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Long-Term Effects of Bone Marrow Transplantation in Dogs with Mucopolysaccharidosis I

Mike A. Breider, Robert M. Shull, and George Constantopoulos

From the Department of Pathobiology, College of Veterinary Medicine, University of Tennessee, Knoxville, Tennessee, and the Developmental and Metabolic Neurology Branch, National Institute of Neurological and Communicative Disorders and Stroke, National Institute of Health, Bethesda, Maryland

The therapeutic effects of allogeneic bone marrow transplantation (BMT) in a canine model of mucopolysaccharidosis I (MPS I) were investigated. Long-term post-BMT pathologic and biochemical studies were performed on three groups of dogs: 1) MPS I-affected dogs that did not receive BMT, 2) MPS I-affected dogs that received total body irradiation followed by an allogeneic BMT, and 3) normal, unaffected dogs that served as BMT donors. All dogs were necropsied at approximately 20 months after BMT. The severity of MPS I-related lesions in the dogs receiving BMT was greatly diminished. These dogs had only slight cardiac valvular thickening, no meningeal thickening, no renal tubular epithelial vacuolation, decreased neuronal vacuolation, decreased corneal stromal vacuolation, and greatly diminished arterial medial thickening. The severity and incidence of degenerative arthropathy also were decreased in BMT dogs, however, vertebral lesions were similar to nontransplanted, affected dogs. Chondrocytes of both MPS I-BMT and MPS I-no BMT groups had similar marked cytoplasmic vacuolation, except for MPS I-BMT chondrocytes near the articular surface, which had more normal morphology. Ultrastructurally, the liver and kidney tissue in BMT recipients had no appreciable lysosomal accumulation of GAGs. These morphologic findings were supported by near normal levels and electrophoretic patterns of glycosaminoglycans (GAG) in most tissues of BMT recipient dogs. This study demonstrates that BMT is capable of substantially diminishing the severity of MPS I-related lesions in this canine model. (Am J Pathol 1989, 134:677–692)

Mucopolysaccharidosis I (MPS I), caused by an autosomal recessive deficiency of the lysosomal enzyme alpha-L-iduronidase, is known to exist in humans, dogs, and cats.[1–4] In each species, there is impaired degradation of the uronic acid containing glycosaminoglycans (GAG) heparan sulfate and dermatan sulfate, which accumulate in lysosomes of cells in many tissues. MPS I exists in humans with at least three phenotypic subtypes: Hurler syndrome (MPS I-H), the most severe form; Scheie syndrome (MPS I-S), the mildest form; and Hurler-Scheie syndrome (MPS I-H/S), a disease of intermediate severity.[5] An important factor that distinguishes MPS I-H from the other clinical syndromes is occurrence of progressive mental retardation due to central nervous system (CNS) involvement. Canine affected with MPS-I have biochemical parameters similar to MPS I-H/S, although CNS storage of GAG is more comparable with that in human MPS I-H.[6] Reduced mentation is, of course, of less consequence and more difficult to evaluate in dogs than in children.

Nonspecific treatment protocols, specific enzyme replacement therapy, and fibroblast or amniotic cell transplantation have generally produced disappointing results in MPS I and other related lysosomal storage disease.[7–10] Since the early 1980s, bone marrow transplantation (BMT) therapy in lysosomal enzyme deficiency diseases has gained favor at some institutions because this procedure theoretically provides the patient with a self-renewing population of cells capable of producing the deficient enzyme.[11–13] Furthermore, progeny of hematopoietic stem cells gain access to many tissues. Because BMT is associated with significant morbidity and mortality, it is critical that this procedure be evaluated as thoroughly as possible in various metabolic diseases before it can be widely accepted as prudent therapy. The use of available animal models of lysosomal storage disease is attractive to develop protocols for clinical trials involving human subjects. To this end, we have performed littermate BMT in five

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Address reprint requests to Robert Shull, DVM, Department of Pathobiology, College of Veterinary Medicine, University of Tennessee, Knoxville, TN 37901-1071.

dogs affected with MPS I. A previous report presented short-term findings in these dogs with an emphasis on early effects within the CNS.[14] This paper reports more complete necropsy and biochemical findings on two of three long-term survivors and compares these findings with those of two affected untransplanted and two unaffected control dogs of the same ages.

Materials and Methods

Experimental Animals and Transplantation Protocol

Six dogs from two separate litters (designated litters A and B) were included in this study. Two of the dogs (3A and 3B) were unaffected littermates to the MPS I-affected dogs and served as bone marrow donors and normal heterozygous controls. Four dogs were affected with MPS I (1A, 1B, 2A, and 2B), confirmed by the absence of a-L-iduronidase activity in leukocytes and serum. Two affected dogs (2A and 2B) were transplanted at 5 months of age with bone marrow from unaffected litter mates (dogs 3A and 3B, respectively).

Before transplantation, the two recipient dogs were given total body irradiation (TBI) of 7.5 to 8.5 Gy in a single dose (0.20 Gy/min) from a cobalt 60 source. Within 2 hours of TBI, recipients were transfused with 2.22 to 3.04 $\times 10^8$ nucleated bone marrow cells/kg body weight from littermate donors, chosen by two-way mixed leukocyte culture (MLC) nonreactivity and dog leukocyte antigen (DLA) identity with the recipient. Oral food and water was withheld until day 6. To prevent potential graft *vs.* host reactions, methotrexate (0.25 mg/kg, intravenously) was given on days 1, 3, 6, 11, and then weekly until day 102. Broad spectrum antibiotics were administered parenterally until the blood neutrophil counts returned to 2000 cells/cu mm. MPS I-affected and the donor littermates were maintained as control animals, but were not given TBI or methotrexate. All animals were killed approximately 20 months after transplantation.

Pathologic Examination

Before necropsy the animals were given general anesthesia and samples of cerebral cortical gray matter were taken for determination of tissue GAG concentrations and for transmission electron microscopy (TEM).

Tissue specimens from liver, kidney, and cornea were collected immediately after euthanasia for both GAG level determination and TEM. Tissues for TEM were immersed in 3% glutaraldehyde (in 0.1 M sodium cacodylate buffer, pH 7.4) for 2 hours at 4 C. They were then postfixed in 1% osmium tetroxide for 1 hour and embedded in Epon 812. Thin sections were stained with lead citrate and uranyl acetate and examined with a Philips 201 electron microscope. Other tissues for light microscopy were fixed for 48 hours in 10% buffered formalin, embedded in paraffin, cut into 6 μ sections, and stained with hematoxylin and eosin (H & E). Alcian blue and toluidine blue stains were not performed on formalin-fixed tissue because tissue GAG is usually removed during processing; however, the quantification and characterization of cytoplasmic storage material was done using biochemical analysis.

Morphometric Analysis

To morphologically quantitate the degree of cellular swelling due to GAG deposition, the mean diameter of renal distal tubular epithelium was determined for each dog. All morphometric measurements were made with a Leitz light microscope and Zeiss VideoPlan Image Analyzer. The distal tubular epithelial cell mean diameter was determined by measuring >400 random cell profiles and graphically calculating the mean cell diameter using a method described previously.[15] The morphometric data was analyzed statistically using Duncan's multiple range test.

Biochemical Analysis

Tissues taken for biochemical analysis were quick-frozen in liquid nitrogen, stored at −70 C, and later analyzed for GAG content using a method described previously.[16] The GAG levels were expressed as a percent of lipid free dry weight and the means of each treatment group were compared using Duncan's multiple range test.

To characterize the GAG present in tissue, electrophoretic separation was performed on 2.5 × 15.2 cm strips of cellulose polyacetate (Sepraphore III, Gelman Sciences, Inc., Ann Arbor, MI), using the system 0.1 M cupric acetate-acetic acid, pH 3.6, at constant current of 0.5 mA/cm for 2 hours,[17] and/or the system 0.1 M barium acetate, pH 8.0, constant current 1 mA/cm for 4.5 hours.[18] Samples of GAG (about 1 μg uronic acid) were applied to the strips at the side of the cathode. Staining was done with alcian blue without prior drying as follows: the strips were immersed in a staining solution containing 0.2% alcian blue in 0.05 M magnesium chloride, 0.025 M sodium acetate, 50% vol/vol ethanol-water, and were allowed to soak for 30 minutes. The strips were then destained in 3 changes, 15 minutes each, of 0.05 M magnesium chloride, 0.025 M sodium acetate in 50% vol/vol ethanol-water. The destained strips were blotted to remove excess buffer and were dried.

Glycosaminoglycans were identified by their mobility and by their susceptibility to the enzymes chondroitinase AC, chondroitinase ABC, testicular hyaluronidase and hyaluronidase from *Streptomyces hyalurolyticus,* as described previously.[19] Hyaluronidase for *Strep. hyalurolyticus* was assayed in 0.02 M sodium acetate-acetic acid buffer, pH 6.0, in 0.15 M NaCl.

Results

Clinical Observations

MPS I, Without BMT

By 9 to 12 months of age both dogs demonstrated lameness and reluctance to ambulate. The carpal and tarsal joints were hyperextended due to apparent laxity of distal limb tendons and ligaments. This resulted in metacarpal and metatarsal planter surface callus formation. Both dogs had bilateral cornea clouding and a central opaque focus. No behavior modifications were evident to indicate a state of demention, although mental development is difficult to evaluate in canine patients.

MPS I, With BMT

In each recipient peripheral leukocyte a-L-iduronidase levels were comparable with donor values 3 months after BMT. This indicates that essentially 100% of recipient leukocytes were of donor origin. Clinical improvements of MPS-I related disease were apparent in both transplant recipients. The urinary GAG levels were comparable to donor dog levels. Improvement in overall mobility of the two recipients was seen and both dogs were active and apparently free of pain. The carpal and tarsal joints had a normal angle, without the hyperextension evident in the MPS-I-no BMT dogs. The corneas of both dogs had a mild degree of corneal cloudiness, however, which was much less than the MPS-I-no BMT dogs and lacking a central opaque focus.

MPS I, Unaffected

These two dogs appeared normal clinically.

Pathologic Findings

MPS I, Without BMT

Both affected control dogs had the most severe gross lesions of all dogs examined (Table 1). The synovia of all examined joints were markedly thickened and dark tan. The articular cartilage of most joints had thinning or ebernation. Coalescing osteophytes were on the periphery of most articular surfaces. Numerous intervertebral (IV) spaces were collapsed and there was associated vertebral osteosclerosis and ventral spondylosis in the cervical and thoracic regions. Both animals had generalized skeletal muscle atrophy. The pulmonic, aortic, and atrioventricular (AV) valves in each were moderately thickened, nodular, and gelatinous. The wall of the aorta in dog 1B was approximately 1.5 times the thickness of the aortas at the same site (1 cm distal of the aortic valve) from MPS I-unaffected dogs. The left and right ventricular walls were thinner than control dogs, suggestive of a mild ventricular dilatation. All lymph nodes were enlarged to 3 to 4 times normal size and were pale tan on cut surface.

Microscopic changes in both dogs were similar to those reported previously for dogs with MPS I.[2] The leptomeninges of the brain and spinal cord were markedly thickened due to the accumulation of vacuolated mesenchymal cells. These cells extended into the sulci and parenchyma as perivascular infiltrates filling Virchow-Robbins space. Occasional cerebral cortical neurons were swollen and had clear vacuolated cytoplasm and marginated nuclei. The spinal cord of one dog had generalized, mild-to-moderate axonal degeneration, predominantly in the ventral and lateral funiculi characterized by axonal swelling, loss of axons, dilated empty myelin sheaths, and occasional gitter cells within myelin sheaths.

The corneas of both dogs had pronounced vacuolation of stromal cells expanding the substantia propria. The mesenchymal cells underlying the bulbar conjunctiva had the greatest degree of vacuolation and also associated linear subepithelial mineral deposits. The lamina propria fibers were disrupted, undulating, and separated by vacuolated stromal cells. The iris, choroid, and sclera contained numerous similarly vacuolated mesenchymal cells. The epithelium lining the iris were also vacuolated.

Changes in the musculoskeletal system consisted of numerous collapsed intervertebral spaces and associated dorsal and ventral prolapse of IV disk material. The collapsed IV spaces and adjacent vertebral osseous tissue had marked proliferation of fibrous connective tissue, irregular cartilage proliferation and thickening of vertebral bone trabeculae. Most joints contained marked synovial villous hyperplasia characterized by numerous villous projections lined by swollen vacuolated synovial cells (Figure 1A). Chondrocytes in the trachea, articular cartilage, costochondral cartilage and IV spaces were swollen, had vacuolated cytoplasm, and completely filled lacunae (Figure 2A).

The cardiovascular system changes consisted of marked expansion of arterial tunica media due to vacuolated smooth muscle cells (Figure 3A). The medial thickness of the left coronary artery in affected-no BMT dogs was 1.5 to 2 times the thickness of the coronary arteries

Table 1. *Gross Lesions of MPS I-Affected Dogs, After and Without BMT**

Group & litter no.	Lymph node enlargement (× normal size)	Cornea opacity	Degenerative joint lesions	Vertebral collapse	AV valve thickness
MPS I-affected; no BMT					
1A	3–4	Bilat +++	+++	C_{2-4}, T_{1-9}	+++
1B	3–4	Bilat +++	+++	C_{5-7}, T_{2-8}	+++
MPS I-affected; given BMT					
2A	–	Bilat +	–	T_{1-5}, L_7–S_1	+
2B	–	Bilat +	+	C_{5-6}, T_{3-9}	+

* Necropsies performed on day 628 (litter A) and day 594 (litter B) after BMT. Donor dogs kept as normal controls showed no gross lesions.
+++, severe; ++, moderate; +, mild; –, no lesions evident.

in controls. All cardiac valves were markedly thickened due to increased loosely arranged vacuolated mesenchymal cells (Figure 4A). The medullary areas of all lymph nodes were expanded due to numerous vacuolated macrophages distending medullary sinuses.

Mesenchymal cells in most organs and epithelial cells in the liver, kidney, and adrenal gland contained varying amounts of cytoplasmic vacuolation as described previously.[2] Most hepatocytes and biliary ductular epithelial cells were extremely swollen and vacuolated. The hepatic sinusoids were compressed in most areas due to the enlarged hepatocytes, although lobular architecture was retained. The renal distal tubular epithelium contained marked cytoplasmic vacuolation and cellular swelling (Figure 5A). The seminiferous tubules in testis contained numerous vacuolated Sertoli cells and germinal epithelial cells. The ovaries in the other dog contained numerous primary, secondary, and tertiary follicles, however, there were several large focal accumulations of vacuolated mesenchymal cells that expanded the internal ovarian architecture.

Ultrastructure of the liver (Figure 6A), cornea, and kidney demonstrated epithelial and mesenchymal cellular elements containing distended lysosomes filled with granular material similar to those reported previously in canine MPS I.[2] Neurons and astrocytes in the brain had similar lysosomal accumulations. Femoral articular chondrocytes were characterized by cytoplasmic expansion and lysosomal distension (Figure 7A).

MPS I, Receiving BMT

The right and left stifle of one dog had ruptured cruciate ligaments but no evidence of degenerative joint changes. Numerous IV disk spaces were collapsed and there was associated vertebral osteosclerosis and spondylosis in both dogs (Table 1). One dog also had a mild degree of dorsal IV disk prolapse associated with collapsed IV spaces. The AV, aortic, and pulmonic valves of both dogs were slightly more thickened than those of the normal control dogs. There was no apparent thinning of the right or left ventricular wall. The aortic wall thickness (2 to 3 mm) was similar to that of control dogs (2 to 3.5 mm). A 3 cm in diameter, well-delineated, dark red mass filled the fourth ventricle of one dog's brain (dog 2A).

Both transplanted dogs had minimal meningeal thickening with only occasional vacuolated mesenchymal cells. There was also minimal neuronal vacuolation or distension in one dog; however, the other BMT dog had a moderate degree of neuronal cytoplasmic vacuolation. Detailed descriptions of CNS changes have been reported recently.[20] One dog had a disseminated ependymoma apparently originating in the fourth ventricle, seemingly unrelated to MPS I. This neoplasm consisted of masses of epithelial lined, papilliferous structures in both lateral, third, and fourth ventricles, and disseminated throughout the subarachnoid area of the spinal cord.

The MPS I-related ocular lesions were much less severe in affected-BMT dogs than in affected-no BMT dogs. The cornea of the affected-BMT dogs had only occasional stromal cells with cytoplasmic swelling and vacuolation, usually confined to areas immediately adjacent to Descemet's membrane. The mesenchymal cells of the ocular tunics, iris, epithelial cells, and retinal ganglion cells lacked evident cytoplasmic vacuolation.

Musculoskeletal changes consisted of IV space collapse and similar vertebral microscopic changes as described for the affected, untransplanted dogs. Although gross skeletal changes were less severe than in affected-no BMT dogs, the chondrocytes in affected BMT dogs still demonstrated cellular swelling and cytoplasmic vacuolation (Figure 2B). Chondrocytes near the articular surface of the distal femoral cartilage were smaller, however, and had minimal cytoplasmic vacuolation. In the uncollapsed IV spaces there were occasional nests of vacuolated chondrocytes associated with cleft like defects in the nucleus pulposus and annulus fibrosis. The synovial membranes appeared normal in both dogs (Figure 1B)

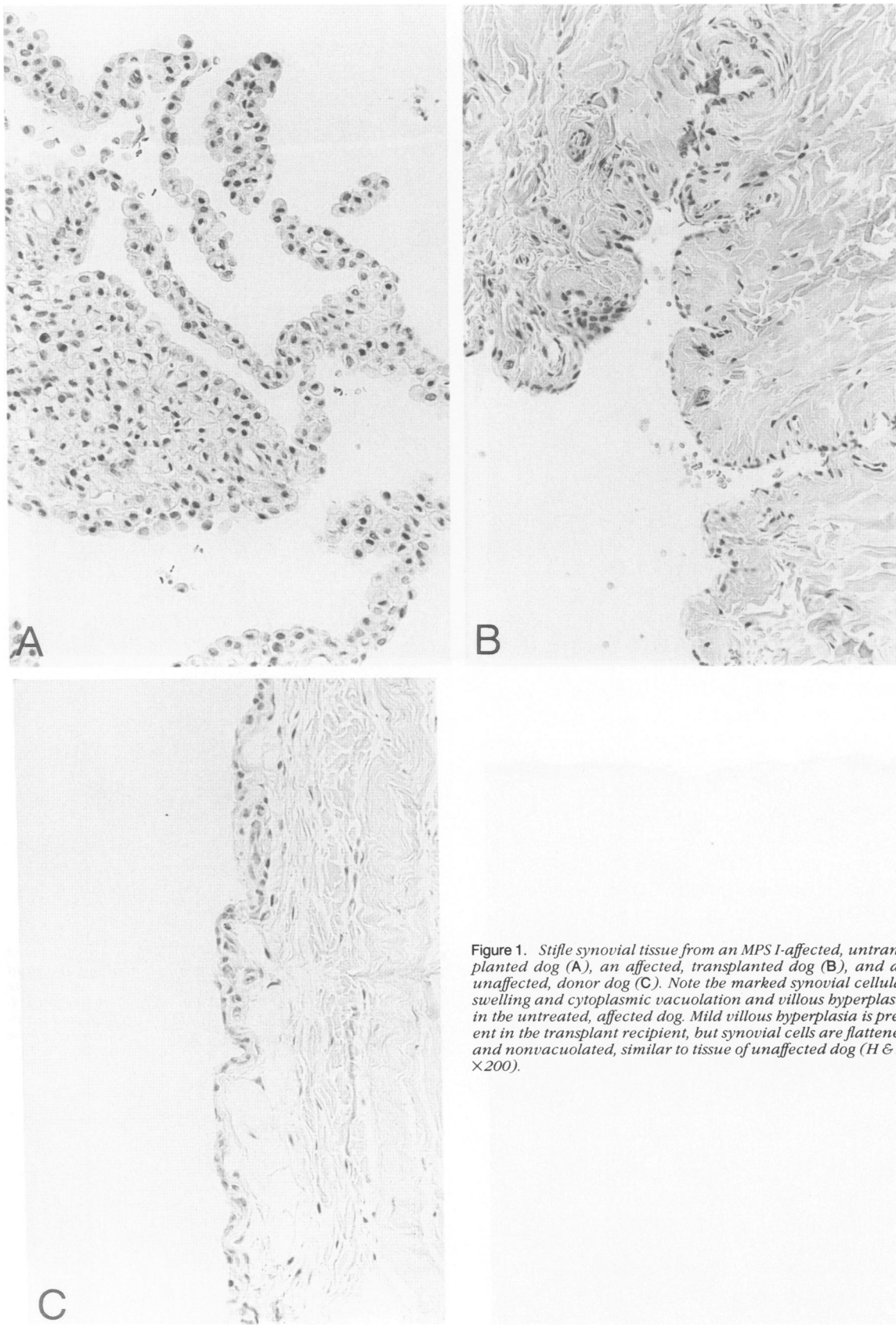

Figure 1. *Stifle synovial tissue from an MPS I-affected, untransplanted dog (A), an affected, transplanted dog (B), and an unaffected, donor dog (C). Note the marked synovial cellular swelling and cytoplasmic vacuolation and villous hyperplasia in the untreated, affected dog. Mild villous hyperplasia is present in the transplant recipient, but synovial cells are flattened and nonvacuolated, similar to tissue of unaffected dog (H & E, ×200).*

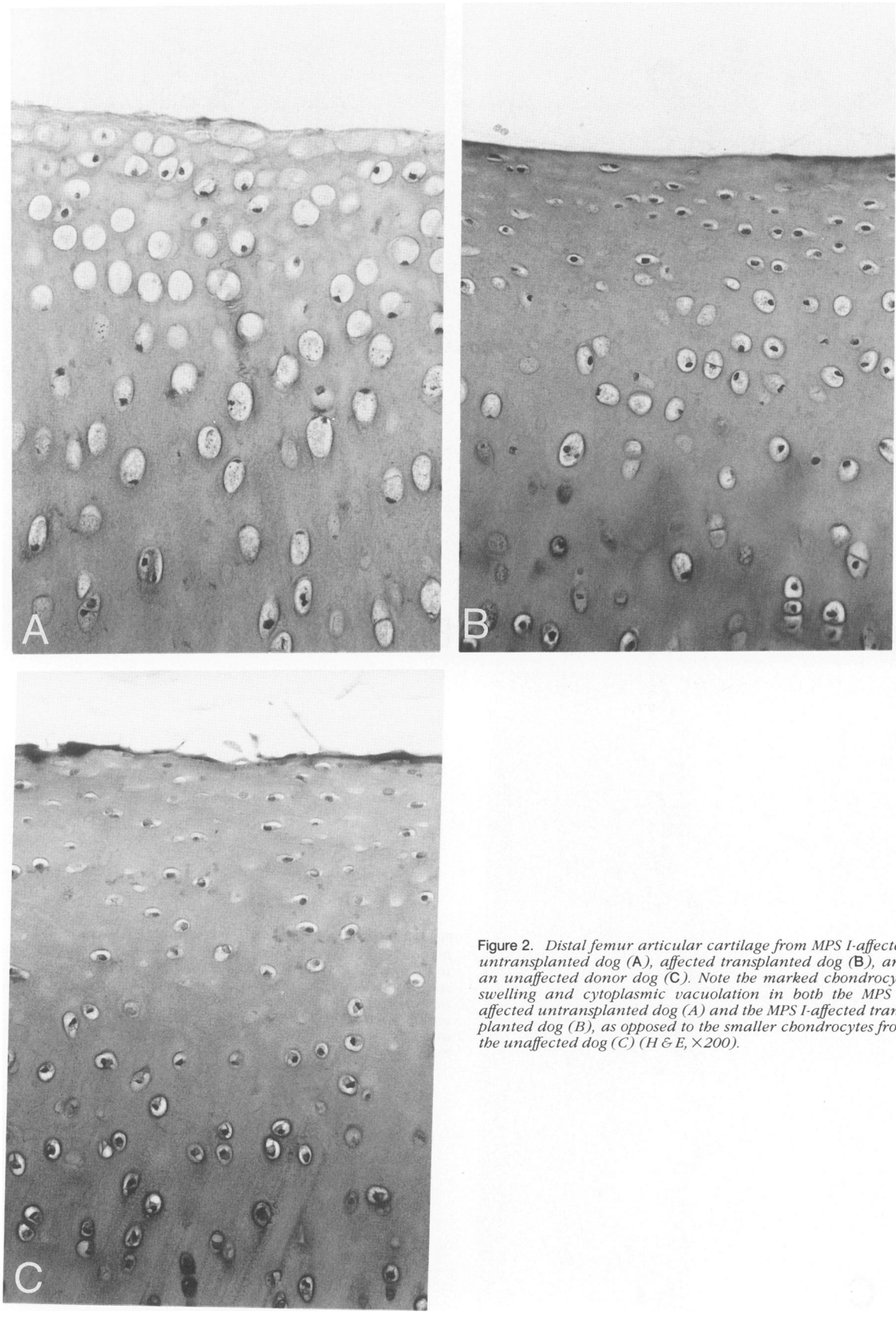

Figure 2. *Distal femur articular cartilage from MPS I-affected untransplanted dog (A), affected transplanted dog (B), and an unaffected donor dog (C). Note the marked chondrocyte swelling and cytoplasmic vacuolation in both the MPS I-affected untransplanted dog (A) and the MPS I-affected transplanted dog (B), as opposed to the smaller chondrocytes from the unaffected dog (C) (H & E, ×200).*

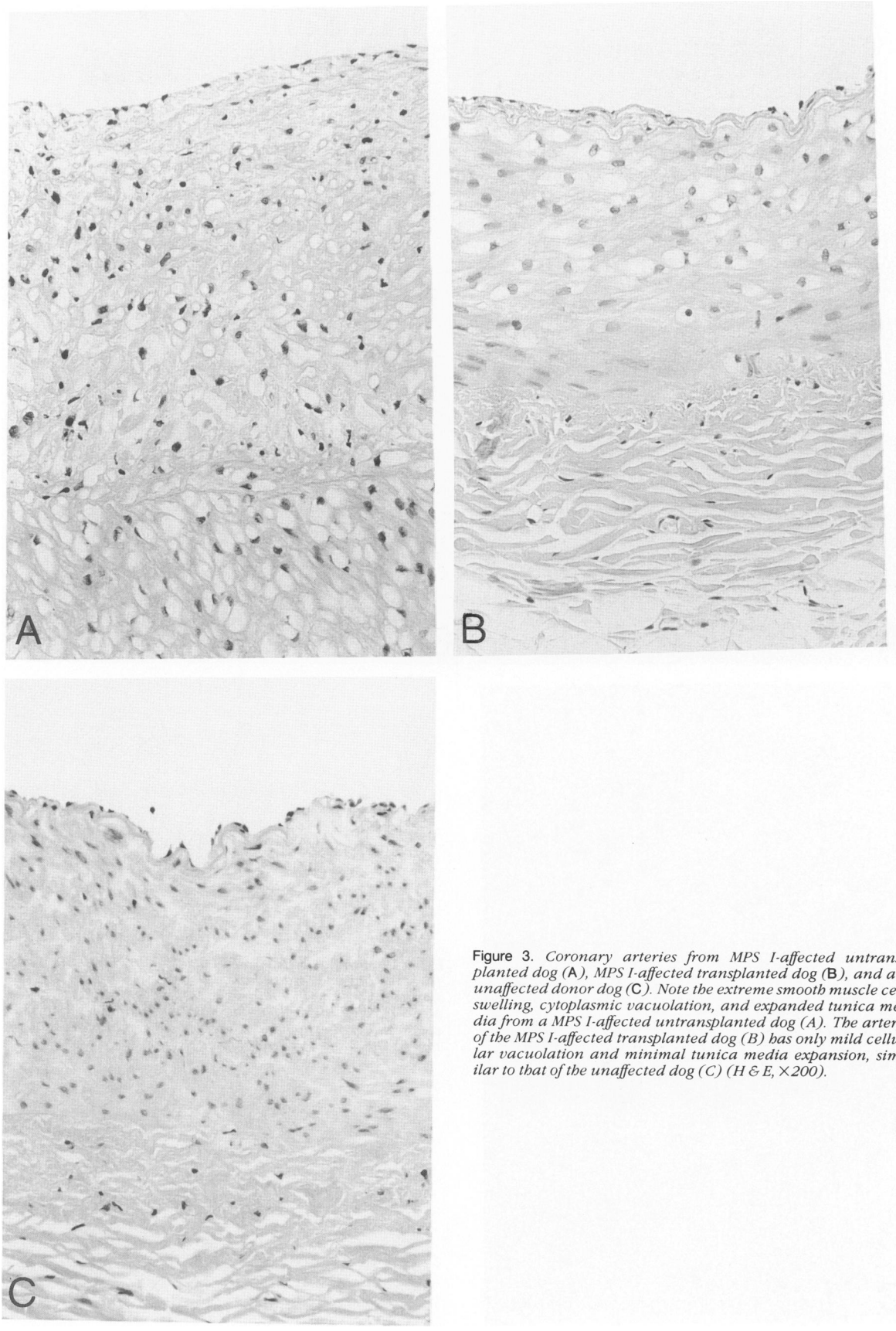

Figure 3. *Coronary arteries from MPS I-affected untransplanted dog (A), MPS I-affected transplanted dog (B), and an unaffected donor dog (C). Note the extreme smooth muscle cell swelling, cytoplasmic vacuolation, and expanded tunica media from a MPS I-affected untransplanted dog (A). The artery of the MPS I-affected transplanted dog (B) has only mild cellular vacuolation and minimal tunica media expansion, similar to that of the unaffected dog (C) (H & E, ×200).*

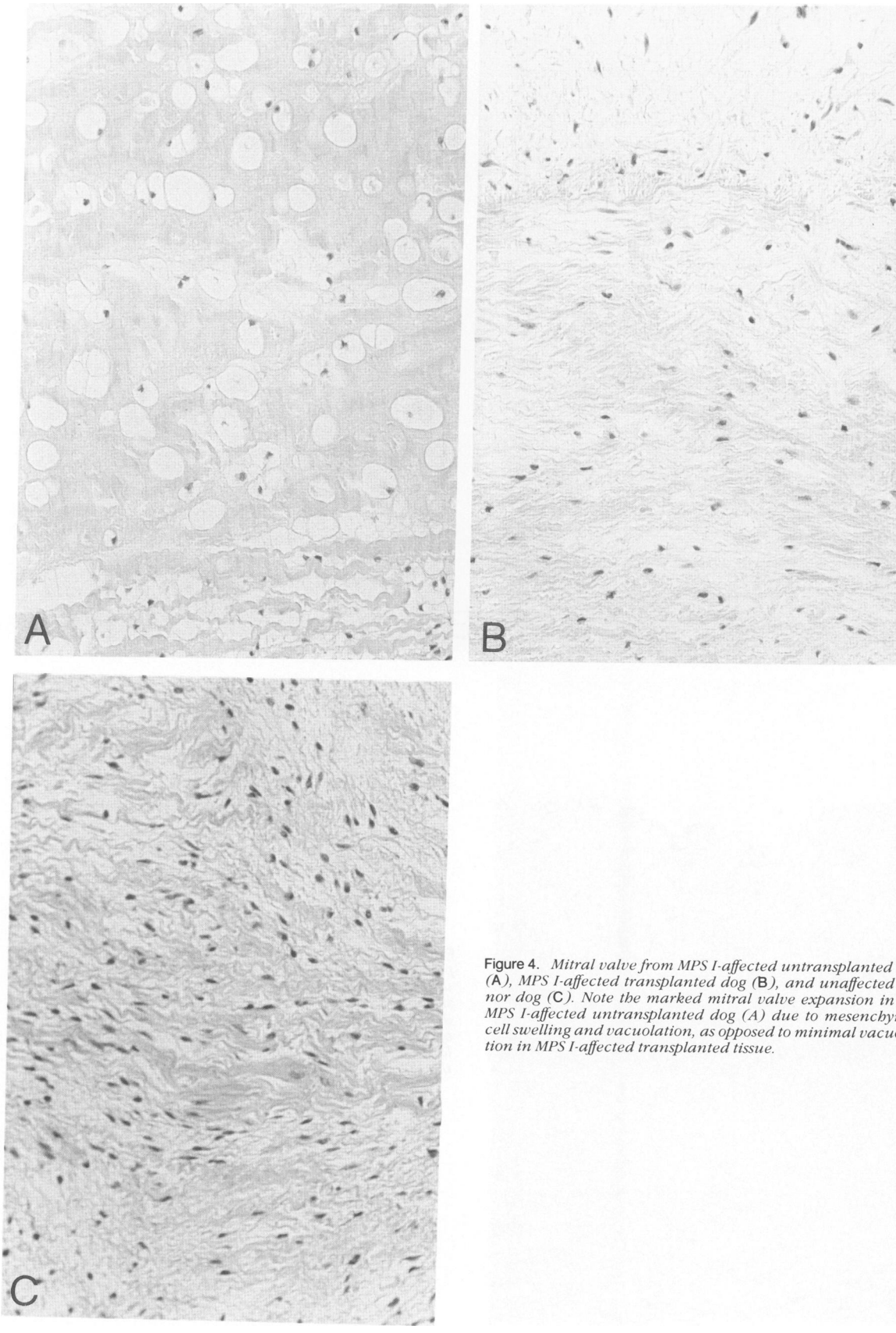

Figure 4. *Mitral valve from MPS I-affected untransplanted dog (A), MPS I-affected transplanted dog (B), and unaffected donor dog (C). Note the marked mitral valve expansion in the MPS I-affected untransplanted dog (A) due to mesenchymal cell swelling and vacuolation, as opposed to minimal vacuolation in MPS I-affected transplanted tissue.*

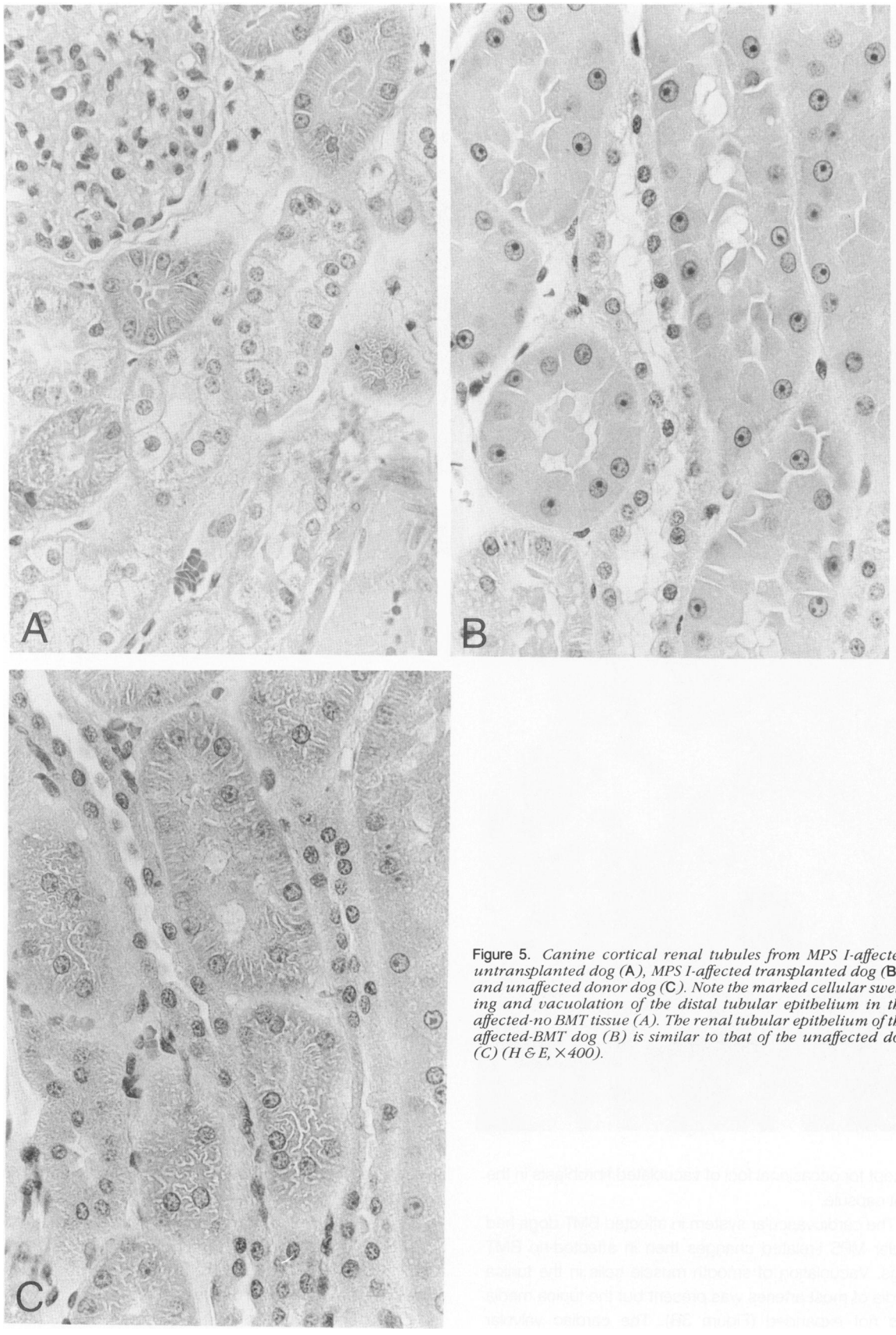

Figure 5. *Canine cortical renal tubules from MPS I-affected untransplanted dog (A), MPS I-affected transplanted dog (B), and unaffected donor dog (C). Note the marked cellular swelling and vacuolation of the distal tubular epithelium in the affected-no BMT tissue (A). The renal tubular epithelium of the affected-BMT dog (B) is similar to that of the unaffected dog (C) (H & E, ×400).*

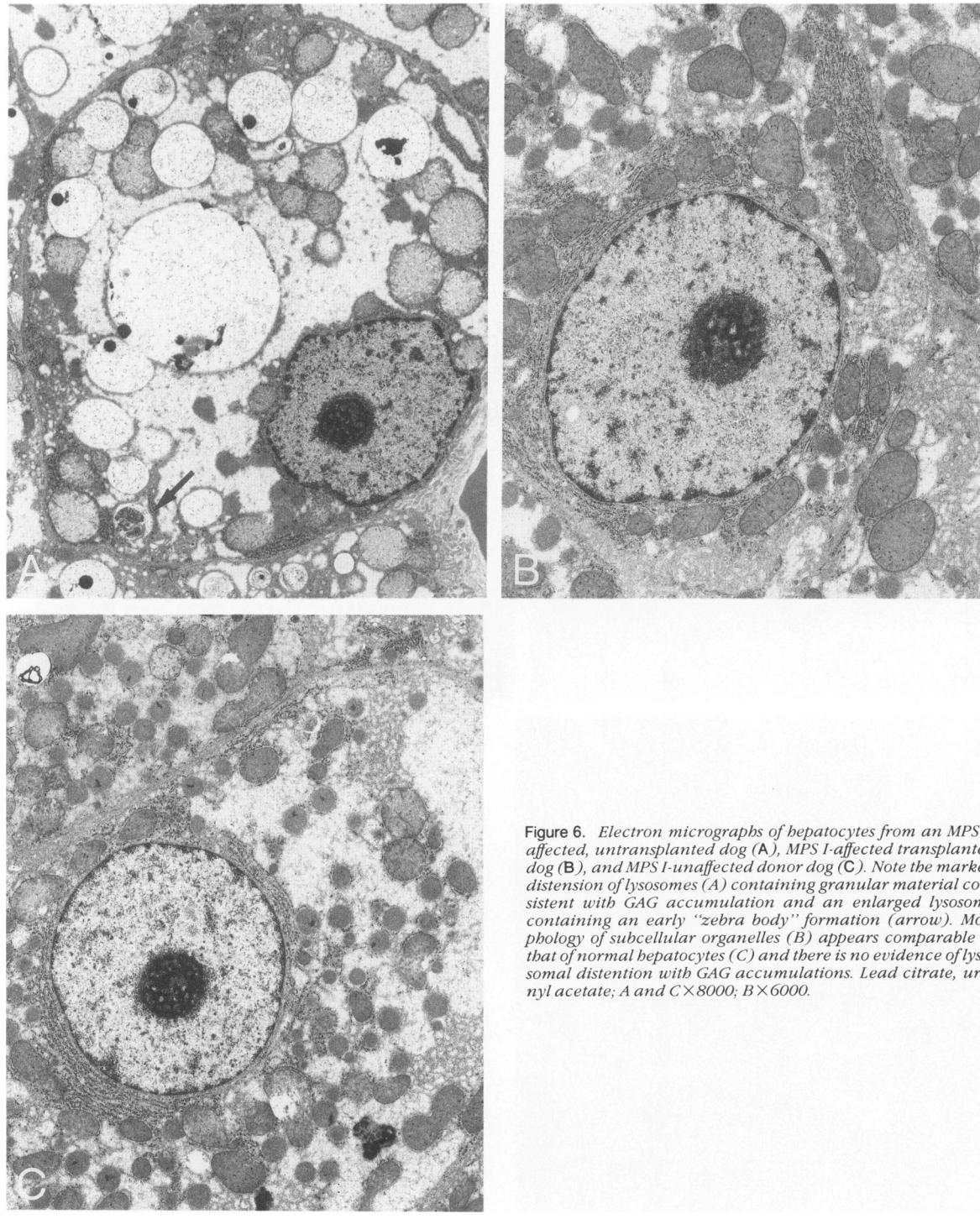

Figure 6. *Electron micrographs of hepatocytes from an MPS I-affected, untransplanted dog (A), MPS I-affected transplanted dog (B), and MPS I-unaffected donor dog (C). Note the marked distension of lysosomes (A) containing granular material consistent with GAG accumulation and an enlarged lysosome containing an early "zebra body" formation (arrow). Morphology of subcellular organelles (B) appears comparable to that of normal hepatocytes (C) and there is no evidence of lysosomal distention with GAG accumulations. Lead citrate, uranyl acetate; A and C×8000; B×6000.*

except for occasional foci of vacuolated fibroblasts in the joint capsule.

The cardiovascular system in affected BMT dogs had milder MPS I-related changes then in affected-no BMT dogs. Vacuolation of smooth muscle cells in the tunica media of most arteries was present but the tunica media was not expanded (Figure 3B). The cardiac valvular leaflets were similar to those of MPS I-unaffected animals and did not contain vacuolated mesenchymal cells, as evident in MPS I-affected, untransplanted dogs (Figure 4B).

The lymph nodes of both dogs did not contain vacuolated cells as did those in untransplanted dogs. Renal distal tubular epithelium also lacked cytoplasmic vacuolation

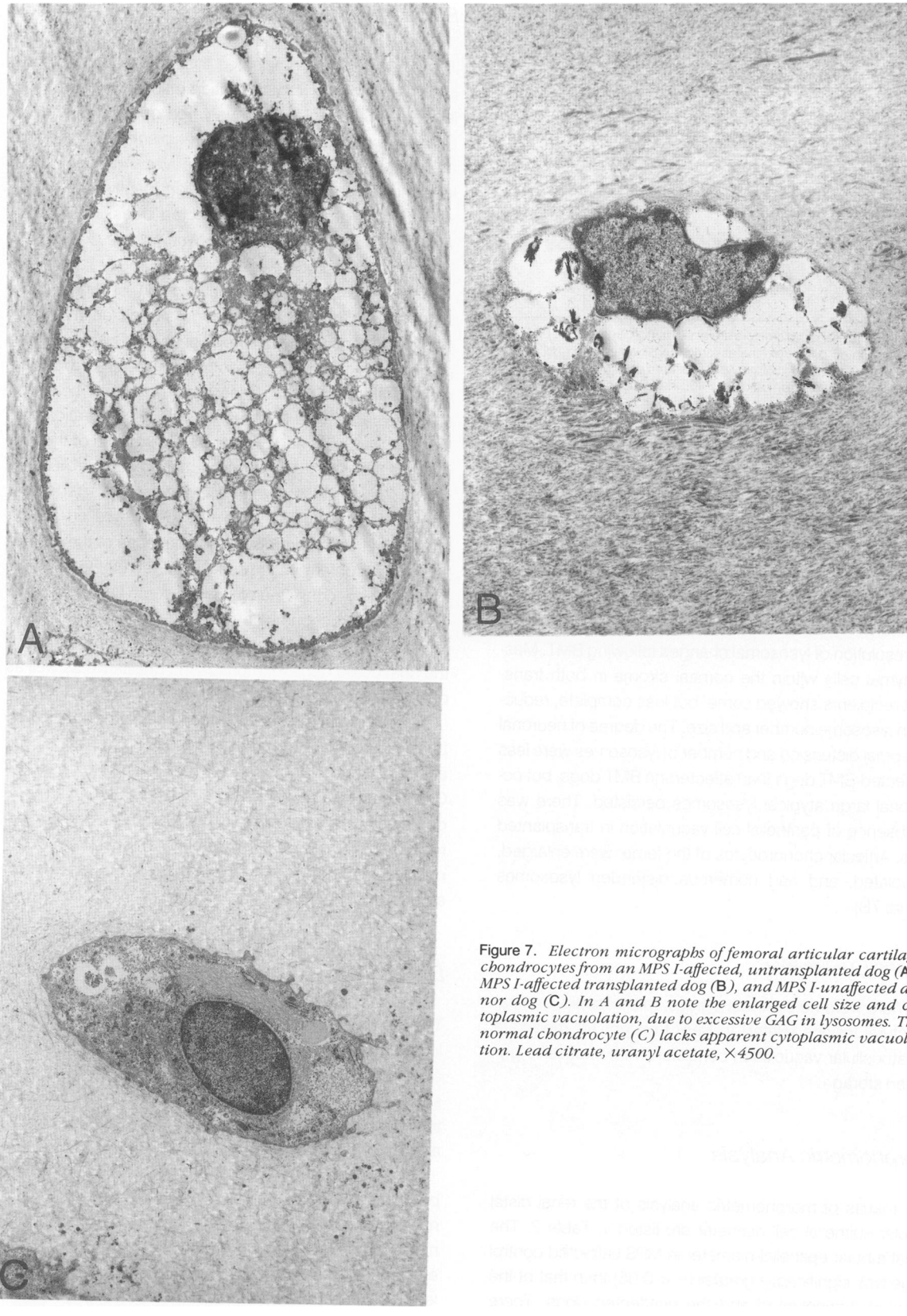

Figure 7. *Electron micrographs of femoral articular cartilage chondrocytes from an MPS I-affected, untransplanted dog (A), MPS I-affected transplanted dog (B), and MPS I-unaffected donor dog (C). In A and B note the enlarged cell size and cytoplasmic vacuolation, due to excessive GAG in lysosomes. The normal chondrocyte (C) lacks apparent cytoplasmic vacuolation. Lead citrate, uranyl acetate, ×4500.*

Table 2. *Morphometric Analysis of Renal Distal Tubular Epithelial Cell Diameters in MPS I-Affected, Untransplanted; MPS I-Affected, Transplanted; and Unaffected Donor Dogs**

Group and litter no.	Renal distal tubular epithelial diameter (μ)†
MPS I-affected; no BMT	
1A	10.7 ± 1.4
1B	11.3 ± 1.8
MPS I-affected; given BMT	
2A	9.3 ± 2.2
2B	9.0 ± 1.4
Unaffected	
3A	8.4 ± 1.1
3B	8.3 ± 1.3

* Mean epithelial cell diameters are significantly different ($P < 0.05$) in the untransplanted dogs from the affected, transplanted and unaffected groups, which were not significantly different from each other.
† Mean and standard deviation of >400 independent measurements.

(Figure 5B). One dog had a moderate, diffuse, membranous glomerulonephritis, probably unrelated to MPS I. Hepatocytes of both dogs were moderately vacuolated but this was due to excessive glycogen accumulation, verified with periodic acid-Schiff (PAS) stain.

By electron microscopy hepatocytes in donor (Figure 6C) and recipient (Figure 6B) liver were virtually indistinguishable, reflecting marked clearance of stored GAG and resolution of lysosomal changes following BMT. Mesenchymal cells within the corneal stroma in both transplant recipients showed some, but less complete, reduction in lysosome number and size. The degree of neuronal lysosomal distension and number of lysosomes were less in affected-BMT dogs than affected-no BMT dogs, but occasional large atypical lysosomes persisted. There was an absence of perithelial cell vacuolation in transplanted dogs. Articular chondrocytes of the femur were enlarged, vacuolated, and had numerous distended lysosomes (Figure 7B).

MPS I, Unaffected

There were no significant gross, microscopic, or ultrastructural lesions related to MPS I except for moderate hepatocellular vacuolation consistent with excessive glycogen storage.

Morphometric Analysis

The results of morphometric analysis of the renal distal tubular epithelial cell diameter are listed in Table 2. The distal tubular epithelial diameter in MPS I-affected control dogs was significantly greater ($P < 0.05$) than that of the affected, transplanted and the unaffected dogs. There was no significant difference in epithelial cell diameter between the transplant recipients and unaffected dogs.

Biochemical Analysis

The levels of GAG in selected tissues for each dog are shown in Table 3. Transplantation resulted in decreased levels of total GAG in kidney, lymph node, liver and, to a lesser extent, cornea in BMT recipients compared to affected, untransplanted dogs. The liver and kidney GAG levels in affected controls were significantly different ($P < 0.05$) from treated and unaffected dogs, while there was no significant difference between tissue GAG levels in the latter two groups.

The electrophoretic characterization of kidney and liver from the three groups of dogs is depicted in Figures 8 and 9, respectively. Kidney tissue GAGs of untransplanted, affected dogs predominantly consisted of dermatan sulfate (DS) and a lesser amount of small molecular size heparan sulfate (HS), as indicated by migration patterns similar to DS and HS standards and loss of the majority of these bands following chondroitanase ABC digestion. It should be noted that electrophoresis for the MPS I-affected, no-BMT dog was performed on $^1/_{20}$ the amount of tissue from other dogs because of the greater total GAG content in its tissues. Kidney GAGs in donor tissues mainly consisted of HS with some hyaluronic acid (HA), DS, and chondroitin sulfate (CS). The electrophoretic patterns of BMT recipients' tissues were similar to the unaffected dogs, demonstrating qualitative as well as quantitative changes after BMT.

Similar findings also were seen in electrophoretic patterns of liver GAGs from the three groups of dogs. The affected, control dogs had a 75-fold increase of stored GAG compared to normal levels. These GAG were predominantly low molecular weight HS and DS. The GAG in recipients' livers were comparable with the pattern of donor livers, predominantly consisting of HS, and lesser amounts of HA and DS.

Discussion

The results of this long-term study demonstrate morphologic and biochemical, beneficial effects of BMT in dogs affected with MPS I. The resolution of GAG storage in tissues of affected dogs nearly 2 years after BMT indicates that clearance of GAG from various tissues reported at 3 and 9 months after BMT has been sustained.[14]

In our study, most organ systems benefited from BMT, but to varying degrees. The hemic-lymphatic, hepatic, renal, ocular, cardiovascular, and nervous systems had almost complete clearance of excess GAG, whereas others, such as the musculoskeletal system, had decreased lesion severity but not complete resolution of the disease.

Arthrodial lesions, such as synovial thickening, articular cartilage ebernation, and formation of osteophytes,

Table 3. *Total Tissue Glycosaminoglycans as a Percent of Lipid-Free Dry Weight in MPS I-Affected, Untransplanted; MPS I-Affected, Transplanted; and Unaffected Dogs*

Group & litter no.	Cornea	Kidney*	Lymph node	Liver*
MPS I-affected; no BMT				
1A	NA	3.39	NA	2.54
1B	2.02	2.67	1.50	2.38
MPS I-affected; given BMT				
2A	0.82	0.36	0.07	0.03
2B	0.96	0.33	0.09	0.08
Unaffected				
3A	0.69	0.15	0.04	0.03
3B	NA	0.12	NA	0.04

NA, not available.
* Affected-no BMT GAG levels are statistically different ($p < 0.05$) from affected-BMT and unaffected GAG levels.

were much less common in affected, transplanted dogs, but there was still collapse of numerous IV disk spaces in both groups. The chondrocyte cytoplasmic vacuolation in affected-BMT dogs indicates that BMT was not successful in completely eliminating GAG deposition in chondrocytes, but there must have been some beneficial effect evidenced by the less severe arthrodial lesions. The decreased severity of arthrodial lesions in affected-BMT dogs may be due to enhanced GAG clearance from superficial articular chondrocytes *vs.* IV space chondrocytes. The normal morphology of synovia and articular chondrocytes near the articular surface in affected-BMT dogs supports the hypothesis that BMT provided for synovial fluid a-L-iduronidase diminishing joint lesions. Unfortunately, articular cartilage GAG levels were not determined to quantitate the chondrocyte clearance of GAG. A detailed radiographic study of these dogs showed that BMT was successful in delaying the onset and reducing the severity of most vertebral and arthrodial lesions, although IV disk collapse did eventually occur in most transplanted dogs.[21] The progression of vertebral lesions may be due to inadequate clearance of chondrocyte GAG, as evidenced by our results, compounded with irreversible changes present in the vertebral column of affected dogs before BMT that progressed to a degenerative lesion despite BMT. These results are similar to those reported in a study of transplanted human infants affected with MPS I, in whom skeletal lesions progressed in spite of visceral clearing of GAG, as in the natural disease.[22]

One of the single most important causes of death in MPS I-affected children is congestive heart failure due to valvular GAG accumulation.[23] At the time of transplantation, ECG and echocardiographic profiles of both the affected-BMT dogs and affected-no BMT dogs were normal (unpublished observations). Later echocardiographic data of these dogs indicated that cardiac function remained normal in affected-BMT dogs several months after transplantation, whereas affected-no BMT dogs had pro-

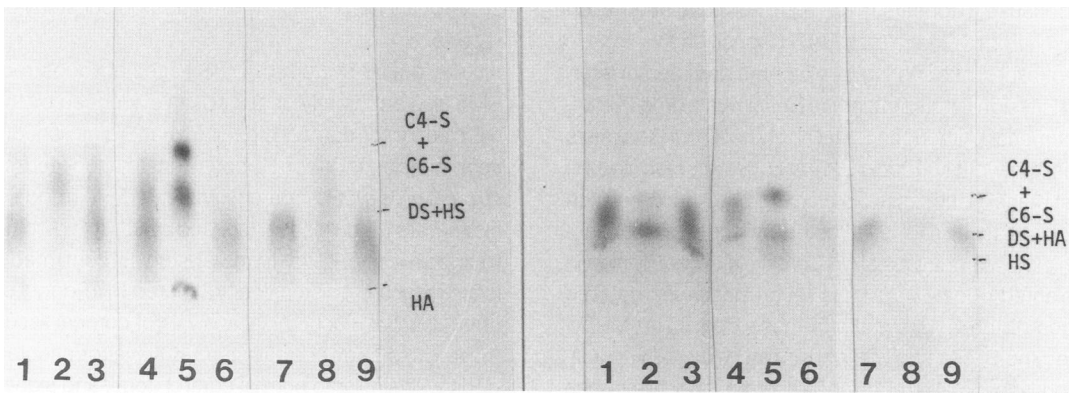

Figure 8. *Cellulose polyacetate electrophoresis of GAGs from canine kidney. Samples are from an unaffected dog (1), an affected, untransplanted dog (2), affected dogs that were given a BMT (3,4), GAG standards (hyaluronic acid, HA; heparan sulfacte, HS; dermatan sulfate, DS; and chondroitins 4 and 6 sulfate, C_{4-5} and C_{6-9}). Lanes 5 and 6 are the same dog as number 4 but after incubation with chondroitinase ABC, and numbers 7, 8, and 9 represent the same dogs as 1, 2, and 3, respectively, but after incubation with chondroitinase ABC. GAGs (1 μg uronic acid) were applied on cellulose polyacetate strips (sepraphore III), and were subjected to electrophoretic separation using the cupric acetate system (left panel) and the barium acetate system (right panel), at a constant current 0.5 mA/cm for 2 hours. Staining was done with alcian blue.*

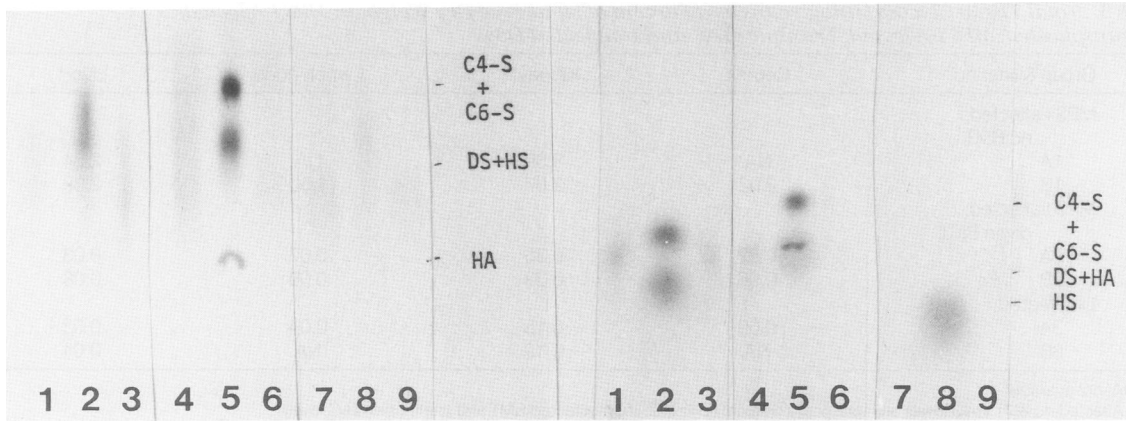

Figure 9. *Cellulose acetate electrophoresis of GAG from canine liver. The bottom legend identifies GAG samples taken from unaffected dog (1), affected-no BMT dog (2), affected-BMT dog (3), affected-BMT dog (4), and GAG standards (HA, HS, DS, and C_{3-4}) (5). Number 6 is the same as number 4 after incubation with chondroitinase ABC, and numbers 7, 8, and 9 represent the same GAGs as 1, 2, and 3, respectively but after incubation with chondroitinase ABC. GAG (1 μg uronic acid) were applied on cellulose polyacetate strips (sepraphore III), and were subjected to electrophoretic separation using the cupric acetate system (left panel) and the barium acetate system (right panel), at a constant current 0.5 mA/cm for 2 hours. Staining was done with alcian blue.*

gressively severe cardiac disease. Although clinical signs of congestive heart failure were not evident in the affected-no BMT dogs, echocardiograms demonstrated right ventricular enlargement and mitral valve regurgitation. These clinical findings are supported by gross and microscopic findings demonstrating that transplanted dogs had only mild valvular thickening and decreased arterial medial thickening compared to the untransplanted, affected dogs.

The most dramatic effect of BMT in clearing stored GAG was observed in lymphoid tissue where almost complete absence of foamy macrophages and mesenchymal cells was observed. Kidney and liver were also cleared of GAG deposits, indicated by microscopic, ultrastructural, and biochemical evidence. Clearance of renal distal tubular GAG storage may be important in the course of disease because it appears that diminished tubular function can result from MPS I-related GAG deposition.[24]

Central nervous system involvement and mental retardation is a significant problem in Hurler syndrome (MPS I-H) in humans.[5] Although mental ability is difficult to assess in dogs, our work suggests BMT in MPS I-affected dogs is at least partially successful in clearing GAG accumulation from brain tissue. This is indicated by decreased meningeal thickness and diminished CNS cellular vacuolation in transplanted dogs. A previous report describes detailed changes of the CNS system in these dogs. The MPS I-affected, no-BMT dogs had consistent neuronal vacuolation and lysosomal "zebra body" formation while in one MPS I-affected, BMT recipient dog neuronal vacuolation was minimal.[20] The other MPS I-affected, BMT recipient dog still demonstrated a moderate degree of neuronal cytoplasmic vacuolation. Both BMT recipient dogs, however, did demonstrate an absence of CNS glial cell vacuolation. GAG levels in transplant recipients' brain tissue was similar to that of unaffected dogs. Transplanted dogs also had detectable levels of a-L-iduronidase in brain tissue, although it is speculated that this may have been due to blood contamination in the tissue. These results suggest that BMT may indeed result in some clearing of GAG from MPS I-affected dogs' brains following BMT but in a varying degree.

The ependymoma in one of the MPS I-affected BMT dogs was an unusual finding. A previous report details four of seven MPS I-affected cats having meningomas at postmortem examination.[25] The whorled or sheetlike arrangement of the neoplastic cells in these cases, however, was unlike the papilliferous growths seen in our case. The radiation preceding the BMT may have been a significant initiating factor, but CNS tumors have not been seen routinely in other irradiated dogs in our study or other reports.

The ocular system of MPS I-affected dogs receiving BMT also apparently benefited, as evidenced by the clinical clearing of corneal cloudiness and diminished microscopic lesions. Although occasional vacuolated corneal stromal cells were apparent in affected-BMT dogs, the lesions were much less severe and clinically did not appear to impair vision. Ultrastructural studies confirmed diminished lysosomal distension in affected-BMT dogs' corneal stromal cells. Biochemical analysis of corneal tissue from these dogs showed that the GAG level of affected-BMT dogs was similar to normal control dogs, and significantly lower than affected-no BMT dogs (unpublished results).

There is a good correlation between reduced GAG levels and decreased microscopic lesions related to MPS I in transplant recipients. Electrophoretic profiles of tissue GAG from affected control dogs and unaffected dogs

were similar, suggesting that normal metabolic GAG clearance was reestablished in most tissues after transplantation. The actual mechanism of GAG removal from tissues following BMT is not well defined. It has been proposed that combinations of the following four different mechanisms may contribute to GAG removal after BMT: 1) plasma catalysis of GAG in the vascular compartment by circulating enzyme; 2) cell transplantation from marrow stem cells into recipient tissue; 3) enzyme replacement to enzyme deficient lysosomes; and 4) metabolic filtration of plasma borne substrate by resident donor origin cells.[22]

The mechanism of plasma catalysis of GAG by circulating enzyme is probably not important in this disease because of the low activity of aiduronidase at blood pH.[22] Cell transplantation could be an important mechanism, evidenced by near normal GAG levels and lack of MPS I-related lesions in organs that were probably seeded by donor hematopoietic cells such as lymph nodes and liver Kupffer cells. However, this would not explain the lack of GAG deposition in other sites such as synovia, kidney, cornea, and brain, where hematopoietic cells will not colonize readily. In these sites, enzyme replacement to enzyme-deficient lysosomes or metabolic filtration of plasma-borne substrate by resident donor origin cells may be more important.

Similar but more limited studies of effects of BMT have been done in children with MPS I.[22,26] Results of these studies are encouraging and similar in some respects to our's in dogs. In several children treated with BMT there has been effective visceral clearing of GAG, but musculoskeletal lesions of MPS I persisted resulting in a crippling condition. Preliminary computerized tomography and magnetic resonance imaging in one group of transplanted patients showed no progression of MPS I-related CNS lesions after transplantation.[22] The improvement in one MPS I-H affected infant was not sustained because of lack of long-term donor cell survival, and accumulation of GAG ensued.[27]

Bone marrow transplantation therapy has been used in several lysosomal storage diseases in both humans and animal models with varying degrees of success.[27-32] Children affected with MPS VI, MPS I-H, or metachromatic leukodystrophy, have demonstrated restored enzyme activity and clinical improvement followed with BMT. Animal models of lysosomal storage disease have obvious benefits for studying the therapeutic efficacy of BMT. Results of BMT in several animal models[28-32] have been encouraging and studies of gene replacement protocols will probably be conducted in the next several years.

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