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A Murine Model of Mucopolysaccharidosis VII

Gross and Microscopic Findings in Beta-Glucuronidase-Deficient Mice

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This report describes the clinical and pathologic alterations found in mice that have a recessively inherited, essentially complete deficiency of the lysosomal enzyme beta-glucuronidase. Affected animals have a shortened life span and are dysmorphic and dwarfed. Abnormal gait and decreased joint mobility correlate with glycosaminoglycan accumulation in articular tissue and cartilaginous and bony lesions result in extensive skeletal deformation. In these enzyme-deficient animals, lysosomes, distended by fine fibrillar and granular storage material, are particularly prominent in the macrophage system but also occur in other tissues including the skeletal and central nervous systems. The clinical and pathologic abnormalities in these mutant mice closely parallel those identified in humans with mucopolysaccharidoses (MPS). Therefore, these mice provide a well-defined genetic system for the analysis of the pathophysiology of mucopolysaccharidosis type VII, which has many features in common with the other MPS. The mutant mice provide an attractive animal model to test potential therapies for lysosomal storage disease. (Am J Pathol 1990, 136:207–217)

The mucopolysaccharidoses (MPS) are inherited disorders of lysosomal degradation of glycosaminoglycans (formerly called mucopolysaccharides). Mucopolysaccharidosis type VII (MPS VII) is a storage disease caused by a severe deficiency of the lysosomal enzyme beta-glucuronidase (EC 3.2.1.31). The enzyme deficiency, inherited as an autosomal recessive trait, results in glycosaminoglycan (dermatan sulfate, heparan sulfate, and chondroitin sulfate) accumulation in lysosomes in leukocytes, macrophages, brain, lung, liver, skin, and kidney. Affected patients have a variable phenotype that usually includes coarse facies, growth and mental retardation, corneal clouding, hepatosplenomegaly, and skeletal deformities—collectively referred to as dysostosis multiplex.[1–11]

There are few effective therapies for patients with lysosomal storage diseases. Clinical variability, common in the genetically heterogeneous MPS, makes evaluation of therapeutic effects difficult. Such phenotypic differences can be eliminated in in-bred animal models that also allow study of a meaningful number of subjects; thus, animal models are useful in the investigation of experimental treatments for the lysosomal storage diseases. Several animal models of MPS have been described, including a canine model of MPS VII.[12,13]

We identified a murine MPS model with morphologic, genetic, and biochemical characteristics that closely mimic those of human MPS VII.[14] Tissues from these mice have less than 1% of normal beta-glucuronidase activity and the enzyme deficiency is inherited as an autosomal recessive trait. Although newborn mutant mice appear normal, weanlings and older animals are dwarfed and have facial dysmorphism. Male and female mice are affected similarly and mutant mice die prematurely, usually before 8 months of age. We now describe the morphologic alterations that develop during the course of the disease produced by beta-glucuronidase deficiency in this murine model.

Materials and Methods

Twenty-two mice of the *B6.C-2*^{bml}*/ByBir-gus*^{mps}*/+* mutant strain obtained from The Jackson Laboratory (Bar Harbor, ME) were studied morphologically. Tissue from

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13 affected animals and nine phenotypically normal littermates killed at term gestation to 197 days of age were examined by light or electron microscopy, or both. The phenotypically normal animals included heterozygotes and homozygote normals. The older affected animals were discernable because of their facial and skeletal dysmorphism, which was first apparent at weaning (3 weeks). Newborn animals too young to show the gross phenotypic abnormalities associated with beta-glucuronidase deficiency were identified as enzyme deficient using a fluorometric assay with 4-methylumbelliferyl-beta-D-glucuronide as the substrate.[15] Body weights of animals studied morphologically and organ weights from another group of ten affected animals and their phenotypically normal littermates at 32 to 76 days of age were compared using the paired *t*-test.

Peripheral blood smears from selected affected and unaffected animals were stained with Wright-Giemsa stain. For light microscopy, tissues were fixed in 10% neutral buffered formalin, processed routinely, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H & E). Mowry's colloidal iron stain and alcian blue-periodic acid-Schiff (PAS) stain at pH 2.5 were used to identify glycosaminoglycans histologically.[16,17] Frozen tissue embedded in OTC compound (Miles Scientific, Naperville, IL) was sectioned and stained with a histochemical technique to demonstrate acid phosphatase.[18] Bone for light microscopic examination was fixed, immersed in 7% nitric acid for one day, rinsed in tap water and processed routinely. Ultrastructural study was performed on tissue minced and fixed in 2% glutaraldehyde in 0.1M cacodylate buffer, pH 7.4, postfixed in 1% osmium tetroxide, stained *en bloc* with 0.5% uranyl acetate, dehydrated, and embedded in Spurr's Medium. One micron thick sections were stained with toluidine blue; ultrathin sections were stained with uranyl acetate-lead citrate and examined with a Joel 100 CX transmission electron microscope.

The skeletons of a mutant and an unaffected 8-month-old littermate were cleared and stained with alizarin red.[19] Skeletons were radiographed at 48 kV with 0.8 MAS using high speed Gaveart Radiograph film in a detail cassette at a 40-inch focal film distance. Bones from a 8-month-old mutant and an unaffected littermate were cleared of soft tissue by digestion in buffered Proteinase K (Bethesda Research Laboratory, Gaithersburg, MD) and manual cleaning.

Results

Clinical and Gross Morphologic Findings

After they reached 3 weeks of age, affected mice had facial dysmorphism and were growth retarded, being visibly smaller and weighing less (unaffected range, 10 to 41.9 g; mean, 27.1 g; SE, 2.6; affected range, 5.6 to 25.2 g; mean, 19.5 g; SE, 1.9, $P < 0.01$) than their unaffected littermates (Figures 1 and 2). Affected animals older than 12 weeks had a hobbled gait, suggesting joint dysfunction, and were lethargic, with a limited and sluggish startle response. Detailed neurologic examination was not performed. At necropsy, the liver and spleen of mutant animals did not appear grossly enlarged compared with control animals; however, spleens from affected animals were heavier than those of littermates (unaffected range, 53 to 80 mg; mean, 63 mg; SE, 2.9; affected range, 57.2 to 101 mg; mean, 78 mg; SE, 4.7; $P < 0.05$); weights of livers and hearts were not significantly different in control and affected animals. Gross examination of cardiac valves failed to identify a lesion. Axial and appendicular skeletons of affected animals were markedly malformed with shortened, broadened long bones and ribs apparent grossly and radiographically (Figures 3 and 4).

Figure 1. *A 2-month-old beta-glucuronidase deficient male animal* (right) *has a broad flattened face with short nose and is smaller than an unaffected littermate* (left).

Microscopic Pathology

The joints of large limbs of weanling and older affected animals showed synovial proliferation with occasional villous projections and vacuolated synovial cells. The articu-

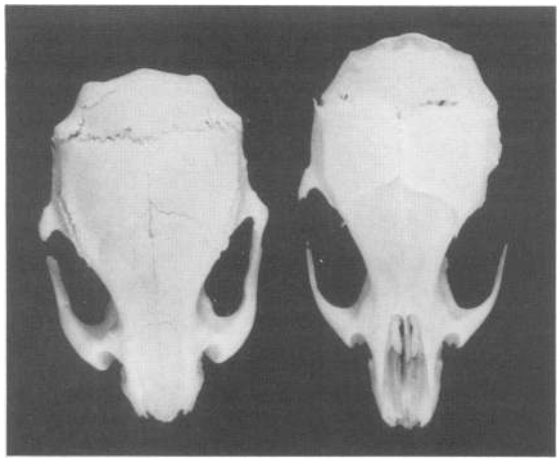

Figure 2. *The facial dysmorphism in enzyme-deficient animals is due to malformation of the skull bones with broad, shortened maxillas and mandibles and widened zygomatic arches. The zygomas are extremely delicate bones and were lost in processing in three of the four zygomatic arches.* (left: *affected 8-month-old male animal;* right: *unaffected littermate).*

lar cartilage was irregular and hypercellular, and chondrocytes were enlarged and vacuolated (Figure 5A and B). There were occasional articular–synovial synechiae. The growth plates were widened, hypercellular, and irregular. Chondrocytes, osteoblasts surrounding diaphyseal bone trabeculae, and cells lining bone marrow sinusoids contained clear cytoplasmic vacuoles readily apparent in toluidine blue-stained, plastic-embedded sections. Ultrastructurally, the vacuoles had a single membrane and some contained a fine fibrillar and granular matrix with occasional very electron dense granules as well as linear and circular membrane fragments. When granular material was lost during processing, the distended lysosomes ultrastructurally appeared empty (electron-lucent) (Figure 5C).

H & E-stained, paraffin-embedded coronal sections of the brain showed no neuronal loss, necrosis, or gliosis. Similarly, there was no neuropil pallor or vacuolation, cell loss, or gliosis in the white matter.

In contrast to the paraffin sections, plastic-embedded sections of brain revealed cytoplasmic vacuolation of varying degrees involving neurons, glia, and cells in the leptomeninges. Areas sampled included cerebral cortex and subcortical white matter, hippocampus, central grey matter, and cerebellar cortex. Generally, two areas were sampled in any individual animal, allowing analysis of the general pattern of storage. Neuronal vacuolation was widespread in the cerebral neocortex and appeared mod-

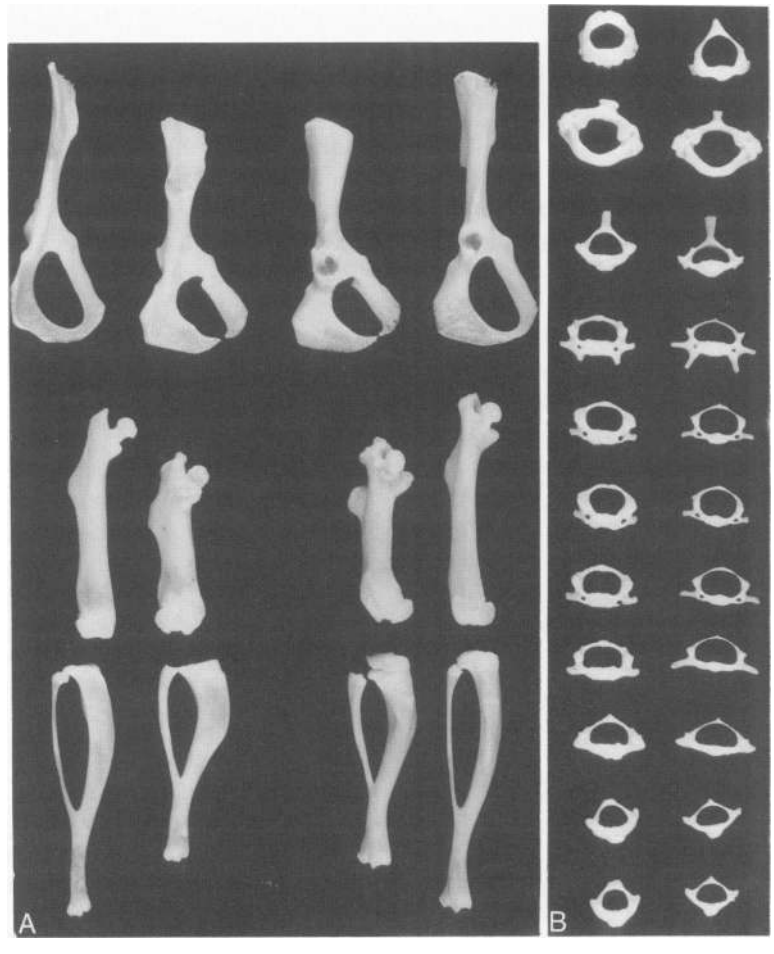

Figure 3. **A:** *The femurs, tibias, and fibulas of a 8-month-old unaffected male mouse (lateral) are normally formed and easily contrasted with the shortened and thickened long bones obtained from a beta-glucuronidase-deficient littermate (medial). The iliac bones in affected animals are hypoplastic and thick.* **B:** *The vertebrae from an affected 8-month-old mouse (left) are thickened and misshaped, compared to those from the normal littermate.*

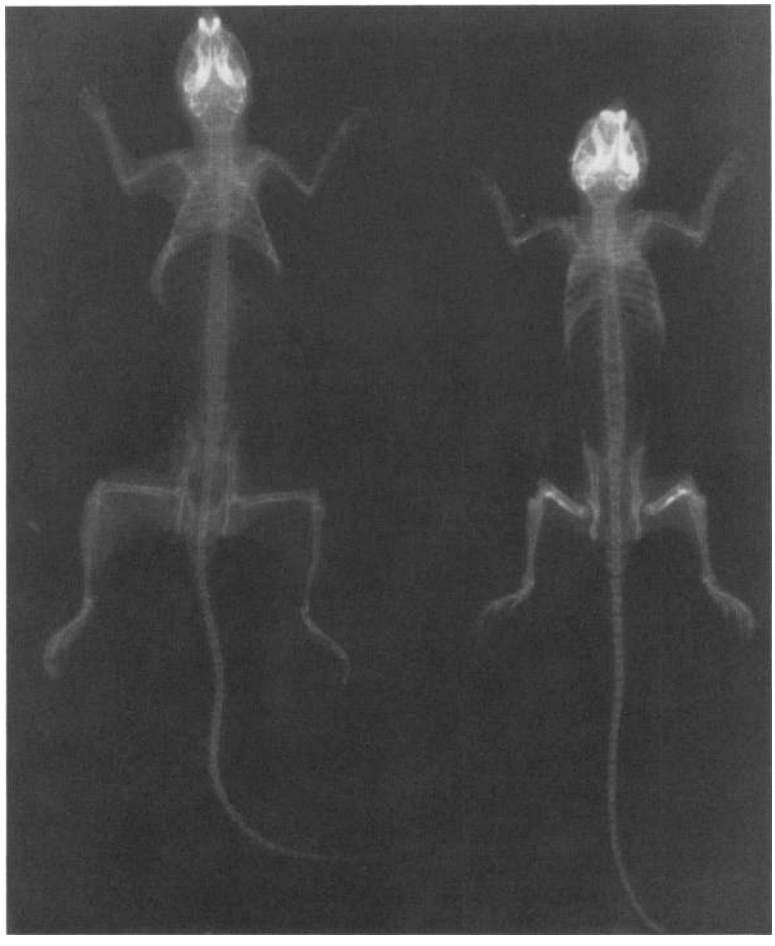

Figure 4. *The spine is shortened and vertebrae are malformed in enzyme-deficient animals. A narrow rib cage with broad ribs, flared metaphyses, enlarged weight-bearing joints, destruction of the coxofemoral joints and sclerotic femurs also characterize the skeleton of affected animals (***right:** *affected 8-month-old animal,* **left:** *unaffected littermate).*

erately extensive in the deep grey matter. The cerebellum, in comparison, was less severely affected. Only scattered vacuolar aggregates were present in the molecular and granular cell layers, and in most instances, the lack of a single clearly associated nucleus made determining the affected cell type problematic. Several Purkinje cells contained one to three discrete rounded structures with vacuolated to granular contents that did not distend their cytoplasm. Glia within the Purkinje cell layer, however, were affected extensively. A single sample of the hippocampal dentate fascia revealed that virtually all neurons contained marked cytoplasmic vacuolation. In general, glial and mesenchymal, ie, perivascular and leptomeningeal cell lysosomal storage, was not as uniform but was more prominent than that in the affected neurons. Only occasionally did sparce vacuolation appear in endothelial cells. Ultrastructurally, cortical neurons and glia had fibrillogranular storage material similar to that described above. In addition, lamellar "zebra bodies" occurred in lysosomes in central nervous system neurons (Figure 6A and B).

In peripheral nerves, endoneural fibroblasts and rare Schwann cells showed moderate vacuolization with fibrillogranular storage but myelin sheaths and axons showed no ultrastructural alteration. The cytoplasm of corneal substantia propria fibroblasts in affected mice was distended by vacuoles (Figure 7). In mature animals the retinal pigment epithelium and ciliary epithelium also contained lysosomal storage material.

Lysosomal storage also was marked in the macrophage system. Splenic sinusoidal-lining cells were distorted by vacuoles in animals of all ages (Figure 8). These cells had colloidal iron and alcian blue-positive cytoplasm. Occasional splenic lymphocytes, endothelial cells, and fibroblasts in connective tissue trabeculae also contained vacuoles apparent in plastic-embedded tissue. Hepatocytes in adult animals had a small amount of primarily pericanalicular vacuolar storage material. These vacuoles were smaller and less numerous than those in the markedly swollen Kupffer cells that encroached on the hepatic sinusoids (Figure 9). Acid phosphatase and glycosaminoglycans were demonstrable histochemically in Kupffer cells and pericanalicular hepatocyte cytoplasm in affected mice.

Endocardial lining cells contained occasional small vacuoles but subendocardial and myocardial perivascular histiocytes as well as atrioventricular valve fibroblasts and aortic medial cells had distended foamy cytoplasm with fibrillogranular, membranous, and electron-dense lyso-

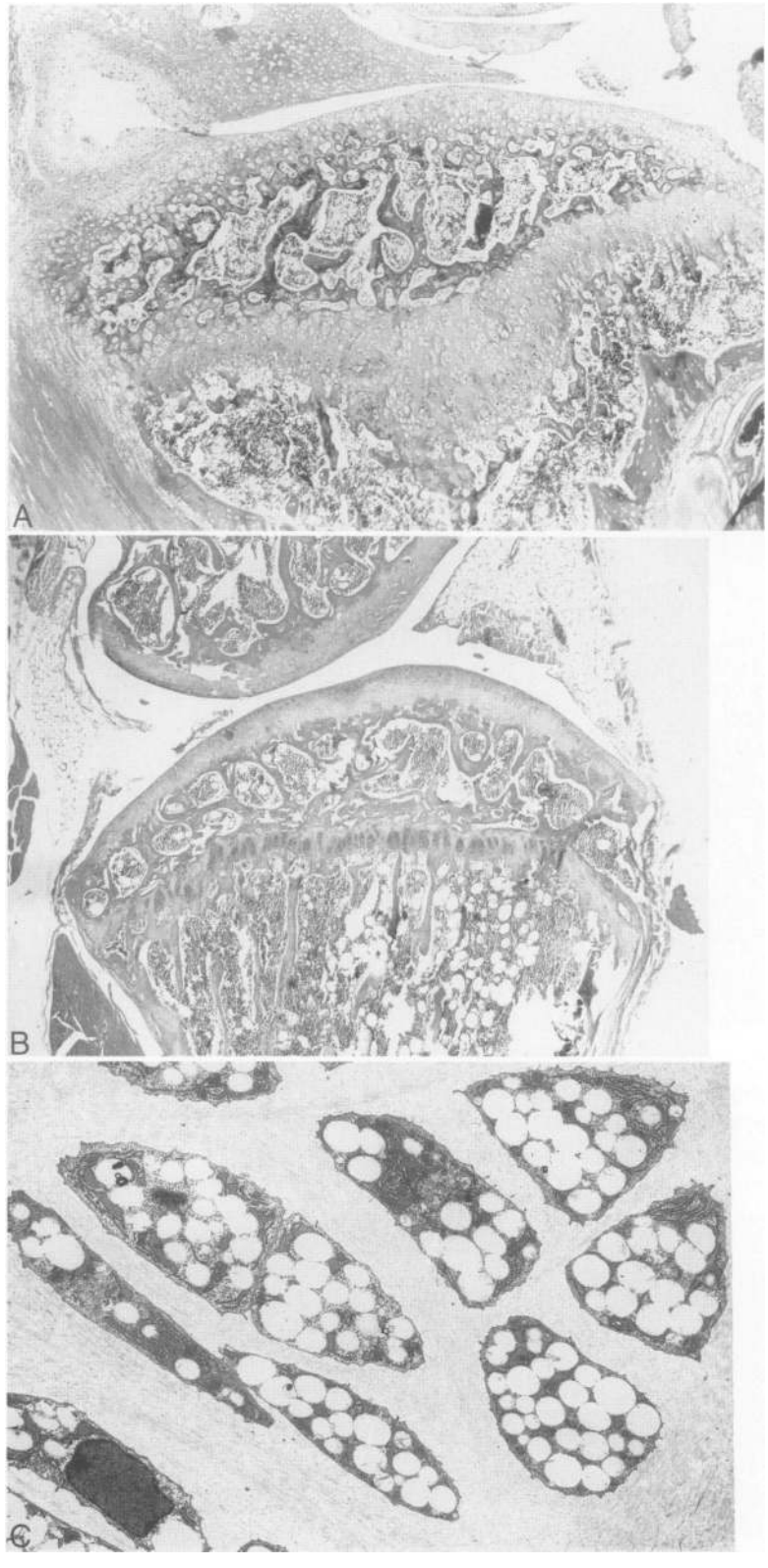

Figure 5. *The articular surface of a large limb joint (stifle) from an affected 3-month-old male mouse* (A) *shows extensive vacuolization of chondrocytes, an irregular growth plate, and proliferated synovium, in contrast a similar joint in a normal littermate* (B). *Ultrastructurally, vacuoles in chondrocytes from a 22-day-old affected mouse* (C) *were empty or contained minimal fibrillar material (*A and B: *hematoxylin and eosin, ×10;* C: *uranyl acetate-lead citrate, ×4000).*

somal storage. Myocardial fiber sarcoplasm in affected mice showed no significant vacuolar change and was histologically indistinguishable from normal myocardium.

Glomerular visceral and parietal epithelial cells contained numerous vacuoles and mesangial cells showed occasional enlarged lysosomes that did not distort cell shape. The proximal renal tubule epithelial cells also had moderate storage (Figure 10). Skin fibroblasts were distended by lysosomal storage. Cutaneous endothelial cells showed rare enlarged lysosomes but epithelial cells

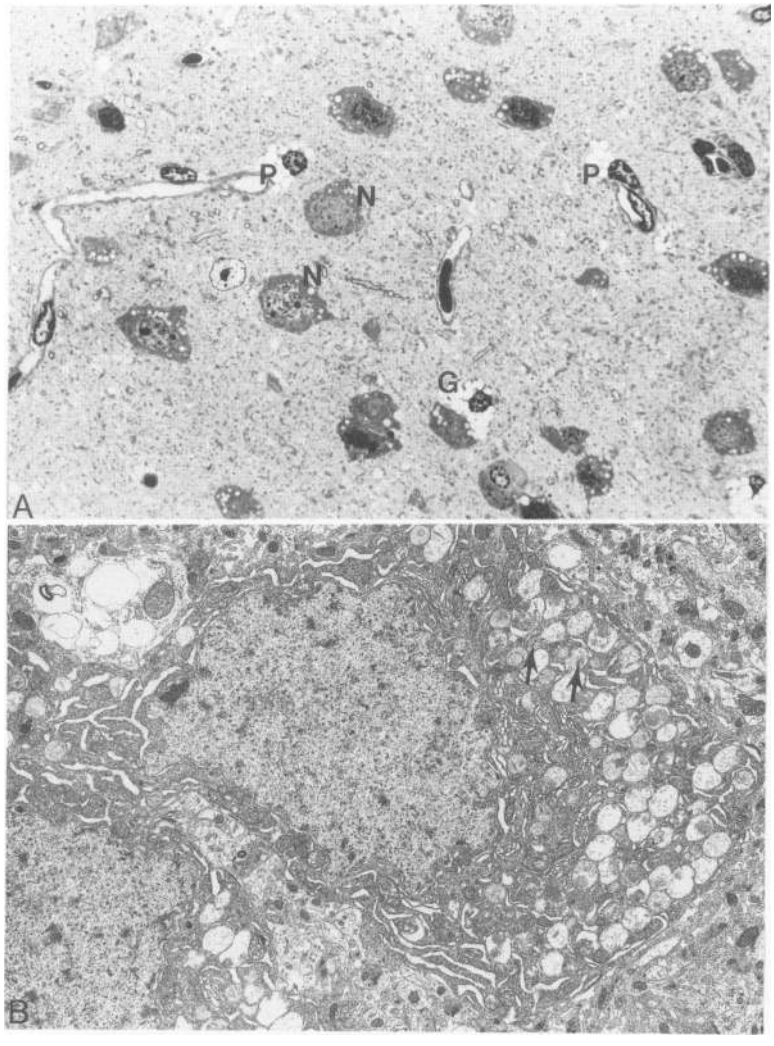

Figure 6. **A:** *Perivascular cells (P) as well as glial cells (G) in the central nervous system of a 6-month-old mutant male are distended by vacuoles. Neurons (N) show smaller and less prominent vacuoles.* **B:** *Vacuoles in a neuron from a 6-month-old mutant male contain both fibrillogranular and lamellar (arrows) storage material ultrastructurally (***A:** *toluidine blue, ×100,* **B:** *uranyl acetate-lead citrate, ×2600).*

showed no definable lesions. Peripheral blood lymphocytes from affected animals had clear cytoplasmic vacuoles with rare coarse basophilic granulation within the vacuoles. Peripheral polymorphonuclear leukocytes contained lilac-colored granules that failed to stain with toluidine blue.

Figure 7. *Fibroblasts (arrow) in the corneal stroma from a 6-month-old affected male animal are vacuolated. The glycosaminoglycan accumulation in the corneal fibroblasts was not sufficient to cause corneal clouding apparent with slit-lamp examination (toluidine blue, ×157).*

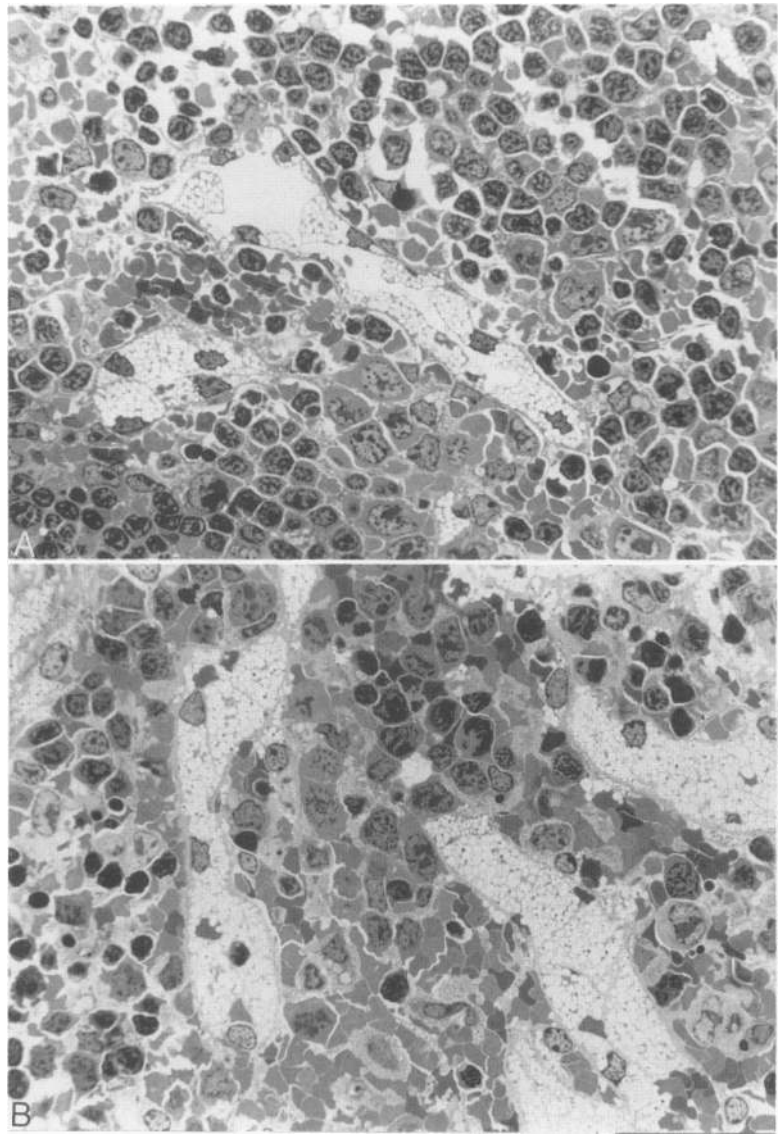

Figure 8. A: *The splenic sinusoidal-lining cells in a newborn pup with beta-glucuronidase deficiency are distended by cytoplasmic vacuoles. Ultrastructurally these vacuoles contain fine fibrillogranular storage material identical to that seen in other organs and in older, enzyme-deficient animals.* B: *The spleen from a 6-month-old affected male shows similar, although more swollen sinusoidal-lining cells (*A and B: *toluidine blue, ×100).*

Pathology in Newborn Mice

The newborn enzyme-deficient animals, identified by the presence of less than 1% of normal beta-glucuronidase activity in liver and kidney, had no gross dysmorphism. Lysosomal storage material was apparent histologically, however, based on toluidine blue-stained, 1-μ thick sections and ultrastructural examination (Table 1). Bones obtained from mutant newborn mice and examined by light microscopy after paraffin embedding could not be distinguished from those of newborns with normal beta-glucuronidase activity; however, the synovium of enzyme deficient newborns was more vacuolated than that of normal littermates. Kupffer cells, splenic sinusoidal-lining cells (Figure 8A), and central nervous system glial cells in newborns contained vacuoles that were best demonstrated in plastic-embedded tissue. Overall, lysosomal storage material was less than that seen in weanlings and older affected mice (Table 1, Figure 8A and B). In contrast to the older animals, newborn mutant mice had minimal or no lysosomal storage material in hepatocytes, glomerular epithelial cells, neurons, and retinal pigment epithelial cells.

Discussion

Lysosomal storage diseases result from inherited deficiencies of individual enzymes involved in degradation of macromolecules in lysosomes. The chemistry of the stored product, for example, lipid or glycosaminoglycan, depends on which degradative pathway is disrupted. The tissues most affected by the enzyme deficiency are those in which the turnover of that particular macromolecule is normally the highest. The clinical severity is influenced by the degree of enzyme deficiency, ie, whether the deficiency is complete or partial. Beta-glucuronidase would

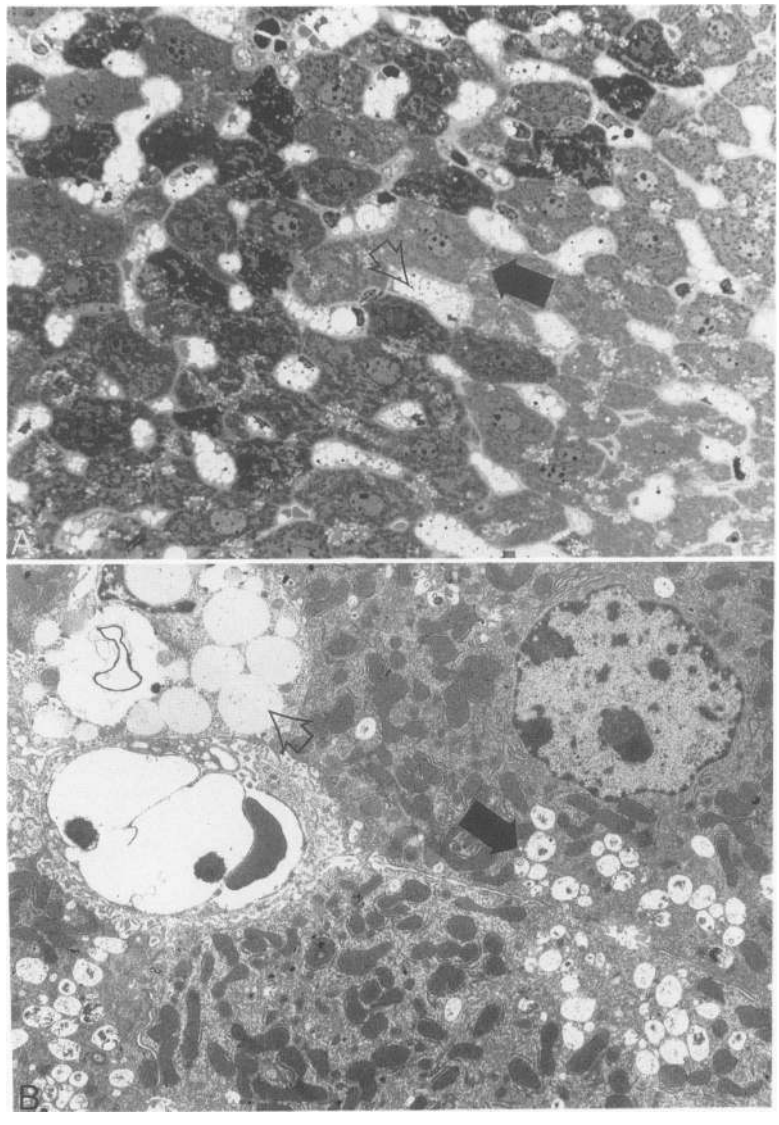

Figure 9. *Hepatocytes from a 6-month-old affected male have a slight amount of lysosomal distention in the pericanalicular region (bold arrows). Kupffer cells are distended by vacuoles (open arrows). (A: toluidine blue, ×100, B: uranyl acetate-lead citrate, ×2600).*

be expected to be required for the turnover of the glycosaminoglycans with beta-glucuronide containing residues, for example, dermatan sulfate, heparan sulfate, chondroitan sulfate, and hyaluronic acid.

The murine lysosomal storage disease produced by beta-glucuronidase deficiency shares many clinical features with human MPS VII, including growth retardation, facial dysmorphism, skeletal deformities, destruction of weight bearing joints, cardiac valvular involvement, splenomegaly, and a shortened life span.[12] Hepatomegaly, common in humans with MPS VII, is not as prominent in the MPS VII mouse. Typically, patients with MPS VII are mentally retarded. Mentation in animals is difficult to evaluate but adult mutant mice become progressively lethargic, relatively insensitive to startle, and sluggish in their locomotion. Whether this behavioral change is a consequence of progressive mental impairment due to lysosomal storage in the central nervous system is unknown. A shortened life span is a consistent feature of murine MPS VII, but the cause of death is uncertain; death usually follows a 1-to-2-week period of rapid weight loss.

Although histopathologic observations in human MPS VII are limited,[1-7] alterations reported are similar to those seen in MPS I, II, and VI, which have been studied extensively[12] The abnormalities of the large joints in the MPS VII mouse are analogous to those leading to joint dysfunction in several human MPS storage diseases, including MPS VII. Radiographic study and histologic examination of paraffin-embedded material allowed definition of these bone lesions. One micron thick sections of plastic-embedded tissue and electron microscopy were necessary for the precise localization and characterization of lysosomal storage, however.

The lysosomes distended with fibrillogranular material in these MPS mice are morphologically similar to the altered lysosomes seen in human MPS. The stored material

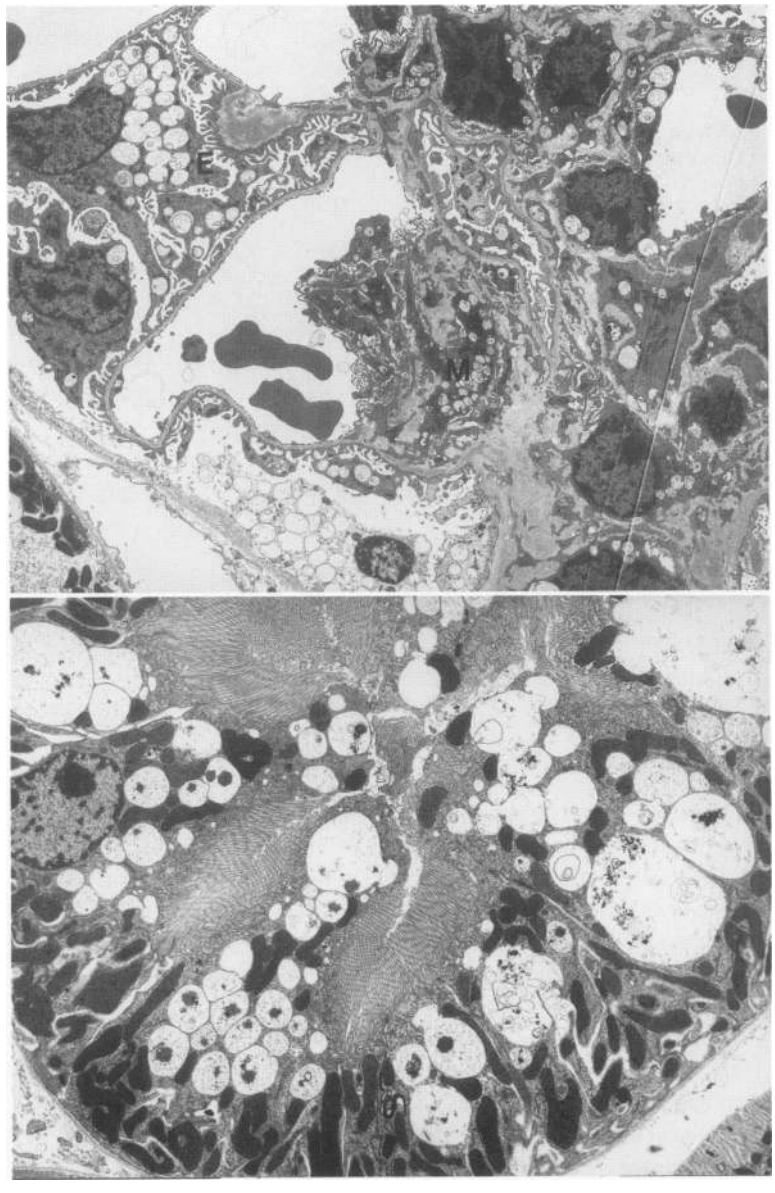

Figure 10. **Top:** *A glomerular visceral epithelial cell (E) from a 3-month-old affected male mouse is distended by cytoplasmic vacuolization. Glomerular endothelial cells show no significant lysosomal storage but occasional distended lysosomes are apparent in the mesangial cells (M).* **Bottom:** *Proximal renal tubule epithelial cells from the same mouse also contain prominent lysosomes not apparent in unaffected control animals (***top and bottom:** *lead citrate-uranyl acetate;* **Top:** *×2000,* **Bottom:** *×6000).*

presumably represents uncatabolized glycosaminoglycans because histochemical techniques indicate that glycosaminoglycans are increased in cells, including Kupffer and splenic sinusoidal-lining cells, with very distended lysosomes. In addition, liver and spleen from affected animals contain greatly elevated amounts of cetyl-pyridinium chloride precipitable glycosaminoglycans that are currently being characterized (Kyle J, unpublished observation). Storage material in the mouse cardiac valves, central nervous system, and cornea is also similar to that encountered in humans with MPS. In the kidney and liver, epithelial cells accumulate lysosomal storage material after birth. The hepatic pericanalicular lysosomal distention correlates with the previously defined localization of beta-glucuronidase in the murine liver.[20] MPS VII is one of the human MPS disorders with basophilic granulation of peripheral blood leukocytes[6] and similar granular inclusions occur in circulating leukocytes in MPS VII mice.

As in human MPS VII, beta-glucuronidase deficiency in the mouse is inherited as an autosomal recessive trait. The murine mutation for this deficiency maps to the distal end of chromosome 5 within 3.7 centimorgans of the structural gene for beta-glucuronidase.[14] The human structural gene maps to the homologous region of chromosome 7.[21] Individuals with MPS VII probably have distinct structural gene mutations that produce different degrees of enzyme deficiency and result in the variable clinical findings. MPS VII patients have 0.1% to 4% of normal beta-glucuronidase activity.[4] The murine MPS mutants we studied have less than 1% of normal beta-glucuronidase in all tissues examined[14] and have consistent and dramatic clinical and pathologic evidence of lysosomal stor-

Table 1. *Lysosomal Storage in Murine MPS VII*

Organ	Newborn (N = 3)	Weanling (N = 2)	Adult (N = 7)
Brain			
Neurons	±	1+	1+
Glial and perivascular cells	2+	3+	3+
Heart			
Valve fibroblasts	NE	NE	3+
Subendocardial, perivascular cells	NE	NE	3+
Spleen			
Sinusoidal-lining cells	2+	3+	3+
Endothelial cells	1+	1+	2+
Kidney			
Glomerular epithelial cells	±	1+	2+
Proximal tubule epithelium	±	1-2+	2+
Interstitial fibroblasts	±	2+	3+
Liver			
Hepatocytes	0	0	2+
Kupffer cells	2+	2-3+	3+
Eye			
Corneal fibroblasts	1+	2+	3+
Retinal pigment epithelium	±	±	2+
Bone			
Chondrocytes	NE	3+	3+
Osteoblasts	NE	2+	2+

0, no storage apparent; ±, equivocal lysosomal distention; 1+, minimal cytoplasmic vacuolization; 2+, moderate cytoplasmic vacuolization; 3+, cells distended by vacuoles; NE, not examined.

age disease. As in humans, murine obligate heterozygotes have approximately 50% of normal beta-glucuronidase levels and no clinical abnormalities. The minimum enzyme level required to escape the consequences of lysosomal storage is uncertain but a mouse strain with 20% of normal beta-glucuronidase has no clinical symptoms or pathologic alterations.[13]

Therapy for MPS has been mainly supportive. Recently, clinical improvement after bone marrow transplantation has been reported in patients with MPS and other lysosomal storage disorders;[12,22] however, evaluation of this experimental therapy has been hampered by small numbers of affected patients with early diagnosis, lack of HLA-identical marrow donors and the genetic heterogeneity that characterizes these enzyme deficiencies. The wide spectrum of clinical severity typical of many of the lysosomal storage diseases makes it difficult to know whether a patient responded to therapy or simply had a mild, slowly progressive form of the disease. For these reasons animal models with genetically defined lysosomal storage diseases have been sought to test therapies. Several animal models for MPS have been identified, including a canine model for MPS VII,[12,13] and bone marrow transplantation has been accomplished in animal models of MPS I and MPS VI.[23,24]

The murine model of MPS VII we describe provides a genetically well-defined, homogeneous inbred population of enzyme deficient animals without the genetic and phenotypic variability typical of the human disorder. Availability of a breeding colony of these animals allows experiments to be designed using an adequate number of mutant and control subjects and the short murine lifespan makes detection of therapeutic effect on survival practical. This murine model can be used to analyze the pathophysiology of MPS and investigate experimental therapies including enzyme replacement by direct enzyme infusion, syngeneic bone marrow, or organ transplantation, and correction of the defect by gene transfer to somatic cells or to zygotes (transgenic mice). The prevention or correction of the morphologic changes we describe will be the basis for evaluating response to a given therapeutic approach. The inferences drawn from such studies may be generalizable to many other lysosomal storage disorders.

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