Light- and Electron-Microscopic Histochemistry of Fabry’s Disease

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A histochemical study was performed on light- and electron-microscopic level in a case of Fabry’s disease. The patient underwent kidney transplantation for renal failure and died of heart failure 6 months later. Patient’s tissues were studied at the light- and electron-microscopic levels with various embedding and staining techniques for lipids and carbohydrates. Two peroxidase-labeled lectins (from Ricinus communis and from Bandeiraea simplicifolia) known to have affinity for α- and β-D-galactose, were strongly reactive with the storage material on frozen sections. The ultrastructural and extraction tests showed that the typical granules had a variable reactivity and morphologic characteristics in different cells, probably reflecting different composition. A small number of typical deposits were also observed in the transplanted kidney. This is the first reported case of recurrence of the storage disease in the allograft. Of interest was also the fact that the patient’s blood inhibited normal α-galactosidase activity, suggesting a possible inhibitor-related mechanism in the pathogenesis of the recurrence. (Am J Pathol 1981, 103:247–262)

FABRY’S DISEASE is a storage disease characterized by lysosomal accumulation of specific neutral glycosphingolipids in most tissues and fluids of affected individuals. The major accumulated glycosphingolipid has been identified as globoglycosylceramide (Galα1-4Galβ1-4Glucβ1-1’cer), and its accumulation ascribed to a defective activity of the lysosomal hydrolase, α-galactosidase A. These neutral glycosphingolipids are deposited in all areas of the body, occurring predominantly in the lysosomes of endothelial, perithelial and smooth muscle cells of blood vessels, and to a lesser extent in histiocytic and reticular cells in connective tissue. The progressive accumulation of the spherocristalline glycolipid deposits leads to clinicopathologic involvement of almost every system.

Early diagnosis is often elusive, and definite diagnosis requires demonstration of the enzymatic deficiency. Most frequently, however, the diagnosis is suspected from histologic, histochemical, or ultrastructural examination of biopsied renal tissue or skin. Identification of the stored material by histochemical techniques is plagued with difficulties. First, the glycolipid is almost entirely extracted by xylene; thus the use of paraffin sections is prevented. Second, there are no absolutely specific histochemical tests for glycolipids. The most selective method is the periodic acid–Schiff (PAS) modification of Adams; it consists of blocking the ethylene bonds of unsaturated fatty acids and the 1-amino, 2-hydroxyl groups (which may also reach with PAS) before the PAS staining. Parallel control sections after lipid extraction are also required. However, it may be impossible to distinguish the Fabry glycosphingolipid substrates from the glycolipids that accumulate in other glycosphingolipidoses and gangliosidoses. Moreover, frozen sections must be used, with consequent loss of fine cellular details.

Similarly, at the electron microscopic level, the type-

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myelin-like structure, although suggestive, is not pathognomonic of Fabry’s disease. Moreover, the need of previous fixation with osmium (a strong oxidative agent) greatly hampers the usefulness and specificity of many electron-microscopic histochemical techniques for carbohydrates, most of which are based on the use of periodic acid, which is also an oxidative agent.

In view of these limitations, we studied the use of alternative procedures to identify the glycosphingolipids in Fabry’s disease. We report here the evaluation of methacylate embedding mixtures for the preservation of glycolipids and the use of osmium postfixation for light- and electron-microscopic histochemistry of neutral glycolipids. In addition, we describe the use of two peroxidase-labeled lectins (from Ricinus communis and Bandeiraea simplicifolia) that bind specifically with β- and α-linked galactose moieties, respectively, and permit selective characterization of complex glycoconjugates in this and possibly other storage diseases. Finally, these techniques were used to examine the lesion in a case of considerable interest. The patient, who died 6 months after a renal allotransplantation, showed evidence for glycosphingolipid deposition in the donor kidney, suggesting a recurrence of the disease, an event that so far has not been reported.

Materials and Methods

Clinical History

The patient, a 36-year-old male, had acroparesthesias since childhood. He was admitted to the hospital with chronic renal failure, proteinuria, and hypertension. The diagnosis of Fabry’s disease was suggested by the histochemical and ultrastructural studies of a renal biopsy. The diagnosis was confirmed by the demonstration of deficient α-galactosidase A activity in plasma and leukocytes. The patient was maintained on chronic dialysis until a renal cadaver allograft was performed. Two months after transplantation, the patient experienced graft rejection that responded to treatment. Six months later, the patient died with bronchopneumonia and heart failure. An autopsy was performed within 1 hour of death.

Tissue was obtained at the time of renal transplantation and at autopsy. The following procedures were used for light-microscopic study:

1) Frozen sections were cut from unfixed or formalin-fixed (24 hours) tissue.
2) Fixation in sodium acetate buffered formalin, or in phosphate-buffered (pH 7.2, 0.1 M) 4% paraformaldehyde.
3) Fixation as in 2. Embedding in glycol methacrylate-butoxyethanol mixture (JB4 mixture, Poly-

Assay of α-galactosidase A activity in tissues and fluids was carried out by the method of Desnick et al.10 The neutral glycosphinigolipids in renal tissues was quantitated by gas chromatography according to the previously reported procedures.18,19 Electrophoretic analyses of purified normal α-galactosidase A20 in heparinized plasma from normal individuals and hemizygotes with Fabry's disease were carried out as previously described.21

**Results**

The results of the light and electron microscopic observations with various procedures are summarized in Table 1.

**Light-Microscopic Observations (Figures 1–12)**

No major differences were observed between the histochemical behavior of formalin and paraformaldehyde fixed tissue. The aldehyde fixation had little influence on the reactivity of the storage material compared with that of unfixed tissues. The only exception seems to be the lectin affinity, which was much stronger in unfixed tissue. With these methods all the inclusions appeared strongly reactive, but other structures were also stained. In particular, red cells and some cell coats (brush borders in the kidney) reacted with the Bandeiraea lectin.

After formalin fixation and methacrylate embedding, the glycolipid droplets were almost completely lost from the vessel walls and glomerular podocytes but were largely retained in kidney tubules and interstitial cells (Figures 1 and 7). Both types of droplets were removed by chloroform–methanol (Figure 8). This variation in behavior was dependent upon embedding procedures and was also reflected in other histochemical tests: vascular wall droplets (mainly within the myocytes) were the only ones faintly stained with the performic acid–Schiff sequence, which was otherwise negative (Figure 9). It must be stressed that all the droplets were completely extracted by chloroform–methanol (2:1, vol/vol) (Figure 11). A very few granules remained in some vascular walls after this procedure, but those were consistent with lipofuscin bodies on both the light- and electron-microscopic levels and will not be considered further.

Embedding in methacrylate proved successful in retaining some of the inclusions, but only if dehydration was carried out in pure glycomethacrylate, avoiding any contact with ethanol or other alcohols. When the embedding mixture used for ultrastructural studies contained butyl as well as methyl-methacrylate, the storage material was not adequately preserved. Similar results were obtained with standard Epon. Osmium postfixation, as expected, had several effects on the preservation and reactivity of the lipids. After 50 minutes of postfixation, the inclusions appeared in unstained sections as pale brown bodies, when compared with the intense black of the adipose tissue. They were no longer extractable, even with hot chloroform–methanol or xylene.

Osmium induced Schiff positivity in many tissue structures and in particular in the storage inclusions, basement membrane, and collagen without pretreatment with periodic acid. Such Schiff positivity was completely abolished by 2-hour incubation with phenylhydrazine. After osmium fixation, the PAS reac-

| Fixation and Embedding | PAS modified | Chloroform-methanol-PAS | PASM | Oil red O | Sudan black | Oil red O-birefringence | Auto-fluorescence | Lectin 1 (Ricinus) | Lectin 2 (Bandeiraea) | PAT-CHSP | H2O2-PTA | Perfor- | Acid- | Schiff |
|------------------------|-------------|-------------------------|------|-----------|-------------|------------------------|----------------|----------------|----------------|---------|---------| formic | acid- | Schiff |
| Unfixed—frozen sections| +           | +                      | -    | +         | +          | +                      | +             | +             | +             | 0       | 0       | ±       |       |       |
| Formalin—frozen sections| +           | +                      | -    | +         | +          | +                      | -             | +             | +             | 0       | 0       | ±       |       |       |
| Formalin—paraffin     | -           | -                      | -    | -         | -          | -                      | -             | +             | -             | 0       | 0       | -       |       |       |
| Formalin—glycolmethacrylate| ±   | ±                      | -    | ±         | ±          | ±                      | ±             | +             | -             | 0       | 0       | ±       |       |       |
| Formalin—osmium paraffin| +           | 0                      | +    | +         | +          | +                      | -             | -             | 0             | 0       | 0       | 0       |       |       |
| Formalin—osmium glycolmethacrylate| +           | 0                      | +    | +         | +          | +                      | -             | -             | -             | 0       | 0       | 0       |       |       |
| Parafomaldehyde—Epon (EM) | 0           | 0                      | 0    | 0         | 0          | 0                      | 0             | 0             | 0             | -       | -       | 0       |       |       |
| Parafomaldehyde—osmium—Epon (EM) | 0           | 0                      | 0    | 0         | 0          | 0                      | 0             | 0             | 0             | +       | 0       |       |       |       |

Reactivity of storage granules with various fixation, embedding, and staining procedures: + = positive or strongly positive; ± = positive in some areas, negative in others (see text); - = negative; 0 = not performed.
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tions, with or without previous phenylhydrazine treatment, were indistinguishable. Fat stains gave very strong reactions after osmium fixation: red with oil red O and bluish-black with Sudan black, against a brown background (Figures 3 and 10).

Hydrogen peroxide bleaching of the sections removed completely the reduced osmium, without reducing the stainability with oil red O, and apparently increasing its specificity (Figure 4). A beneficial effect was also observed with hematoxylin counterstain, which otherwise was rather faint. Hydrogen peroxide treatment also enhanced the Schiff positivity in the sections. The significance of this effect is not clear and is currently being investigated. The PAS modification of Adams (chloramine T—performic acid—phenylhydrazine—PAS) was virtually indistinguishable from the standard PAS when performed after formalin fixation on either frozen sections or methacrylate embedded tissues.

The storage material was observed by light microscopy in almost all the organs examined. In the kidney, glomerular, tubular (mainly distal), and interstitial cells and vascular walls were involved. In the liver, the deposits were mostly in Kupffer cells, but occasionally also in the hepatocytes. Other organs with striking accumulations of the Fabry substrate were the intestines (both muscularis layers and ganglion cells), myocardium, vascular endothelium of skin, and the adeno- and neuro-hypophysis.

**Electron Microscopy (Figures 13–19)**

The inclusions showed heterogeneous composition
(Figure 13). With both the silver proteinate and phosphotungstic acid techniques, some areas were heavily stained, with occasional lamellar structures (Figure 16), while others were almost completely free of stain (Figures 14–16). A thin reactive layer was evident around some of the granules, suggesting the presence of a coated membrane. With the H$_2$O$_2$–PTA sequence, only structures known to contain carbohydrates were stained. In addition to the inclusions, glycogen, collagen, and cell coats were stained (Figure 15). The granules were also stained by the PASM procedure (Figure 17). The inclusions had a regular periodicity of about 60 Å when examined at high magnification (Figure 18). Ultrastructural study revealed, after extensive search, a few inclusions in several endothelial cells of the intertubular capillaries in the transplanted kidney (Figure 19). These inclusions had the typical appearance of those found in patient's own organs, including the characteristic periodic structure (Figure 19). The inclusions were not visible by light microscopy.
Biochemical Studies

Table 2 shows the levels of the individual neutral glycosphingolipids in the renal allograft and kidneys from normal individuals. In the allograft, the levels of globoglycosylceramide and galabiosylceramide, the substrates which accumulate in Fabry kidney, were not significantly different from their respective normal mean values. In an effort to find a circulating inhibitor of normal α-galactosidase A activity, mixtures of purified plasma α-galactosidase A and heparinized plasma from the recipient as well as other Fabry patients with renal transplants and normal control subjects were assayed. As shown in Table 3, the recipient's plasma inhibited the purified activity by 43%, whereas 19–20%, 13–20%, and 12–20% inhibition were found in the mixtures containing plasma from other Fabry transplant recipients (RG, DG), other Fabry hemizygotes, and normal individuals, respectively. None of the medications which the recipients
received inhibited α-galactosidase A activity. Therefore, the possibility that the recipient had produced antibodies to the normal enzyme was evaluated. As demonstrated previously, when purified α-galactosidase A from normal human spleen was electrophoresed in the presence of rabbit antihuman splenic or plasma α-galactosidase A, the mobility of the enzyme was retarded. Analogously, when the purified plasma enzyme was mixed with the recipient’s plasma, it was also retarded (Figure 20; lane 3). In addition, the apparent activity was reduced, as assessed by the brightness of the activity band (lane 3), compared with the activity observed in the other lanes, consistent with the results of the in vitro assays (Table 2). In contrast, neither normal plasma (lane 2) nor plasma from a nontransplanted Fabry hemizygote had any effect on the mobility or apparent activity of the added enzyme (lane 4). Treatment with antibody (lane 5) also retarded the mobility of a portion of the added enzyme, while treatment with 10 times this amount of antibody yielded a complex that was immobile (lane 6) and probably a precipitate. In each case, the more antibody (or recipient plasma) present in the mixture, the greater the retardation of the enzymes’ electrophoretic mobility.

**Discussion**

The purpose of this study is to characterize the storage material in Fabry disease by means of light-and electron-microscopic histochemistry. The Fabry substrates are complex glycosphingolipids with terminal α-galactosyl moieties. From a histochemical point of view, these substrates are therefore expected to display (and indeed do display) the staining characteristics of both lipids and carbohydrates.

Since in formalin-fixed paraffin-embedded tissue the glycolipids are completely extracted, we use alternate fixation and embedding procedures.

Osmium tetroxide has long been known to be an excellent fixative for lipids in general and for the polyunsaturated forms in particular. Similar observations have been made with the use of potassium dichromate as a fixative for preservation of lipids, including those of Fabry disease. Our results are in agreement with these data. Osmium, both in methacrylate and in paraffin sections, preserves material that otherwise would be extracted by solvents used for the processing of tissue (alcohol, xylene, methacrylate itself). After osmium fixation, lipids are no longer extractable, even in hot chloroform-methanol. Thus, after osmium postfixation, the distribution of storage granules can be studied in paraffin or methacrylate sections with better resolution than in frozen sections. However, the specificity of almost all subsequent histochemical tests is considerably reduced. In fact, osmium induces a diffuse Schiff positivity, acting on vicgylcol groups and on double bonds of unsaturated lipids; it also induces a Sudan black stainability of tissues, even when lipids have been previously extracted.

We attempted to restore some of the specificity of the PAS reaction after osmium fixation, using a phenylhydrazine blockade or carbonyl groups. Since this...
Figure 13—Pituitary gland. Paraformaldehyde fixation; osmium postfixation; Epon embedding; uranyl and lead "stain." Note the heavy accumulation of storage material in the parenchymal cells. Note also the nonhomogeneous morphologic appearance of the granules (x 6900) (Compare with Figure 14.) Figure 14—Liver. Kupffer cell; paraformaldehyde fixation; osmium postfixation; Epon embedding; hydrogen peroxide-phosphotungstic acid "stain." Note the nonhomogeneous reactivity of the vacuolar material with this staining procedure, considered selective for carbohydrates. Note also the thin-layer (coat) lining the lysosomes. (x 18,000)
Figure 15—Same procedure as in Figure 14. Note the selectivity of the stain for carbohydrates. Only glycogen, collagen, and Fabry inclusions are stained. The treatment with hydrogen peroxide has the effect of etching the epoxy resin and also removes the interference of the osmium electron density. (× 19,800) Inset—Higher magnification of a storage vacuole. Note the lamellar pattern. (× 110,000)
blockade, which suppresses all osmium-induced Schiff positivity, is periodic-acid-resistant, \(^{26}\) we believe that when performed before the PAS sequence, it can improve the specificity of the PAS reaction. On the other hand, every fixative interferes with the PAS specificity: even formalin reacts with lipids and can introduce unspecific Schiff positivity. \(^{27, 28}\) In a similar way, we tried to improve the selectivity of oil red O stain in osmicated tissues. Hydrogen peroxide bleaching of the sections completely removed the brown background color of the granules, probably by reoxidizing and solubilizing the osmium. No structures other than the granules stained with this technique. Although we cannot prove the specificity of the method, since, as mentioned before, lipids are no longer extractable, the selectively appeared to be excellent. However, hydrogen peroxide bleaching did not restore the eosinophilia of tissues lost through the osmium-induced deamination. \(^{23}\)

In summary, although it carries several theoretical shortcomings, osmium postfixation is useful in the study of the morphology of the glycolipid granules. The specificity of some of the histochemical reactions can be partly restored by appropriate blocking and bleaching treatments. The substitution of paraffin with methacrylate as embedding medium also proved to reduce to a significant degree the loss of lipids, even in nonosmicated tissues. These findings are not surprising. Although methacrylate has been shown to extract some phospholipids more than ethanol, \(^{29}\) it has been used to preserve lipids in a case of Faber granulomatosis \(^{30}\) and it has been reported that all the conventional lipid histochemistry tests were positive with methacrylate embedding. \(^{31}\) Methacrylate embedding also offers the advantage of allowing the use of thin sections with excellent resolution of details and minimal distortion.

The histochemistry of Fabry's disease has been described in several reports, some of which are quite detailed. \(^{1, 32-33}\) Our results on frozen, as well as methacrylate-embedded, tissues confirm the previous data and are consistent with the glycolipid nature of the storage material.

It is of great interest that preservation of glycolipids with methacrylate embedding affected various cells in a different manner, suggesting different solubility and/or composition of the lipid moiety in different cells. Also consistent with the variable composition of the storage granules was the finding of a performic acid–Schiff positivity in the most easily extractable (arterial wall) granules. Altogether, however, the performic acid–Schiff reaction was faint or absent in this case, even on frozen sections. This is in keeping with the biochemical results of Bagdade et al. \(^{34}\) which revealed that 70% of the lipids extracted from the lung of a Fabry's case were saturated.

The findings of more than one kind of lipid in this disease is not surprising. Storage of nonspecific lipids in lipidoses may be due to physicochemical binding of other lipids by the primary deposits. Cholesterol, for instance, has been found in Tay-Sachs disease. \(^{8}\) It is also known that a primary enzymatic defect can greatly influence the activity of other enzymatic complexes, particularly in lysosomes. \(^{35}\) We also confirmed the positivity of the PAS reaction as modified by Adams. \(^{1, 36}\) In spite of the doubts expressed about the necessity of this time-consuming procedure, \(^{37}\) we consider it the most specific test for glycolipids presently available.

In order to characterize more specifically the carbohydrate moiety of the glycolipids, we used two peroxidase-labeled lectins, known to react specifically with D-galactose. As expected, the two procedures showed strong reactivity of the granules. In fact, Fabry's substrates contain two terminal molecules of a- and \(\beta\)-linked galactose. Lectin from Ricinus (RCA 120) is known to display a selective affinity for D-galactose, \(^{38}\) and lectin from Bandeiraea simplicifolia is highly reactive with \(\alpha\)-linked D-galactose. \(^{39}\) In a detailed study at the light-microscopic level, using a technique similar to the one used by us, Yamada and Shimizu \(^{19}\) showed that peroxidase-labeled lectin from Ricinus specifically reacts with galactose in histologic sections. A similar technique has been used by Bretton and Bariéty \(^{40}\) to stain glomeruli of the normal rat at the ultrastructural level.

So far no report has been published on the use of peroxidase-labeled lectin from Bandeiraea in human tissue. However, Peters and Goldstein \(^{44}\) described the use of a fluorescein conjugate to stain a galactopyranoside group. The fact that chloroform–methanol extraction completely abolishes all reactivity of the granules in our case supports the view that they are galactolipids. It is interesting to note that besides the storage granules, we found staining of red blood cells and the brush border of the proximal kidney tubules. These structures possibly represent the substrate of \(\alpha\)-galactosidase.

In conclusion, although techniques using these two lectins are still in an experimental phase, we believe that they represent the most specific histochemical test to demonstrate the presence of carbohydrates in complex lipid molecules. Moreover, this approach allows the specific determination of monosugars, and it could be therefore a very useful tool in the further characterization of other glycolipid storage diseases on histologic level, with obvious practical diagnostic advantages.
Figure 16—Pituitary gland. Paraformaldehyde fixation; osmium postfixation; Epon embedding; periodic acid-thiocarbohydrazide; silver proteinate. Note the strong reactivity of the inclusion material as well as the selectivity of the procedure. However, after osmium postfixation, this, like any other procedure involving periodate oxidation, loses some of its specificity. (x 24,000) Figure 17—Kidney. Paraformaldehyde fixation; osmium postfixation; Epon embedding; periodic acid-silver methenamine. Note the reactivity of the vacuolar inclusions in the vascular wall. (x 5300)
Figure 18 — Pituitary gland. Paraformaldehyde fixation; osmium postfixation; lead stain. Note the typical, although not specific, periodicity (60 Å) of the storage material. (×350,000)

Figure 19 — Transplanted kidney. Paraformaldehyde fixation; osmium postfixation; Epon embedding; uranyl and lead stain. Ultrastructural investigation proved the only reliable method of detecting the recurrence of the disease in the transplanted kidney. Note the small vacuoles in the endothelial cell of an intertubular capillary. (×5900) Inset—Higher magnification of another vacuole. (×67,000)
Our ultrastructural studies confirmed the result already reported by others and reviewed by Desnick et al.4 The histochemical tests reported here, periodic acid-thiocarbohydrazide-silver proteinate and periodic acid-silver methenamine, suffer from some of the limitations already discussed, since they were performed on osmium-fixed tissues. However, the Thiéry method showed considerable selectivity for the storage granules. The phosphotungstic acid stain (PTA) was apparently the most selective for carbohydrate-containing structures. The pretreatment with hydrogen peroxide both etched the Epon, allowing a more intense staining with phosphotungstic acid, and bleached the osmium, reducing the interference caused by its electron density. Although PTA positivity has been assumed to indicate the presence of mucopolysaccharides in Fabry's disease,42 this is not supported by other available literature. The use of this method, that of hydrogen peroxide-phosphotungstic acid, could turn out to be the easiest and one of the more reliable ways of studying glycolipids at the ultrastructural level in Fabry's disease.

Table 2—Levels of Neutral Glycosphingolipids in Transplanted and Normal Kidneys

<table>
<thead>
<tr>
<th>Source</th>
<th>Lactosyl-ceramide</th>
<th>Galabiosyl-ceramide</th>
<th>Globo-glycosyl-ceramide</th>
<th>Tetra-hexosyl-ceramide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allograft (this case)</td>
<td>0.12</td>
<td>0.13</td>
<td>0.39</td>
<td>0.52</td>
</tr>
<tr>
<td>Normal kidney</td>
<td>Mean</td>
<td>0.16</td>
<td>0.10</td>
<td>0.35</td>
</tr>
<tr>
<td>Range (n = 3)</td>
<td>0.09-0.25</td>
<td>0.03-0.15</td>
<td>0.25-0.40</td>
<td>0.21-0.55</td>
</tr>
</tbody>
</table>

The reoccurrence of Fabry's disease in a renal allograft has not been previously reported.43-50 Actually, kidney transplantation has been regarded by some as a possible way of correcting the enzymatic deficiency through enzyme replacement by means of an implant of a normal organ which continually produces the endogenous activity of a-galactosidase A.43,44

The amount of storage material found at autopsy in the transplanted kidney was very limited. In fact, only electron-microscopic investigation proved successful.

Table 3—Inhibition of Normal a-Galactosidase A by Normal and Fabry Hemizygote Plasma

<table>
<thead>
<tr>
<th>Source</th>
<th>Case</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal:</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CK</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>DB</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>GP</td>
<td>15</td>
</tr>
<tr>
<td>Fabry hemizygotes:</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>JC</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>BC</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>FS</td>
<td>20</td>
</tr>
<tr>
<td>Fabry hemizygotes (transplanted):</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>RG</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>DG</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>This case</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>This case†</td>
<td>43</td>
</tr>
</tbody>
</table>

* Plasma samples were adjusted to pH 7.2 with HCl following the addition of 1/10 volume of 0.25 M sodium HEPES, pH 7.2. The buffered plasma samples (22 µl) plus purified human splenic α-galactosidase A (5 µl, 4 nmoles of artificial substrate cleaved per hour) were mixed together and incubated with 150 µl of artificial substrate solution (5 mM 4-methylumbelliferyl-a-D-galactopyranoside in 0.15 M citrate-phosphate buffer, pH 4.6). The 100% control solution contained instead of plasma, 22 µl of 1 mg/ml human serum albumin in 25 mM sodium HEPES, pH 7.2.
† Dialyzed.

Figure 20—Cellulose acetate electrophoresis of plasma a-galactosidase A in the presence of normal and Fabry plasma and anti-a-galactosidase A. The electrophoretic method and fluorescent activity stain were as described previously. In each lane, 1 µl of solution was applied and electrophoresed which contained 1 unit of human plasma a-galactosidase A activity mixed with 1) 1mg/ml human serum albumin in 25 mM HEPES, pH 7.2; 2) normal human plasma containing 25 mM HEPES and adjusted to pH 7.2; 3) Fabry plasma, case FR, buffered as in 2; 4) Fabry plasma, case BC (nontransplant patient); 5) 0.3 g rabbit antihuman splenic a-galactosidase A; and 6) 3.0 g rabbit antihuman splenic a-galactosidase A.
in detecting the typical myelinlike structures. This is in keeping with the fact that the increase in Fabry's substrate in this organ was below the levels of biochemical detection. It is interesting that the cells most involved at this early stage were the endothelial cells of the intertubular capillaries, which are most likely to come in contact with the highest levels of Fabry's substrate present in the circulating blood. Although this finding remains unexplained, there are several possible explanations. The cells degraded capacity may have been overwhelmed by the large amount of substrate. The endothelial cells in which the storage material was observed may have populated the vascular wall from endogenous reticuloendothelial stem cells. Thus, they would be deficient in α-galactosidase activity and store the substrates. Alternatively, the substrate may have accumulated due to the presence of an inhibitor to normal α-galactosidase A. Support for this latter hypothesis is based on the finding of a circulating inhibitor in the recipient's plasma (Table 3), which behaved on electrophoresis in a way similar to that of anti-α-galactosidase antibodies. This hypothesis is not inconsistent with the fact that the recipient experienced a rejection episode 2 months after transplantation, which may have resulted in the formation of anti-α-galactosidase antibodies. Further studies to confirm the immunoglobin nature of the circulating inhibitor are in progress.

References


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