Cardiac Valvular Anomalies in Fabry Disease
Clinical, Morphologic, and Biochemical Studies

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SUMMARY The cardiovascular abnormalities were investigated in two unrelated hemizygous males with Fabry disease who had clinical mitral insufficiency. Postmortem examination of their hearts revealed anatomic, ultrastructural and biochemical abnormalities resulting from defective activity of the lysosomal enzyme, α-galactosidase A. The ultrastructural and biochemical studies demonstrated the marked accumulation of the major glycosphingolipid substrate, trihexosyl ceramide, in the lysosomes of all the cardiac tissues examined; the greatest concentrations were found in the mitral valve and left ventricular myocardium. Intriguingly, digalactosyl ceramide, a glycosphingolipid substrate not detectable in normal lung, vessel or cardiac tissues, was found increased only in the lung and right heart tissues. Morphologic and chemical examination of cardiac and systemic vessels demonstrated accumulation of trihexosyl ceramide in lysosomes of the vascular endothelium. These studies demonstrate that the progressive accumulation of trihexosyl ceramide in the lysosomes of the cardiac structures and vascular system leads to the multiple cardiovascular manifestations of Fabry disease.

FABRY DISEASE, an X-linked disorder of glycosphingolipid metabolism, results from the defective activity of the enzyme, α-galactosidase A, a lysosomal hydrolase necessary for the catabolism of the glycosphingolipids, trihexosyl and digalactosyl ceramides.1-4 The disorder is characterized by the marked accumulation of trihexosyl ceramide predominantly in endothelial, perithelial and smooth muscle cells of blood vessels and to a lesser degree in connective tissue, histocytes and reticular cells. Clinical manifestations in affected hemizygous males occur during childhood or adolescence with the onset of episodic acroparesthesias, the appearance of cutaneous lesions (angiokeratomas), and the finding of an increased erythrocyte sedimentation rate. Often these observations have led to the misdiagnosis of rheumatic fever and the initiation of penicillin prophylaxis.

With increasing age, cardiac disease becomes manifest in most hemizygous males; systemic hypertension, myocardial ischemia and infarction and congestive heart failure are common cardiac complications of Fabry disease. Heterozygous female carriers of this X-linked gene are usually asymptomatic or have mild symptoms of the disease; however, rarely they show clinical1,5 and chemical6 manifestations as severe as hemizygous males, including severe cardiac involvement.8

Pompen and co-workers first described the pathologic involvement of the myocardium and vasculature in two hemizygous male siblings in 1947.9 Subsequently, Ferrans and associates described the histochemical and ultrastructural abnormalities in the heart of a severely affected heterozygous female.6 In addition, these authors reviewed the cardiac manifestations of Fabry disease and suggested the need for further studies of the valvular involvement.

In this report we describe the cardiac findings in two hemizygous males with Fabry disease, with particular emphasis on the clinical, morphologic and biochemical involvement of their valvular abnormalities. We demonstrate that the valvular anomalies in this disease result primarily from the accumulation of trihexosyl ceramide in hypertrophied lysosomes of the cardiac valves.

Materials and Methods

Patient Material

Two hemizygous males with primary cardiac involvement were evaluated at the University of Minnesota Hospitals. Clinical hemizygosity for Fabry disease was confirmed biochemically. The levels of α-galactosidase A activity were determined in plasma, isolated leukocytes, isolated erythrocyte membranes or cultured skin fibroblasts by the methods of Desnick et al.4 The concentrations of trihexosyl ceramide were determined in plasma6 and urinary sediment.10,11

Biochemical studies were performed on cardiac and lung tissues obtained within one hour of death from patient 1 and from age and sex-matched individuals who died from noncardiac causes.

Histochemical and Ultrastructural Studies

For light microscopy, small portions of various cardiac tissues were fixed in buffered 10% formalin and sections were stained with hematoxylin-eosin, Sudan black B, and periodic acid-Schiff before and following digestion with diastase. For electron microscopy, portions of fresh cardiac and vessel tissues were fixed in Millonig's buffer containing 1.25% glutaraldehyde and 1.25% osmic acid. After one hour, the tissues were dehydrated in graded alcohols and propylene oxide and then embedded in Epon. Thin sections were cut on an LKB ultra-microtome, contrasted with uranyl acetate and lead citrate and examined in a Phillips 200 electron microscope.
Glycosphingolipid Analyses

Cardiac tissue and vessel glycosphingolipids were isolated and quantitated essentially by the procedures of Desnick et al. Total lipid extracts from individual tissues were isolated by the following procedure. Each specimen was homogenized in a Waring blender with 100 ml of methanol, then 200 ml of chloroform was added. Following homogenization, the chloroform-methanol suspension was put in an Erlenmeyer beaker under nitrogen and allowed to further extract on a shaker-bath at room temperature overnight. The suspension was then suction-filtered over sintered glass and the residue was re-extracted as above, the filtrates combined, and the water-soluble contaminants removed from the total lipid extract by the Folch procedure. The neutral glycosphingolipids were then isolated by silicic acid column chromatography and the phospholipid contaminants were removed by mild-acid hydrolysis; the individual glycosphingolipids were identified by thin layer chromatography, eluted and subsequently quantitated by gas-liquid chromatography as previously described.

Case Summaries

Two unrelated hemizygotes with Fabry disease were evaluated at the University of Minnesota Hospitals. The clinical diagnosis of each was biochemically confirmed by the demonstration of deficient \( \alpha \)-galactosidase A activity and the accumulation of trihexosyl ceramide in plasma and various tissues as shown in table 1.

Patient 1

C.B., a hemizygous male with Fabry disease, who had been followed at the University of Minnesota Hospitals for more than 20 years, died at age 55 years. From the age of five years he had noted angiokeratoma on his scrotum, lower abdomen and iliosacral regions. Although he never experienced acroparesthesias, painful Fabry episodes or renal insufficiency, he had the other characteristic manifestations of the disease including bilateral Fabry corneal dystrophy, bilateral lymphedema, lipid-laden, PAS-positive bone marrow macrophages and birefringent Maltese crosses in his urinary sediment.

When evaluated in 1967 at age 50 years, a grade II/VI apical systolic murmur was present which radiated to the left axilla and was considered to represent mitral insufficiency. There were no signs of congestive cardiac failure. During the next five years, his condition deteriorated in several respects. Congestive cardiac failure was diagnosed at age 52 years. Subsequently, he developed subacute bacterial endocarditis secondary to \( \alpha \)-streptococci which was treated successfully with intravenous penicillin. During this period he had repeated respiratory infections and ultimately developed chronic respiratory insufficiency. Three cerebrovascular accidents left him with residual mild left-sided weakness. On two occasions he was hospitalized for myocardial infarction. Over the last years he had become very confused and was diagnosed as having organic brain syndrome.

His final admission was for cough, fever and dysphagia. Physical examination showed him to be in moderate respiratory distress; massive lymphedema of the legs and scrotum was present. The blood pressure was 140/80 mm Hg. Moderate weakness of the left upper and lower extremities was noted. On cardiac examination, no thrills or heaves were present. A grade IV/V holosystolic murmur was again heard at the apex and radiated to the axilla. A grade II/VI aortic ejection systolic murmur was heard at the base of the heart and radiated to the neck. No hepatosplenomegaly was present. The electrocardiogram showed a pattern of left ventricular hypertrophy and an old anterolateral myocardial infarction. Thoracic roentgenograms revealed cardiomegaly, a right lower lobe infiltrate and a right pleural effusion. Thoracentesis was performed and 330 cc of thick, amber fluid was removed. He was treated with methacillin and gentamicin for the pneumonia. His condition deteriorated and he died shortly after admission.

Patient 2

J.C., a 47-year-old hemizygous male, experienced an episode of severe pain, generalized malaise and weakness of the extremities at age 12 years which was diagnosed as rheumatic fever. During the next several years, he had recurrent episodes of burning pain, particularly in the lower extremities, associated with fever. At that time he noted the development of angiokeratoma on his abdomen and scrotum and a decreased ability to perspire. He sought no medical attention until two of his brothers who had died from renal insufficiency were diagnosed pathologically as hemizygous for Fabry disease. He was evaluated at age 42 years. The diagnosis of Fabry disease was suspected clinically because of the history and the presence of the characteristic corneal

<table>
<thead>
<tr>
<th>Table 1. Diagnosis of Fabry Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Source</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Hemizygeotes</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Normal Adult</td>
</tr>
<tr>
<td>Range</td>
</tr>
<tr>
<td>N</td>
</tr>
</tbody>
</table>

*Levels of \( \alpha \)-galactosidase A and B activities determined in above sources by methods of Desnick et al.4

†Concentrations of trihexosyl ceramide determined in plasma by the method of Vance et al.5 and in urinary sediment by method of Desnick et al.6,11
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FIGURE 2. Left side of the heart in patient 2 showing thickening and moderate intrachordal hooing of the mitral valve. Chordae tendineae appear normal. Papillary muscles are thickened and short.

Light Microscopic Findings

The light microscopic findings were similar in both patients. Central clear areas were seen throughout the myocardium which were associated with peripheral displacement of the myofibrils. In patient 1, several areas of myocardial necrosis and hemorrhage were present. The small arterial vessels showed medial hypertrophy and marked vacuolation of the smooth muscle cells.

In both cases, light microscopy of the mitral and tricuspid valves showed numerous lipid-laden cells, embedded within the fibrous tissue. The presence of excessive deposits of lipid in the leaflets was confirmed by positive staining with Sudan black B.

Ultrastructural Findings

The electron microscopic examination of the cardiac tissues from both hemizygotes with Fabry disease demonstrated similar changes. Ultrastructural examination of both tricuspid and mitral valves revealed massive accumulation of lipid within single membrane-lined organelles as shown in figures 3 and 4. Examination of the myocardial capillaries showed extensive deposition of osmophilic concentric lamellar configurations in the lysosomes of hypertrophied endothelial cells similar to that seen in capillaries in other tissues (fig. 5). These laminated bodies have the same myelin-like configuration characteristic of trihexosyl ceramide lipid deposition in lysosomes as has been observed in other tissues.1

Biochemical Findings

Table 1 shows the levels of α-galactosidase A and B activities in the plasma, leukocytes, isolated erythrocyte mem-

FIGURE 3. Electron photomicrograph of a section of the mitral valve from patient 1 showing the concentric lamellar inclusions in the lysosomes (arrows) of fibrocytes (× 22,000).
branles and myocardium of patients 1 and 2 and normal individuals. No α-galactosidase A activity was detected in these sources from the hemizygous males compared to those of normal individuals. The levels of trihexosyl ceramide in plasma and urinary sediment were increased significantly in patients 1 and 2, thereby confirming the enzymatic diagnosis.

Table 2 shows the concentrations of neutral glycosphingolipids in the cardiac valves from patient 1 and from age-matched normal male controls. The levels of the ac-
was not present. The right pulmonary artery measured 6 mm in diameter and the arterial structure from which it originated measured 2.5–3 mm in diameter. A thrill was not palpable in the right pulmonary artery at the time of operation, suggesting that the right ductus had closed. A 3 mm, end-to-side anastomosis was made between the right pulmonary artery and the ascending aorta (fig. 3). Immediately after completion of the anastomosis, a thrill was present in the right pulmonary artery and the arterial pO₂ increased to, and remained in the range of 35–50 throughout the remainder of the patient’s hospitalization. When observed six months after operation, the patient was acyanotic.

Comment

Origin of one pulmonary artery from the homolateral ductus arteriosus is not an unusual congenital anomaly but bilateral origin of the pulmonary arteries from the homolateral ducti arteriosi is rare. To our knowledge, the first report of this condition was that of Murray and associates, who described two cases.

In a review of 262 patients with discontinuity between the heart and the pulmonary arteries, Berry and associates reported that only nine patients did not have continuity between the right and left pulmonary arteries, and in only four of these patients was there bilateral origin of the pulmonary arteries from the homolateral ducti. In the 180 pathological specimens of truncus arteriosus communis...
cumulated substrate, trihexosyl ceramide, were 5370, 4380, 3830, and 3550 nanomoles/g wet weight in the mitral, aortic, tricuspid, and pulmonary valves, respectively; these values were more than 245-, 175-, 225-, and 185-fold greater than the respective normal adult mean levels. The concentrations of glucosyl ceramide in the valves of the hemizygote were within normal range. However, there were consistent nonspecific increases in the levels of the glycosphingolipids distal and proximal to the metabolic block; the levels of lactosyl and trihexosyl ceramides in the valves from patient 1 were 4 to 18- and 4 to 7-fold greater than the respective normal adult mean values.

Similarly, the concentrations of the neutral glycosphingolipids in the atria, ventricles, selected vessels and lung tissue from patient 1 and normal adult individuals are shown in table 3. Each tissue analyzed from the hemizygote had a marked accumulation of the Fabry glycosphingolipid. In the right and left atria and the right and left ventricles the concentrations of trihexosyl ceramide were approximately 450-, 190-, 565- and 650-fold greater than the mean levels in the respective myocardial tissues from normal adult hearts. Small, nonspecific increases in the levels of the other glycosphingolipids in the myocardial tissues from the hemizygote were observed compared to the respective mean adult normal values.

The cardiac vessels studied were also found to have markedly increased levels of trihexosyl ceramide; the levels in the aorta, pulmonary artery and coronary artery were more than 25-, 85- and 245-fold greater than the normal mean adult values, respectively. In addition, the trihexosyl ceramide level in the lung tissue from patient 1 was more than 15-fold greater than the normal mean value. The concentrations of the other neutral glycosphingolipids in these sources were within or only slightly greater than their respective normal adult ranges (table 3).

In addition to the marked accumulation of trihexosyl ceramide in each tissue analyzed from patient 1, a selective deposition of the glycosphingolipid substrate, digalactosyl ceramide, was found. Table 4 shows that digalactosyl ceramide was detected only in the right heart structures and lung from patient 1 with decreasing levels in the direction of blood flow. The concentrations in the right atrium, tricuspid valve, right ventricle, pulmonary artery and lung tissue were 268, 113, 61, 55 and 11 nanomoles/g wet weight, respectively. Galactosyl ceramide was also found in the tricuspid valve and pulmonary artery from patient 1 (table 4). Digalactosyl ceramide structures: Glucosyl Ceramide = Galβ1-4Glcf1-1-ceramide, lactosyl ceramide = Galα1-4Galβ1-1-ceramide, trihexosyl ceramide = Galα1-4Galβ1-1-4Galβ1-1-ceramide, and tetrahexosyl ceramide = Galα1-4Galβ1-1-4Galβ1-1-4Galβ1-1-ceramide.

TABLE 2. Concentrations of Neutral Glycosphingolipids in the Cardiac Valves from a Hemizygote with Fabry Disease and Normal Individuals*:

<table>
<thead>
<tr>
<th>Source</th>
<th>Glucosyl Ceramide</th>
<th>Lactosyl Ceramide</th>
<th>Trihexosyl Ceramide</th>
<th>Tetrahexosyl Ceramide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitral Valve</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient 1</td>
<td>253</td>
<td>271</td>
<td>5570</td>
<td>292</td>
</tr>
<tr>
<td>Normal mean</td>
<td>210</td>
<td>216</td>
<td>26</td>
<td>26</td>
</tr>
<tr>
<td>and range (N = 3)</td>
<td>(120-242)</td>
<td>(20-38)</td>
<td>(17-32)</td>
<td>(30-60)</td>
</tr>
<tr>
<td>Aortic Valve</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient 1</td>
<td>246</td>
<td>383</td>
<td>4380</td>
<td>273</td>
</tr>
<tr>
<td>Normal mean</td>
<td>185</td>
<td>21</td>
<td>25</td>
<td>41</td>
</tr>
<tr>
<td>and range (163-202)</td>
<td>(12-35)</td>
<td>(20-40)</td>
<td>(31-56)</td>
<td></td>
</tr>
<tr>
<td>Tricuspid Valve</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient 1</td>
<td>131</td>
<td>80</td>
<td>3830</td>
<td>156</td>
</tr>
<tr>
<td>Normal mean</td>
<td>121</td>
<td>17</td>
<td>17</td>
<td>38</td>
</tr>
<tr>
<td>and range (105-149)</td>
<td>(12-25)</td>
<td>(11-20)</td>
<td>(29-55)</td>
<td></td>
</tr>
<tr>
<td>Pulmonary Valve</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient 1</td>
<td>276</td>
<td>249</td>
<td>3550</td>
<td>295</td>
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<tr>
<td>Normal mean</td>
<td>248</td>
<td>19</td>
<td>19</td>
<td>45</td>
</tr>
<tr>
<td>and range (196-327)</td>
<td>(31-76)</td>
<td>(15-26)</td>
<td>(32-61)</td>
<td></td>
</tr>
</tbody>
</table>


TABLE 3. Concentrations of Neutral Glycosphingolipids in Hemizygote with Fabry Disease and in Normal Individuals*:

<table>
<thead>
<tr>
<th>Source</th>
<th>Glucosyl Ceramide</th>
<th>Lactosyl Ceramide</th>
<th>Trihexosyl Ceramide</th>
<th>Tetrahexosyl Ceramide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right Atrium</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient 1</td>
<td>175</td>
<td>154</td>
<td>7690</td>
<td>264</td>
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<tr>
<td>Normal mean</td>
<td>25</td>
<td>9</td>
<td>17</td>
<td>20</td>
</tr>
<tr>
<td>and range (23-29)</td>
<td>(6-13)</td>
<td>(11-22)</td>
<td>(12-24)</td>
<td></td>
</tr>
<tr>
<td>Left Atrium</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient 1</td>
<td>147</td>
<td>217</td>
<td>4040</td>
<td>203</td>
</tr>
<tr>
<td>Normal mean</td>
<td>33</td>
<td>21</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>and range (24-39)</td>
<td>(8-19)</td>
<td>(11-34)</td>
<td>(19-45)</td>
<td></td>
</tr>
<tr>
<td>Right Ventricle</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient 1</td>
<td>342</td>
<td>193</td>
<td>11340</td>
<td>460</td>
</tr>
<tr>
<td>Normal mean</td>
<td>31</td>
<td>11</td>
<td>20</td>
<td>26</td>
</tr>
<tr>
<td>and range (22-38)</td>
<td>(7-16)</td>
<td>(12-25)</td>
<td>(14-36)</td>
<td></td>
</tr>
<tr>
<td>Left Ventricle</td>
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<td></td>
<td></td>
</tr>
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<td>Patient 1</td>
<td>191</td>
<td>172</td>
<td>12400</td>
<td>312</td>
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<tr>
<td>Normal mean</td>
<td>43</td>
<td>13</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>and range (22-74)</td>
<td>(10-17)</td>
<td>(16-25)</td>
<td>(32-68)</td>
<td></td>
</tr>
<tr>
<td>Aorta</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient 1</td>
<td>83</td>
<td>76</td>
<td>2580</td>
<td>150</td>
</tr>
<tr>
<td>Normal mean</td>
<td>176</td>
<td>89</td>
<td>95</td>
<td>152</td>
</tr>
<tr>
<td>and range (123-219)</td>
<td>(70-108)</td>
<td>(59-133)</td>
<td>(121-169)</td>
<td></td>
</tr>
<tr>
<td>Pulmonary Artery</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient 1</td>
<td>136</td>
<td>124</td>
<td>2730</td>
<td>172</td>
</tr>
<tr>
<td>Normal mean</td>
<td>76</td>
<td>23</td>
<td>67</td>
<td></td>
</tr>
<tr>
<td>and range (72-86)</td>
<td>(17-28)</td>
<td>(18-40)</td>
<td>(31-102)</td>
<td></td>
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<tr>
<td>Coronary Artery</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient 1</td>
<td>415</td>
<td>258</td>
<td>11280</td>
<td>337</td>
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<tr>
<td>Normal mean</td>
<td>312</td>
<td>57</td>
<td>46</td>
<td>90</td>
</tr>
<tr>
<td>and range (245-364)</td>
<td>(54-62)</td>
<td>(34-59)</td>
<td>(75-112)</td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient 1</td>
<td>98</td>
<td>69</td>
<td>1090</td>
<td>33</td>
</tr>
<tr>
<td>Normal mean</td>
<td>140</td>
<td>135</td>
<td>66</td>
<td>54</td>
</tr>
<tr>
<td>and range (92-206)</td>
<td>(87-175)</td>
<td>(34-102)</td>
<td>(30-88)</td>
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</tbody>
</table>

*See table 2 for neutral glycosphingolipid structures.
†N = 3 for normal mean levels and ranges.

TABLE 4. Concentrations of Galactosyl and Digalactosyl Ceramides in Various Tissues from Patient 1*:

<table>
<thead>
<tr>
<th>Source</th>
<th>Galactosyl Ceramidedef</th>
<th>Digalactosyl Ceramidedef</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitral Valve</td>
<td>n.d.§</td>
<td>n.d.</td>
</tr>
<tr>
<td>Aortic Valve</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Tricuspid Valve</td>
<td>135</td>
<td>113</td>
</tr>
<tr>
<td>Pulmonary Valve</td>
<td>51</td>
<td>n.d.</td>
</tr>
<tr>
<td>Right Atrium</td>
<td>n.d.</td>
<td>268</td>
</tr>
<tr>
<td>Left Atrium</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Right Ventricle</td>
<td>n.d.</td>
<td>61</td>
</tr>
<tr>
<td>Left Ventricle</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Aorta</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Pulmonary Artery</td>
<td>238</td>
<td>55</td>
</tr>
<tr>
<td>Coronary Artery</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Lung</td>
<td>n.d.</td>
<td>11</td>
</tr>
</tbody>
</table>

*Concentrations of galactosyl and digalactosyl ceramides were not detectable in these tissues from three adult normal control individuals.
†The structures of galactosyl and digalactosyl ceramides are Galβ1-1-ceramide and Galβ1-1-4Galβ1-1-ceramide, respectively.
§n.d. indicates that the glycosphingolipid was not detectable in the sample analyzed.
or galactosyl ceramide was not detected in normal cardiac, vessel or lung tissues from three normal adult individuals.

Discussion

Fabry disease is an inborn error of glycosphingolipid metabolism characterized by the deposition of the glycosphin-
golipids, trihexosyl and digalactosyl ceramide, primarily in the cardiovascular-renal system. The primary metabolic
defect resulting in the accumulation of these substrates is the defective activity of the specific, X-linked lysosomal
enzyme, α-galactosidase.13-16 In hemizygous males, the defective activity of α-galactosidase A results in the progres-
sive deposition of trihexosyl ceramide in the secondary lysomes of endothelial, epithelial, and perithelial cells of the
cardiovascular-renal system, particularly in the endothelium of blood vessels. Cardiac involvement also occurs
through the progressive infiltration of glycosphingolipid into myocardial cells and valvular fibroblasts leading to significant
cardiac abnormalities in hemizygotes,17 and some het-
erozygotes.8 Although mitral valvular involvement may be
present in most patients with Fabry disease, the clinical
manifestations of this abnormality have rarely been
described. Furthermore, the relationship between the meta-
bolic defect and the valvular lesion has not been investigated
previously correlating ultrastructural and biochemical
findings.

In our two hemizygous patients, the major cardiac feature
was mitral insufficiency and at postmortem examination
evidence for involvement of both the mitral and tricuspid
valves was found. The major anatomical findings contrib-
uting to the mitral regurgitation were interchordal
hooding of the mitral valves and thickening of the valvular
leaflet and papillary muscles. The tricuspid valves showed
similar changes, but to a lesser degree. The aortic cusps of
patients 1 were markedly thickened and appeared obstructive,
although hemodynamic confirmation was not und-
taken. No gross abnormalities of the pulmonary valve were
present in either of our patients. Gross involvement of the
tricuspid or pulmonary valves has not been reported in the
literature; however, marked lipid deposition in the lys-
somes of these valves has been observed ultrastructurally.18

Histologically, both mitral and tricuspid valves demon-
strated lipid-laden fibrocytes embedded in fibrous tissue;
lipid deposition was indicated by positive staining with
Sudan black B. Other cardiac tissues also showed the stored
material. The myocardial cells showed clear central areas
throughout with peripheral displacement of the myofibris.
The small arterioles showed hypertrophy of the media and
marked vacuolization of the smooth muscle cells.

Ultrastructural examination of cardiac valves revealed
numerous single membrane-lined lysosomes containing con-
centric lamellar lipid-dense inclusions. These inclusion
bodies ranged from 0.1 to 10 microns in diameter and the
lamellar arrangement had a periodicity of approximately 50
to 60 Å (fig. 4).

The biochemical analyses documented the ultrastructural
finding of lipid deposition in the cardiac tissues and vessels
studied. Markedly increased concentrations of the major
accumulating glycosphingolipid, trihexosyl ceramide, were
found in each cardiac valve and other cardiac tissues and
vessels analyzed from patient 1 (tables 2 and 3). The mitral
insufficiency and left ventricular hypertrophy were cor-
related with the chemical and ultrastructural findings; the
mitral valve and left ventricular myocardium from patient 1
contained the greatest concentrations of trihexosyl ceramide
of all the cardiac structures analyzed, levels which were 245-
and 650-fold greater than the respective mean normal adult
values. On ultrastructural examination, the mitral valve (fig.
3 and 4) and left ventricle appeared to have more hyper-
trophied lysosomes containing the lipid-dense Fabry lamel-
lar inclusions than any other cardiac tissues examined.
However, massive amounts of trihexosyl ceramide and num-
eros Fabry inclusions were found in all the cardiac struc-
tures studied. The clinical manifestations certainly result
from the accumulation of trihexosyl ceramide in these struc-
tures, but the mitral valve and left ventricular involvement
in Fabry and other storage diseases (e.g., mucopolysacchari-
doses, types I and II; Gm, gangliosidosis, type 1; and Gm,
gangliosidosis, type 2) presumably results in part from
other contributing factors including the unique hydrody-
namic forces acting on these structures. As with many
storage diseases, mitral insufficiency is a common feature;
the mitral valve and its supporting structures are altered by
the infiltration of the stored material. Since the pressure
difference across the mitral valve is greater than any other
cardiac valve, the stresses on the valve are great and
presumably lead to insufficiency. In addition, because of
these pressure differences and the contour of the left ven-
tricle, mitral insufficiency has greater cardiac manifestations
than similar lesions of other cardiac valves.

The involvement of the vasculature, particularly in the en-
thelium of small vessels, has been demonstrated ultra-
structurally (fig. 5) as well as histochemically. Tri-
hexosyl ceramide which accumulates in the plasma of
hemizygotes and heterozygotes may gain access to the
lysosomal apparatus of the vascular endothelium (and endo-
cardium) by diffusion of the lipophilic substrate or by endo-
cytosis. There was a significant accumulation of trihexosyl
ceramide in the aorta and pulmonary vessels of patient 1
table 3) and when compared to mean normal adult levels
infiltration from the myocardial fibrocytes. With age, the
accumulation in the coronary artery which was more than 245-fold
greater than the mean normal adult level. The accumu-
lation of trihexosyl ceramide in the endothelium of the coro-
ary arteries specifically, and in small vessels systemically,
causes structural narrowing and dilatation of these vessels.

With age, the progressive glycosphingolipid accumulation
eventually leads to ischemia and frank infarcts resulting in
the major clinical manifestations of this disease, including
myocardial and cerebrovascular ischemia and infarction,
renal insufficiency, angio kerasoma, and the chronic, ex-
cruciating acroparesthesias.

An intriguing biochemical finding concerns the glyco-
sphingolipid, digalactosyl ceramide, which was detected
only in the right-sided heart structures and lung tissues of
patient 1 (table 4). This glycosphingolipid is also a substrate
of the defective Fabry enzyme and is a normal component of
human pancreas and kidney. Previously, digalactosyl
ceramide has been found accumulated only in spinal ganglia,
kidney, urinary sediment and pancreas of hemizygotes with
Fabry disease. Although digalactosyl ceramide was not detected in any of the normal heart or lung tissues
analyzed, it was found in the right atrium, tricuspid valve, right ventricle, pulmonary artery and lung tissues from patient 1, in decreasing concentrations, respectively. Since it is unlikely that the intrinsic glycosphingolipid content of the right heart structures would differ from those of the left in Fabry disease, these findings require an alternate explanation. It is temting to speculate that the source of digalactosyl ceramide in the right heart structures of patient 1 was derived from the marked deposition in his kidneys. It is conceivable that the accumulated renal digalactosyl ceramide gained access to the circulation and was delivered directly to the right heart structures and subsequently to the reticulendothelial cells of the lung. This explanation is consistent with the facts that 1) the relative concentration of digalactosyl ceramide in these structures decreased in the direction of blood flow, 2) no digalactosyl ceramide was detected in any of the left heart structures, and 3) a significant concentration of the substrate was detected in the pulmonary tissue from patient 1 (table 4).

These ultrastructural and biochemical findings demonstrate that the valvular and other cardiac abnormalities in Fabry disease result from the pathologic accumulation of neutral glycosphingolipids in the lysosomes of the cardiovascular system. Characterization of these abnormalities has become more important since current investigations attempting to correct the metabolic defect by enzyme replacement therapy4-7 7-9 are encouraging and future trials may document their biochemical and clinical effectiveness. Clearly, early intervention by enzyme therapeutic endeavors will be requisite to potentially alter the morbidity and/or mortality of the cardiovascular abnormalities in these patients.

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