Histochemical Studies of the Myocardium and Conduction System in Acquired Iron-Storage Disease

By Paul F. Schellhammer, M.D., Mary Allen Engle, M.D., and Jack W. C. Hagstrom, M.D.

SUMMARY

The myocardium, all parts of the conduction system, and the S-A node contained iron in six patients with acquired iron-storage disease. The nerve fibers and ganglia contained negligible amounts. No correlation was demonstrated between the amount of histochemically demonstrated iron in the myocardium and conduction system, or the amount of transfused iron, or the amount of quantitatively analyzed myocardial iron and the presence of antemortem arrhythmias and conduction disturbances. The degree of scarring in the S-A node, A-V node, and bundle of His did not parallel the density of iron deposits in these structures.

These observations support the theory that factors other than iron alone are responsible for the cardiac rhythm and conduction abnormalities associated with iron-storage disease.

Additional Indexing Words:
Arrhythmias  Conduction defects  Heart block  Heart failure
Hemochromatosis  Anemia  Hemosiderosis  Blood transfusions  Thalassemia

The pathogenesis of iron-storage disease and its ultimate effect on organ function are still unclear. Much of the controversy would be resolved if there were conclusive evidence that iron is toxic for those tissues in which it is deposited. It has been frequently assumed that iron, when deposited in excessive amounts in parenchymal tissue, has a cytotoxic effect. This is based upon the finding of necrotic foci and scars in viscera in which iron is present. The questions to be answered are whether the presence of excessive iron within cells is the cause of these changes, whether its presence triggers some other mechanism leading to cytolysis, or whether it is a coincidental finding.

The term "hemochromatosis" presented includes two related, but distinct, entities. The first, defined as "idiopathic" or "endogenous" hemochromatosis, refers to the accumulation of intracellular iron by what is believed to be a hereditary aberration in gastrointestinal iron absorption; the second, "exogenous" hemochromatosis, refers to the accumulation of intracellular iron secondary to massive oral intake or to numerous blood transfusions. The latter is often an iatrogenic disorder and, as such, may be preventable.

Despite the disagreement concerning the relationship between iron and tissue destruction, the fact remains that progressive deterioration of cardiac function is a frequent cause of death among persons with both exogenous and endogenous hemochromatosis. Iron deposits in the myocardium have been held responsible.

A clinicopathological study was undertaken by James to investigate the changes present...
in the myocardium and conduction system in the hearts of five patients with hemochromatosis. Two had exogenous, and three had endogenous, hemochromatosis. He found extensive iron deposits in the myocardium and in the atrioventricular (A-V) node of each heart, but noted that the sinoatrial (S-A) node was spared in all cases. He postulated that the large amount of iron within the A-V node was responsible for the cellular necrosis and degeneration and that these changes could account for the antemortem conduction disturbances and arrhythmias. Concerning the absence of iron in the S-A node, he suggested that a difference in local tissue metabolism resulted in the lack of iron deposition.

The purpose of this paper is to report findings from a study of the conduction system and myocardium from the hearts of six young patients with acquired iron-storage disease. The patients studied had no history or anatomic evidence of primary arteriosclerotic, rheumatic, or hypertensive cardiovascular disease. Particular attention was directed to iron deposits and fibrous tissue in the S-A node, A-V node, A-V bundle, right bundle branch (RBB), left bundle branch (LBB), Purkinje fibers, autonomic nerve bundles and ganglia, and in the myocardium. We observed the relationship between the degree of iron deposition and the extent of scarring in these structures. Finally, we attempted to correlate the estimated transfusional intake of iron, the quantitatively analyzed myocardial iron content, and the severity of microscopic iron deposits in the conduction system with the frequency and severity of antemortem arrhythmias and conduction defects.

Methods

Four hearts from patients with thalassemia major (cases 1 to 4) and two from patients with chronic, refractory, regenerative anemia (cases 5 and 6) were studied both in the gross and microscopically. The microscopic changes in the myocardium and in the specialized tissues of the conduction system were studied in detail. The conduction system was dissected from the heart according to the methods described by Hudson and Lev and associates. Step sections of the S-A node, A-V node, bundle of His, and the right and left bundle branches from each heart were made. Sections from the moderator band were taken to demonstrate Purkinje fibers; the posterior walls of the right atria were sectioned to demonstrate autonomic nerves and ganglia. The sections were stained with hematoxylin and eosin, Perls' iron, and the Masson trichrome stains.

All sections were evaluated with respect to the amount of iron deposited in these regions and in the myocardium. Sections of the S-A node, A-V node, bundle of His, and myocardium were evaluated with respect to the amount of scarring. The iron deposits and the extent of scarring were each described as absent, light, moderate, or heavy. There are limitations in the histochemical method employed for the quantitation of iron. Despite the fact that the Prussian blue reaction is a delicate test for the detection of iron, it is not possible to distinguish minor variations in the amount present. Furthermore, it cannot be assumed that all areas of any individual portion of the conduction system are uniformly affected. We noted such variation in the density of stainable iron and attempted to make an average evaluation from the several sections. Aliquots of myocardium of known weight were taken from the lateral wall of the left ventricle of each heart and were analyzed quantitatively for iron content using the technique described by Slavin and Sprague.

The histories of all patients were reviewed. The electrocardiograms in the charts were analyzed for arrhythmias and heart block. These tracings were obtained as clinically indicated; they were not continuous recordings in the days prior to death. The onset and duration of congestive cardiac failure were noted. The number of grams of iron received via transfusions was calculated. While it was possible to ascertain whether a patient had received oral iron therapy, it was not possible to obtain an accurate estimate of the duration and dose.

Results

The results are summarized in the tables. Death was due to congestive cardiac failure in all but one patient (case 6) who died from infection. In the last 1 to 2 years of their lives, four patients (cases 1, 2, 3, and 5) had arrhythmias and two (cases 1 and 2) showed atrioventricular conduction disturbances prior to the administration of digitalis. None had delayed intraventricular conduction. Clinical findings in cases 1 to 5 were
Iron-storage disease

Summary of Clinical Findings

<table>
<thead>
<tr>
<th></th>
<th>Case 1*</th>
<th>Case 2*</th>
<th>Case 3*</th>
<th>Case 4*</th>
<th>Case 5†</th>
<th>Case 6†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at death (yr)</td>
<td>25</td>
<td>20</td>
<td>9</td>
<td>8</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td>Iron I.V. (g)‡</td>
<td>104</td>
<td>5</td>
<td>58</td>
<td>25</td>
<td>29</td>
<td>34</td>
</tr>
<tr>
<td>Congestive heart failure (mo to death)</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>1/4</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Arrhythmia§</td>
<td>Supraventricular PC, PT</td>
<td>PT, A Fib, A Flt</td>
<td>PC, PT</td>
<td>PC, PT</td>
<td>PC, PT</td>
<td></td>
</tr>
<tr>
<td>Ventricular Heart block**</td>
<td>A-V</td>
<td>1*</td>
<td>1*, 2*, 3*</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Thalassemia major.
†Aregenerative anemia.
‡Calculation based on 250 mg Fe in 500 ml of whole blood or in 300 ml of packed cells.
§Key to arrhythmia: A Fib = atrial fibrillation; A Flt = atrial flutter; PC = premature contractions; PT = paroxysmal tachycardia.
**No intraventricular block.

Summary of Histochemical Findings

<table>
<thead>
<tr>
<th></th>
<th>Case 1 I.C.</th>
<th>Case 2 L.G.</th>
<th>Case 3 C.T.</th>
<th>Case 4 I.M.</th>
<th>Case 5 A.K.</th>
<th>Case 6 L.B.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight of Heart (g)</td>
<td>Observed 300</td>
<td>500</td>
<td>330</td>
<td>180</td>
<td>270</td>
<td>140</td>
</tr>
<tr>
<td>Expected</td>
<td>250</td>
<td>300</td>
<td>115</td>
<td>115</td>
<td>115</td>
<td>115</td>
</tr>
<tr>
<td>Iron content of myocardium (mg/g)</td>
<td>2.4</td>
<td>3.1</td>
<td>2.3</td>
<td>0.9</td>
<td>1.9</td>
<td>1.9</td>
</tr>
<tr>
<td>Extent of histologically demonstrated fibrosis (Masson’s trichrome stain)</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>S-A node</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>A-V node</td>
<td>++</td>
<td>++</td>
<td>0</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Bundle of His</td>
<td>++++</td>
<td>++++</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>RBB</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>LBB</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Ganglia</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>++</td>
<td>0</td>
</tr>
<tr>
<td>Nerves</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Purkinje fibers</td>
<td>++</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
<td>†</td>
<td>+++</td>
</tr>
<tr>
<td>Myocardium</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Extent of histologically demonstrated fibrosis (Masson’s trichrome stain) in conductive system and myocardium*</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

*Key: Severity of deposits and of fibrosis is indicated by: 0 = absent; + = light; ++ = moderate; +++ = severe.
†Specimens not detected on repeated section.

Table 1

Table 2

The findings in the gross were uniform; the myocardium of all hearts was brown; all weighed more than expected; and dilatation and hypertrophy were present in all. On microscopic examination, cardiac myofibers from all the hearts were heavily involved with iron. The S-A node in three instances contained iron deposits, moderate
in case 2 and light in cases 1 and 5. All other portions of the conduction system contained moderate to heavy deposits except in case 3 in which iron was minimal in these structures. The autonomic nerve fibers and ganglia were spared in all but cases 1 and 5. In case 5 there were moderate deposits of iron in the ganglia, but not in the nerves. In case 1 deposits were light in ganglia and nerves.

We attempted to correlate the extent of histochemically demonstrable iron deposits in the conduction system and myocardium with the severity and frequency of antemortem arrhythmias and conduction defects. No correlation was possible. Whereas the microscopic findings in the hearts of five patients (cases 1, 2, 4 to 6) were similar, two had neither rhythm nor conduction abnormalities, two had both, and one had supraventricular and ventricular arrhythmias. In the sixth patient (case 3), who had a supraventricular arrhythmia, there was minimal iron in all structures of the conduction system.

We attempted to correlate the extent of scarring in the S-A node, A-V node, bundle of His, and myocardium with the severity of iron deposits in these structures. Scarring was moderate to heavy in the myocardium of all hearts, and was heavy in the S-A node in all but case 3. Scarring was less marked in the A-V node and bundle of His in three instances (cases 4 to 6). Thus, the degree of scarring in each structure did not parallel the density of iron deposits.

Finally, the presence of severity of rhythm and conduction abnormalities did not correlate with either the estimated transfused iron or with the quantitatively analyzed iron in the myocardium.

Discussion

Trousseau, in 1865, first described an example of iron saturation of the tissues25 and Von Recklinghausen, in 1889, first used the term “hemochromatosis” to describe the classic triad of iron-loading disease (cirrhosis of the liver, fibrosis of the pancreas, and bronze pigmentation of the skin).26 Since then many questions have been raised and some of them have been settled concerning the pathogenesis of the condition: the manner in which iron is absorbed,27-33 the situations predisposing to excessive deposition of iron in tissues,36-40 factors that might differentiate dietary from parenteral hemochromatosis,41 the distinction between hemosiderosis and hemochromatosis based on evidence of tissue destruction and scarring in the latter,42 and the progression of dietary hemosiderosis36-39, 43-45 and of transfusional hemosiderosis46-50 to hemochromatosis. Iron-overloading in anemic patients has two bases: not only do they absorb increased amounts of iron, dietary and medicinal, from the gastrointestinal tract,28, 34, 39, 40 but also those kept alive by transfusions accumulate iron from administered blood.41, 49-51

There is still a question, however, concerning whether the iron is cytotoxic, its presence resulting in hepatic, pancreatic, and cardiac failure. Polson22-24 showed that the typical lesions of hemochromatosis failed to develop in animals to whom large amounts of iron were repeatedly administered via various routes. This finding has been criticized by some9, 47 on the grounds that observations for damage extended over too short a period (never greater than 2 years). Nevertheless this work provided some reason to doubt the acceptance of the cytotoxic effects of iron.

MacDonald25 believed that the histological and functional changes seen in hemochromatosis were unrelated to the presence of iron. He claimed that the iron deposits present in hemochromatosis were a coincidental finding in a group of patients who also had cirrhosis and that the two were separate phenomena bearing no causal relationship. He demonstrated that increased iron absorption and deposition occurred in response to hepatic injury.56 He pointed out that “wine, beer, and spirits” contained large amounts of iron.57 He explained the combined presence of iron and cirrhosis on the basis of a common factor resulting in two separate sequelae. He suggested that alcohol and an associated nutritional deficiency were causal.
in both the cirrhosis and the iron-loading. Once cirrhosis had developed, there was an increased avidity for iron which resulted in further tissue deposition. Though perhaps applicable in certain instances, this explanation does not apply to the children reported on in this paper who were under observation at this hospital from infancy.

The present study included four hearts from patients with Cooley’s anemia and two from patients with regenerative anemia. All had received large amounts of transfusional iron except one (case 2). Our data showed that the pattern of iron deposits in five of the six hearts was as follows: the S-A node contained light to moderate iron deposits in three and no iron in two; the A-V node, bundle of His, RBB, LBB, Purkinje fibers, and myocardium contained moderate to heavy iron deposits; the autonomic nerve fibers and ganglia showed negligible iron deposits. Two of these five patients had neither rhythm nor conduction disturbances, two had both, and one had only a supraventricular arrhythmia. In the sixth patient who had a supraventricular arrhythmia, iron was minimal in all structures.

Thus there was no correlation between the presence of recorded antemortem arrhythmias and conduction defects and the extent of iron deposits in the components of the conduction system and myocardium. In addition, our data showed that in all hearts scarring was essentially moderate to heavy in the S-A node and myocardium, but less marked in the A-V node and bundle of His. Thus, the degree of scarring in each structure did not parallel the density of iron deposits found there. Finally, antemortem rhythm and conduction disturbances showed correlation with neither the estimated transfused iron nor the quantitatively analyzed iron in the myocardium. These observations support the theory that factors other than the presence of iron alone are responsible for the cardiac arrhythmias and conduction defects associated with iron-storage disease.

Acknowledgment

Because of their anemia, these patients had been under the long-term care of Dr. Carl Smith and his associates in Pediatric Hematology. Mrs. Eva Krauss and Mr. Julius Mesiar of the Department of Pathology assisted with the specimens and prepared the slides.

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S. Weir Mitchell on The Physician, 1887

Our profession has in its work enough of true difficulties, but we still owe many of our worst errors to want of absolutely complete study of our cases, and with the careless these slips are obvious enough to enable any one who is watchful to sit in judgment on the failures. The more delicate illustrations of the fine union of qualities which attain the highest triumphs are, of course, only seen and comprehended by physicians, whose general opinion on their fellows is in the end almost always a just one. There is a potent combination of alertness in observation, with a never-satisfied desire to know even the trifles of a case, which, with sagacity, gives a medical mental character as rare as it is valuable.

For such men there are no trifles, and, on entering a sick-room, they seem to absorb at a glance matters which escape others, and yet to the end are still so quietly observant and searching that they seem never to be quite content with what they have learned. Not to know surely is to them a form of unhappiness.—S. WEIR MITCHELL: Doctor and Patient, ed. 4. Philadelphia and London, J. B. Lippincott Company, 1904, p. 37.
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