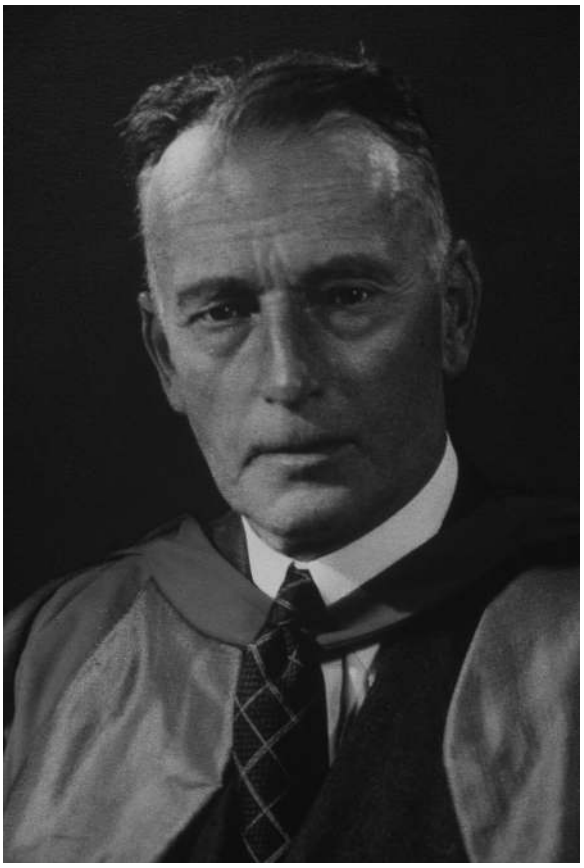

Fig. 1.1. Dr. Joseph H. Sheldon, an English gerontologist, was the author of the first monograph on hemochromatosis; his text was published in 1935.[12,13] Sheldon's observations on 311 cases carefully selected from the literature led him to conclude that iron absorption is increased in persons with hemochromatosis, and to suggest that the hemochromatosis is an inborn error of metabolism that primarily affects men.[13]

derived support of his opinion from Strachan's report of alcohol consumption and nutritional deficits in Native Africans with iron overload.

Many investigators subsequently published evidence of the central role of a heritable factor that increased iron absorption in whites with hemochromatosis, and recognized that the clinical phenotype of hemochromatosis was influenced by age, sex, and other attributes. In contrast, it was apparent in 1964 that the severity of iron overload in sub-Saharan Native Africans was directly related to their rate of consumption of iron contained in traditional beer.[15] In this disorder, hepatic iron concentrations usually exceeded those typical of alcoholism, and most Native Africans with iron overload did not have histologic abnormalities of the liver typical of alcoholism.[11]

Diagnosis of hemochromatosis and iron overload

The 1962 histologic grading method of Scheuer and colleagues, and the availability of reliable clinical measurements of serum iron, total serum iron-binding capacity, and serum ferritin provided the standard basis for ascertaining the iron phenotype of hemochromatosis for more than 30 years. Phenotype variability among probands and the respective family members with hemochromatosis could be demonstrated using these techniques.[16,17] The hepatic iron index helped to distinguish presumed hemochromatosis homozygotes from heterozygotes and persons with alcoholism and other conditions,[18] although validity of the index in evaluating persons with non-*HFE* hemochromatosis or iron overload has not been established. SQUID (superconducting quantum interference device) and magnetic resonance imaging have been used as non-invasive techniques to estimate organ iron content since 1982 and 1983, respectively.[19,20]

Complications of iron overload

Since Troisier's account,[5] it has been generally acknowledged that the liver is the major target organ of iron overload in hemochromatosis (and many other iron overload disorders).[21] Bassett and co-workers demonstrated that the hepatic iron concentrations in hemochromatosis are directly related to the occurrence of cirrhosis.[18] This argues strongly for early diagnosis and treatment of iron overload, although some reports suggest that phlebotomy therapy may reverse cirrhosis in some patients.[22] Diabetes mellitus, once attributed only to iron overload of the pancreatic islets, is now associated primarily with insulin resistance, coincidental inheritance of other diabetogenic genes, and other factors. Accordingly, iron depletion changes diabetes control very little in most patients. Cardiac siderosis with cardiomyopathy and arrhythmias were first described in the 1930s,[9] and occur almost exclusively in persons with early age-of-onset ("juvenile") hemochromatosis due to mutations in the genes that encode transferrin receptor-2, hemojuvelin, hepcidin, or ferroportin. Hypogonadotrophic hypogonadism is widely recognized as the cause of *infantilisme* in children and young

adults with early age-of-onset hemochromatosis, but also explains loss of libido and muscle mass and the development of osteoporosis in some men with *HFE* hemochromatosis. In 1964, Schumacher described a distinctive type of arthropathy that affects some persons with hemochromatosis.[23] Factor(s) other than hemochromatosis alleles or iron overload may cause this condition. The cutaneous abnormalities of hemochromatosis are more diverse than the hyperpigmentation described by Trousseau and Troisier.[24]

Iron overload injures the liver and other organs by causing discrete biochemical and microanatomical lesions.[25] Two major (but not mutually exclusive) mechanisms have been proposed to describe the toxicity induced by iron overload at the cellular and subcellular levels.[25] The oxidative injury hypothesis postulates that iron overload in vivo results in the formation of oxyradicals, with resultant damage to cellular constituents and impairment of cellular function. The lysosomal injury hypothesis proposes that excessive accumulation of iron within lysosomes can lead to lysosomal fragility, impaired lysosomal function, and eventual cellular injury through the release of hydrolytic enzymes and stored iron into the cytoplasm. In addition to lysosomes, iron-induced oxyradicals may damage hepatic mitochondria, endoplasmic reticulum, plasma membranes, and DNA.[25]

Iron is carcinogenic in humans and in laboratory animals.[26] The incidence rate of primary liver cancer in persons with hemochromatosis is high, especially in those with cirrhosis, as reported by Deugnier and colleagues in 1993.[27,28] African iron overload may be a risk factor for esophageal carcinoma. Mechanisms whereby iron may be involved in carcinogenesis include induction of oxidative damage of DNA, facilitation of tumor proliferation, and modifications of the immune system.[26] Common hepatic disorders such as alcoholism, steatosis, and viral hepatitis may augment liver injury and dysfunction in persons with hemochromatosis, and may also enhance liver iron deposition in persons with hemochromatosis or African iron overload.

Iron and immunity

Iron is essential to the normal function of neutrophils, macrophages, and lymphocytes, but iron overload impairs many functions of these cells.[29] Over several decades, there have been case reports of fulminant infections by unusual siderophilic bacteria, fungi, or trypanosomes in persons with hemochromatosis, iron overload, or chronic liver disease.[29,30] Infections are not generally reported as major causes of morbidity or mortality in persons with hemochromatosis, although a re-evaluation of the 1929 work of Strachan revealed that tuberculosis was a significant cause of death in black South Africans with iron overload.[10,11] In 1978, de Sousa and co-workers reported fundamental relationships of hemochromatosis, iron overload, and numbers of CD8+ lymphocytes.[31] In 2003, it was reported that approximately 30% of hemochromatosis probands have a form of heritable antibody deficiency linked to chromosome 6p.[32] In 2005, a significant inverse relationship of total blood lymphocyte counts and severity of iron overload in hemochromatosis probands was described,[33] extending the previous conclusions of de Sousa and co-workers. Altogether, immunity in patients with iron overload encompasses functions of infection resistance, modulation of iron overload, and tumor surveillance, and these may be decreased in some respects in many patients.

Treatment and outcomes

In 1952, Davis and Arrowsmith reported treating three persons with hemochromatosis with repeated phlebotomy.[34] A long-term study published in 1988 demonstrated that the longevity of hemochromatosis patients treated with phlebotomy was greater than that of untreated subjects.[35] Phlebotomy also permits an accurate (although retrospective) quantification of total body iron burdens in patients with hemochromatosis. In a prospective trial, Niederau and colleagues published in 1996 that hemochromatosis patients without hepatic cirrhosis or diabetes mellitus who undergo iron depletion have normal actuarial survival.[36]

In 1962, Sephton-Smith reported the use of the parenteral iron chelator deferoxamine to manage iron overload associated with severe thalassemia.[37] By 1994, it was demonstrated that the early use of deferoxamine in an amount proportional to the transfusion iron load reduces the body iron burden and helps protect against diabetes mellitus, cardiac disease, and early death in patients with thalassemia major.[38] In 1983, Kontoghiorghes and colleagues first reported the treatment of iron overload in thalassemia with the oral iron chelator deferiprone ("L1").[39] In 2003, Galanello and colleagues first reported the

treatment of iron overload in thalassemia with the oral iron chelator deferasirox.[40]

Genetic basis of iron overload syndromes

In 1975, Simon and colleagues reported in a letter that the genetic factor associated with hemochromatosis was closely linked to the human leukocyte antigen (HLA)-A*03 locus on chromosome 6p.[41] By the late 1970s, HLA immunophenotyping was used to identify relatives of probands who also inherited two HLA-linked hemochromatosis alleles, sometimes before iron overload occurred. In 1977, Utah investigators reported the utility of various iron measures as diagnostic criteria for stages of hemochromatosis in patients and relatives.[16] In 1988, Edwards and colleagues used iron phenotyping and HLA typing to screen more than 11 000 white Utah blood donors for hemochromatosis, demonstrating clearly that hemochromatosis is a common heritable disorder expressed more prominently and frequently in men than women.[42]

In the half-century between 1945 and 1995, X-linked sideroblastic anemia, aceruloplasminemia, hereditary hyperferritinemia-cataract syndrome, and ferroportin hemochromatosis were described, and the heritable nature of each condition was proven or strongly suspected at first publication (Table 1.1).

In 1996, Feder and colleagues discovered a previously undocumented atypical major histocompatibility (MHC) class I gene on chromosome 6p by analyzing whites with hemochromatosis. In almost every case, the gene (later named *HFE*) contained the missense mutations C282Y or H63D (Table 1.1).[43] *HFE*, expressed only by certain tissues, encodes a protein that binds transferrin receptor and participates in controlling iron absorption. The C282Y mutation causes a significant disruption in the tertiary structure of HFE protein, and thereby a consequential loss of function. Homozygosity for *HFE* C282Y mutation occurs in approximately 90% of persons with hemochromatosis phenotypes, and this genotype defines "classical" hemochromatosis. As expected, C282Y occurs almost exclusively in whites of European descent. In contrast, the *HFE* H63D mutation occurs in almost all population groups worldwide, although it causes little change in HFE protein structure and thus is infrequently associated with increased iron absorption.

Today, *HFE* genotyping facilitates routine clinical evaluation and population screening for hemochromatosis, especially in European white populations. The discovery of *HFE* also kindled renewed enthusiasm for the study of iron among scientists and clinicians, leading to a "golden age" of discovery of many previously unknown proteins and control mechanisms germane to iron absorption and homeostasis, and to the clinical and genetic characterization of iron overload syndromes less common but no less informative than *HFE* hemochromatosis (Table 1.1).[44]

Population screening for hemochromatosis and iron overload

Severe iron overload is common in series of patients diagnosed to have hemochromatosis in medical care, and thus it was generally presumed for many years that most hemochromatosis (or C282Y) homozygotes would eventually develop injurious iron overload. Accordingly, population screening using iron phenotyping of white populations was promoted to achieve early diagnosis and permit timely treatment to alleviate iron overload.[45] In 2000, Beutler and colleagues reported the results of screening a large multiracial adult population in southern California using iron phenotyping and *HFE* genotyping. They revealed, to the surprise of many, that most C282Y homozygotes do not have symptoms or signs attributable to iron overload, that few have evidence of consequential liver disease, and that life expectancy for most is normal.[46,47] Other large screening studies in Norway, Australia, and North America confirmed the essence of these outcomes.[48–50] Each of two large screening studies in North America, including one that oversampled African Americans, Hispanics, and Asians, demonstrated that few non-whites had evidence of *HFE* hemochromatosis or non-transfusion iron overload.[46,50] In 2006, the US Preventive Services Task Force concluded that "Research addressing genetic screening for hereditary hemochromatosis remains insufficient to confidently project the impact of, or estimate the benefit from, widespread or high-risk genetic screening for hereditary hemochromatosis."[51,52] Regardless, there is substantial and rational support for hemochromatosis screening in certain subpopulations of whites, including selected family members of probands,[16,53,54] men,[42,55] persons with undiagnosed liver disease,[56] and those with serum ferritin >1000 μg/L.[57]

Social issues

After the discovery of *HFE* in 1996, there was heightened concern about the potentially negative social implications of phenotype or genotype diagnoses of heritable disorders, especially one as common as hemochromatosis. In 2003, Shaheen and colleagues reported that insurance denial and increased premium rates were reported commonly by hemochromatosis patients without end organ damage diagnosed in medical care, but the overall proportions with active insurance, good quality of life, and psychological well-being were similar to those of siblings without hemochromatosis.[58] In the HEIRS Study, there were only minor negative emotional responses to the genetic testing.[59,60] The risk of insurance or employment problems 1 year after phenotype and genotype screening for hemochromatosis and iron overload was also very low.[61] In the last decade, there has been much interest in utilizing hemochromatosis patients as blood donors. Altogether, many hemochromatosis patients and their donated units meet current blood bank criteria for acceptability, and policy changes to permit treatment-related blood donations without "labeling" the harvested blood units have been enacted.[62–64]

Conclusions

Pursuit of the etiology and treatment of hemochromatosis first described in western Europeans in the nineteenth century has led to a plethora of basic science discoveries and clinical revelations since 1996, the magnitude of which is unparalleled in the history of iron studies in living organisms. Many other important basic science and clinical truths about hemochromatosis and other iron overload disorders probably remain to be discovered and published. Future directions for clinical and basic science research in hemochromatosis and iron overload are discussed in Chapter 39.

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Chapter 2

Normal iron absorption and metabolism

Iron is essential to life because it is the central oxygen ligand in the heme proteins hemoglobin and myoglobin. Accordingly, there are many interactions between iron homeostasis and oxygen regulation and delivery. Iron is also required for cytochrome P-450 enzyme oxidative metabolism and DNA synthesis. In health, body iron content is controlled by absorption that responds to iron losses and the rate of erythropoiesis. Multiple mechanisms provide functional feedback control of iron homeostasis, tissue oxygen sensing and delivery, and the tempo of red blood cell production. The physiologic capacity to excrete iron is very limited. Thus, body iron content is regulated almost entirely by controlled absorption.[1,2] This chapter reviews the basic physiologic and molecular characteristics of iron metabolism and homeostasis, and their pertinence to iron overload disorders.

Iron physiology

Normal iron homeostasis is maintained by absorption of iron from the diet that precisely balances iron loss, and by controlled iron distribution in the body.[1,3] Normal healthy adults have 4000–5000 mg of iron (Table 2.1). Daily iron loss occurs due to perspiration, desquamation from skin, and minor injuries, and from the gastrointestinal tract. The rate of this unavoidable iron loss is proportional to body iron stores. Women lose additional iron due to menstruation, pregnancy and childbirth, and lactation. Overall, daily iron losses in adult men and post-menopausal women are approximately 1.0 mg and in menstruating women approximately 1.5 mg. The median iron loss ascribable to pregnancy is 500 mg.[4] Iron requirements of growth and development in infants or adolescents may exceed those of normal adults severalfold.

Hemoglobin in erythrocytes constitutes the largest normal body iron pool. Developing erythroid cells in the bone marrow highly express surface transferrin receptors to obtain iron for hemoglobin synthesis. In healthy adults, red blood cell production is ~2 million cells/second. Normal erythropoiesis requires the delivery of ~25 mg Fe daily to erythroid cells via transferrin. This amount far exceeds the amount of iron needed to replenish stores. The quantity of iron in the circulating erythrocyte mass is usually stable, although this may change as an adaptation to altitude, lung disease, cigarette smoking, or other conditions that affect oxygen availability. Erythropoiesis stimulated by blood loss, hemolysis, erythropoietin, or hypoxemia requires an additional 10–40 mg Fe daily. Mature erythrocytes filled with hemoglobin circulate in the blood. Many physiology experiments of past decades sought to identify the "erythropoiesis regulator," a substance now generally acknowledged to be erythropoietin.

Hepatocytes and macrophages (including Kupffer cells) contain storage iron, and represent the second largest normal iron pool. In health, the quantity of iron present in hepatocytes and macrophages is nonetheless relatively small in comparison with the erythroid compartment, especially in women of reproductive age. Amounts of stored iron in these sites sometimes increases with age. Many physiology experiments of past decades also sought to identify the "stores regulator," now acknowledged to be hepcidin.

Myoglobin, the third largest iron pool, contains heme moieties in myoglobin that are necessary for normal muscle and cardiac function. The quantity of iron in myoglobin iron is relatively stable, although it increases with growth and development and decreases with age or catabolism. At high altitude, iron needed for erythropoiesis may be donated by myoglobin.

Typical daily western diets contain 10–20 mg of iron. Many vegetarian diets contain less iron, but the quantity is nonetheless sufficient to replace daily iron losses. There are two major forms of intrinsic food