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**Research Article**

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# Bone Marrow Transplantation in Canine Mucopolysaccharidosis I

## Effects within the Central Nervous System

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### Abstract

Five dogs with mucopolysaccharidosis I, a model of human Hurler/Scheie syndrome, were transplanted with marrow from phenotypically normal littermates at 5 mo of age. At 3 and 9 mo posttransplantation, biopsies of cerebral cortex, liver, and cerebrospinal fluid were obtained. The  $\alpha$ -L-iduronidase levels in these tissues were 0.8–7.4, 26–45, and 6.3–14.9% of the paired donor tissues, respectively. Although iduronidase was present in relatively low levels in the recipients' brains and cerebrospinal fluid at both biopsy times, reduction in brain glycosaminoglycan (GAG) was comparable to that observed in liver. Ultrastructural studies of cells within the transplanted dogs' brains showed less lysosomal distension and storage product than in affected, non-transplanted, littermate controls. The most marked clearing of stored GAG was in cells surrounding blood vessels, but decreased lysosomal storage in neurons and glial cells was also observed. Urinary GAG excretion also decreased to near normal levels by 5 mo posttransplantation.

### Introduction

The initial rationale for attempting transplantation therapy in inherited enzyme deficiency diseases has been supported by recent reports of clinical improvement in patients after transplantation, usually of bone marrow. Whether due to direct replacement of cells within various tissues, enzyme leakage or secretion from transplanted cells with subsequent cellular uptake, or by clearance of circulating substrate, extraneural sites such as liver, spleen, cornea, and joints have been shown to benefit. The number of lysosomal storage diseases in which hematopoietic stem cell transplantation has been attempted is increasing and now includes Hurler (1), Hunter (2), Sanfilippo B (3), Morquio (4), and Maroteaux-Lamy (5) syndromes, Fabry's disease (6), Niemann-Pick disease (6), metachromatic leukodystrophy (7), adrenoleukodystrophy (8), Gaucher's disease (9), and murine Krabbe's disease (10). The degree of clinical improvement varies, those diseases characterized mainly by visceral storage, without neural involvement, generally being more amenable to treatment than those diseases with progressive neurodegeneration. The critical question as to what extent hematopoietic cell transplantation can be expected to lead to improvement in those

diseases with central nervous system (CNS) involvement has yet to be fully answered. Clearly, the blood-brain barrier represents a significant impediment to enzyme transfer within intact cells or free in the blood.

A canine animal model of mucopolysaccharidosis I (MPS I),<sup>1</sup> caused by deficiency of the lysosomal enzyme  $\alpha$ -L-iduronidase (EC 3.2.1.76, herein referred to as iduronidase), has been described in a family of Plott hounds (11, 12). Affected dogs share many clinical signs with affected humans such as corneal clouding, stunted growth, and joint stiffness (13). Human MPS I exists as at least three separate syndromes based on clinical severity ranging from Hurler syndrome (MPS IH), the most severe, to Scheie syndrome (MPS IS), the mildest. An intermediate form, Hurler/Scheie phenotype (MPS IH/S), appears clinically to be most analogous to the canine disease. Affected dogs have CNS glycosaminoglycan (GAG) storage similar to that seen in MPS IH; brain GAG levels in human MPS IH/S have not been reported. In both species there is urinary excretion and lysosomal storage of large amounts of dermatan sulfate (DS) and heparan sulfate (HS). The nature of the enzyme deficiency in dogs is very similar to that in human MPS I where affected individuals often have < 1/2% of normal iduronidase, and secondarily increased  $\beta$ -hexosaminidase and decreased  $\beta$ -galactosidase (14). The lesions within the CNS of both species are also very similar, each having glial and neuronal storage of DS and HS and secondary accumulations of G<sub>M3</sub>, G<sub>M2</sub>, and G<sub>D3</sub> gangliosides (15). Development of lamellar zebra bodies within lysosomes of affected neurons and other cells is seen in both species (12).

When available, animal models of human disease provide the opportunity to study potential treatment protocols in a manner not possible with human patients. The canine MPS I model was used to evaluate the effects of bone marrow transplantation with an emphasis on the effects within the CNS.

### Methods

Five dogs affected with MPS I were transplanted at 5 mo of age with bone marrow from a nonaffected littermate. In three of the five transplants the donor was presumed by its enzyme level to be a heterozygote, but their status has not been confirmed by breeding.

Recipients were given total body irradiation (TBI) of 7.5–8.5 Gy in a single dose (0.20 Gy/min) from a cobalt 60 source. Within 2 h of TBI, recipients were transfused with  $2.22\text{--}4.03 \times 10^8$  nucleated bone marrow cells/kg body weight from littermate donors which were chosen by two-way mixed leukocyte culture (MLC) nonreactivity and dog leukocyte

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1. *Abbreviations used in this paper:* CSF, cerebrospinal fluid; DLA, dog leukocyte antigen; DS, dermatan sulfate; GAG, glycosaminoglycan; HS, heparan sulfate; LFDW, lipid-free dry weight; MLC, mixed leukocyte culture; MPS I, mucopolysaccharidosis I; MPS IH, Hurler syndrome; MPS IH/S, Hurler/Scheie phenotype; MPS IS, Scheie syndrome; TBI, total body irradiation.

antigen (DLA) identity with the recipient. Oral food and water was withheld until day 6 and 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 count returned to 2000 cells/mm<sup>3</sup>. At day 30 each recipient's leukocyte iduronidase level was assayed with the finding of donor levels taken as documentation of marrow engraftment. Control MPS I affected littermates were maintained for comparative purposes, but were not given TBI or methotrexate.

Biopsies of liver and cerebral cortex were obtained from recipients, donors, and littermate affected, nontransplanted controls, at 3 and 9 mo posttransplantation. Cerebrospinal fluid (CSF) was obtained concurrently and assayed for GAG content, iduronidase activity, and examined cytologically with Wright-Giemsa and Alcian blue stains.

Tissue and CSF iduronidase was measured by a fluorimetric assay as previously described (16). Briefly, after homogenization and sonication at 4°C, the tissue homogenate protein concentration was determined by the method of Lowry (17). 40 µg of protein was incubated for 1 h at 37°C with 25 µl of 0.05 mM 4-methylumbelliferyl-iduronide in a 0.4 M formate buffer (pH 3.5). After the addition of 1 ml carbonate-glycine buffer (pH 9.85) liberated 4-methylumbelliferone was read on a fluorimeter and compared with a 1-nmol 4 MU standard. Final results were expressed as nanomoles substrate hydrolyzed per milligram protein per hour of incubation (nmol/mg per h). The CSF assay was identical except that the incubation was 5 h at 25°C and 50 µl of sample was used. All assays were run in triplicate and included both specimen and reagent blanks, which were subtracted to obtain the final result. Before analysis, tissues were stored at -80°C for a maximum of 1 wk.

Glycosaminoglycan levels in tissues and CSF were measured by previously published techniques (18) and expressed as micrograms uronic acid content per 100 µg of lipid-free dry weight (% LFDW) for tissues and micrograms uronic acid per milliliter for CSF. Urine was collected before transplantation (two recipients) and after transplantation (all recipients) for GAG analysis; results were expressed as micrograms uronic acid per milligrams creatinine.

In addition to tissue frozen for biochemical analysis, a portion of each biopsy was minced immediately after collection in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4). Tissues were postfixed in 1% osmium tetroxide for 1 h and subsequently washed, dehydrated, and embedded in Epon 812. 1-µm sections were stained with toluidine blue; thin sectioning was followed by routine staining with uranyl acetate and lead citrate and the sections were viewed on a Philips 201 electron microscope. 10 neuronal profiles from one transplanted dog and its nontransplanted littermate control were photographed and the volume fraction of lysosomes was determined using point counting volumetry (19).

Two-sample paired *t* tests were computed to determine whether biochemical changes seen after transplantation represented statistically significant differences between treated dogs and the normal or affected controls.

## Results

All recipient leukocyte counts returned to the normal range within 20 d and, when assayed for iduronidase at approximately day 30, all had levels corresponding to those of the donor, which indicated that each bone marrow transplant had engrafted. Clinical signs of graft rejection were not seen in any recipient; however, two recipients died before their 3-mo biopsy date. One succumbed to apparent graft-vs.-host disease with hepatic involvement (day 82) and the other to encephalitis believed to have been caused by infection with canine distemper virus (day 88). The three surviving recipients are completely well at 17, 18, and 21 mo posttransplantation showing only premature graying of their hair coat and early closure of epiphyseal growth plates, both presumably direct effects of TBI.

Clinical signs of MPS I, which have developed in the affected controls, have essentially been eliminated in the transplanted

dogs. Most notable in the surviving recipients are decreases in corneal clouding and joint instability, which have caused loss of visual acuity, lameness, and exercise intolerance in the nontransplanted dogs. Mild corneal neovascularization of unknown etiology, which responded to topical corticosteroid treatment, was seen in the recipients. In all recipients the levels of urinary GAG have approached normal values after initial increases in GAG excretion occurred in two dogs (Fig. 1). Detailed reports of clinical, radiographic, and echocardiographic studies will be published elsewhere.

Table I presents tissue and CSF activities of iduronidase at 3 and 9 mo posttransplantation. The two dogs that died before the 3-mo biopsy had tissues collected at necropsy for biochemical analyses. Both dogs had detectable iduronidase activity in brain, liver, and CSF, but are not included in Table I due to their illness at the time of tissue collection. Enzyme was not detectable in the liver, brain, or CSF of nontransplanted, affected dogs at either biopsy time. There appeared to be a correlation between the enzyme activity of the donor animal (litter 1, probable heterozygote; litters 2 and 3, normal) and the levels attained in the recipients.

Recipients' liver, at 3 mo posttransplantation, contained 32–45% and, at 9 mo, 26–38% of donor activity of iduronidase. Cerebral cortical tissues of all recipients contained small, but consistently detectable amounts of iduronidase. Individual recipient brain tissues contained between 0.8 and 7.4% of the concurrent donors activity, with the average values at 3 and 9 mo posttransplantation being 2.8 and 1.0%, respectively. Iduronidase was present in relatively higher concentrations in CSF of the transplanted dogs than in their brain tissue. Between 6.3 and 14.9% of donor iduronidase was found in the recipient's samples; the average 3- and 9-mo posttransplantation activities were 10.8 and 7.2% of donor activities, respectively.

In each recipient a significant reduction in GAG storage was seen when compared with the concurrent level in the affected littermate control. Table II presents the GAG concentrations at

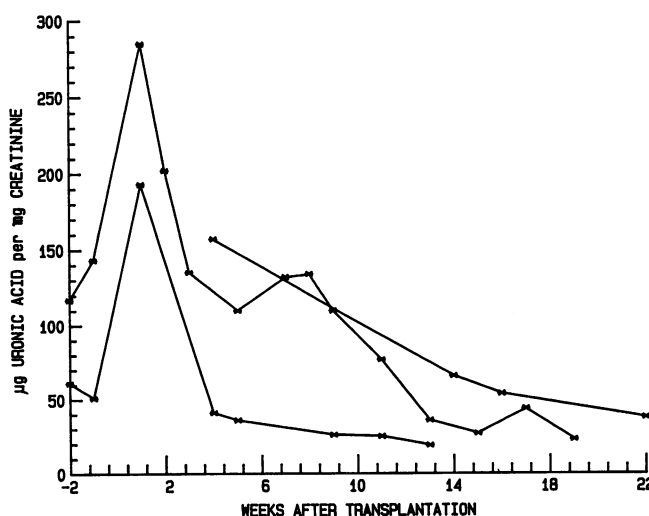


Figure 1. Polymeric urine GAG concentrations in three MPS I dogs given bone marrow transplants from nonaffected littermates. In the two dogs in which sampling began before transplantation, an initial increase in GAG excretion occurred. In all three dogs the excretion of GAG progressively decreased toward normal canine levels (10–30 µg/mg creatinine).

Table I. Canine Tissue Iduronidase Post-Bone Marrow Transplantation

Dog status	Liver		Brain		CSF	
	3 mo	9 mo	3 mo	9 mo	3 mo	9 mo
	<i>nmol/mg protein per h</i>					
MPS I, no transplant	0*	0	0	0	0	0
	0	0	0	0	0	0
	0	0	0	0	0	0
Mean	0	0	0	0	0	0
Marrow donors	1.66	1.58	0.27	0.43	0.49	0.47
	3.14	2.48	0.70	1.27	1.29	1.60
	3.08	2.79	1.12	1.26	3.24	2.23
Mean	2.63	2.28	0.70	0.98	1.67	1.43
MPS I with marrow transplant	0.68	0.41	0.02	0.01	0.02	0.07
	1.00	0.93	0.03	0.02	0.14	0.13
	1.10	1.01	0.02	0.01	0.39	0.14
Mean	0.93	0.78	0.02	0.01	0.18	0.11

\* Zero iduronidase corresponds to levels < 0.005 nmol/mg protein per h.

3 and 9 mo posttransplantation for each group of dogs. Consistent, marked reduction in hepatic GAG storage compared with the nontransplanted affected dogs was observed. At 3 and 9 mo posttransplantation, respectively, the mean GAG in recipient livers were 3.3% ( $P < 0.05$ ) and 3.7% ( $P < 0.05$ ) of the GAG content in the affected control dogs. Hepatic GAG concentrations in the transplanted dogs closely approximated those in the donors by 3 mo posttransplantation and remained stable in both groups for the next 6 mo. Histologic examination of recipients' liver tissues showed marked, diffuse reduction in vacuolation of hepatocytes, macrophages, and cells lining vessels and bile ducts, reflecting the decreased GAG storage found biochemically.

Reduction in CSF GAG levels indicates iduronidase activity in cells bathed by or producing CSF. At 3 and 9 mo, respectively, the mean recipient GAG levels in CSF were 39 and 51% of those in nontransplanted dogs. At each biopsy time the mean GAG level of transplanted dogs was only 1.7 times the donor mean, while comparable values of 4.2 and 3.7 existed for nontransplanted, affected dogs. Mononuclear cells from CSF of affected dogs had marked basophilic granulation of the cytoplasm due to lysosomal GAG accumulation. When stained with Giemsa or Alcian blue stain, these cells had an appearance very similar to mast cells. Corresponding cells in the CSF of transplanted dogs had only a few cytoplasmic granules, indicating significant reductions in lysosome number, size, and GAG storage.

Brain tissue in the recipients also had statistically significant reductions in GAG by 3 mo posttransplantation and remained nearly constant throughout the period of study in the donor animals and recipients (Table II). Compared with the nontransplanted MPS I dogs, the recipient brains had from 30 to 82% reduction in GAG levels. The mean brain GAG concentration in the recipients at 3 and 9 mo (0.13 and 0.15% LFDW) both represented statistically significant decreases ( $P < 0.05$ ) in GAG storage compared with untreated affected dogs and were close to levels in the donors. In one of the nontransplanted dogs the

Table II. Canine Tissue Glycosaminoglycans Post-Bone Marrow Transplantation

Dog status	Liver*		Brain*		CSF†	
	3 mo	9 mo	3 mo	9 mo	3 mo	9 mo
MPS I, no transplant	2.10	2.02	0.27	0.33	1.08	0.91
	1.63	1.76	0.36	0.86	0.87	0.71
	3.50	3.52	0.29	0.30	1.23	1.96
Mean	2.41	2.43	0.31	0.50	1.06	1.19
Marrow donors	0.05	0.05	0.11	0.10	0.19	0.17
	0.03	0.06	0.11	0.12	0.31	0.44
	0.02	0.06	0.13	0.10	0.24	0.45
Mean	0.03	0.06	0.11	0.11	0.25	0.35
MPS I with marrow transplant	0.11	0.09	0.19	0.17	0.46	0.39
	0.10	0.11	0.09	0.15	0.45	0.79
	0.05	0.08	0.12	0.14	0.34	0.65
Mean	0.09	0.09	0.13	0.15	0.42	0.61

\* Percentage of lipid-free dry weight.

† Micrograms uronic acid per milliliter.

GAG concentration in brain increased nearly threefold between 3 and 9 mo, but concurrent brain GAG in the transplanted MPS I dogs either remained stable or increased only slightly.

Ultrastructural studies of the brains of all three treated dogs at 3 and 9 mo posttransplantation showed changes consistent with GAG clearing or reduced storage of GAG within cells of the CNS. Most prominent among the changes within the recipients' CNS was the reduction in lysosomal storage in cells surrounding small blood vessels. The transplanted dogs' perithelial cells (Fig. 2) were markedly less vacuolated than cells around vessels in the nontransplanted dogs (Fig. 3), especially 9 mo posttransplantation. Neurons from nontransplanted MPS I dogs had mild to moderate increases in both lysosomal number and size (Fig. 4 and 5). Early evidence of zebra bodies, which as they progress are typical of advanced disease in humans and dogs, was visible. Neurons of affected, transplanted dogs (Figs. 6 and 7) appeared to have fewer lysosomal changes. Whereas recipient neurons were comparable in appearance to neurons in donor animals, there were mild increases in lysosomal size and number in some glial cells (Fig. 7). Marked distortion of lysosomes in CNS macrophages (Fig. 8), common in the affected control dogs, was not seen in any of the transplanted dogs.

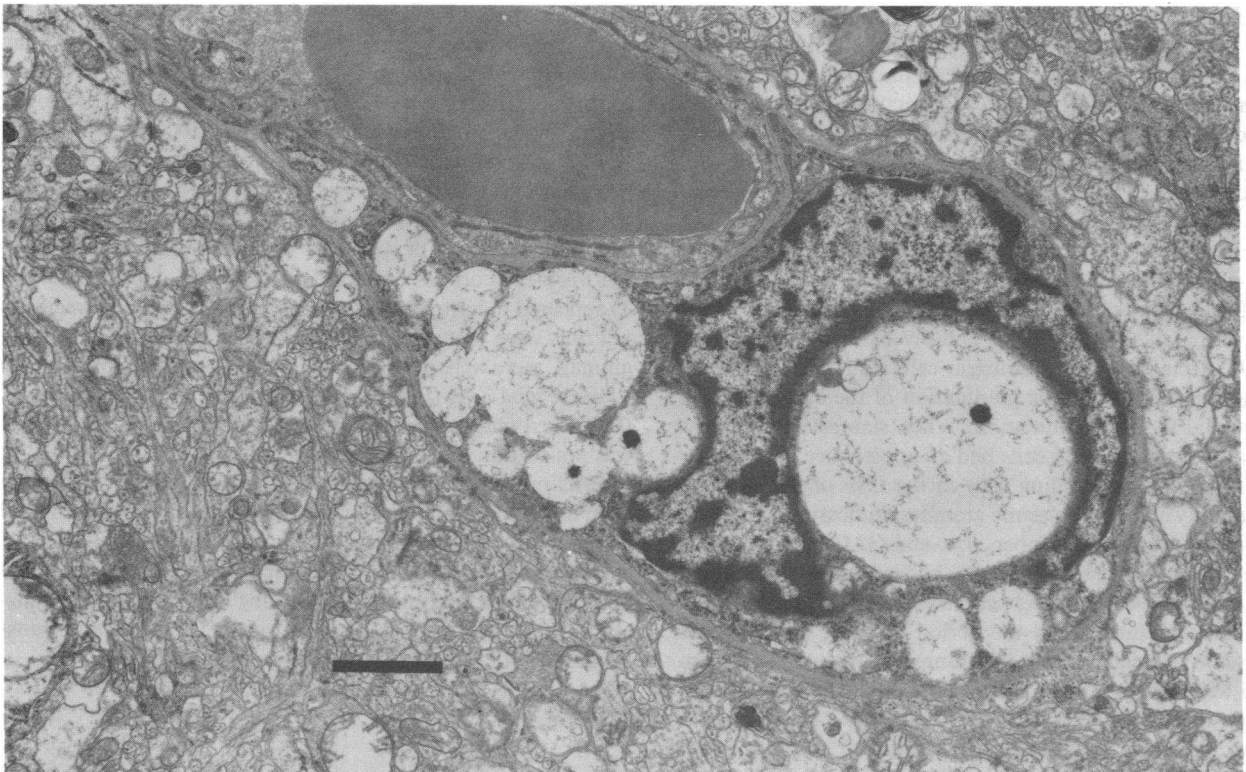
Preliminary morphometric analysis of neuronal lysosomes from one transplanted and one control dog supported the initial subjective contention that significant differences existed 9 mo after transplantation. The volume fraction ( $\pm$ SE) of lysosomes from the affected, nontransplanted dog ( $0.069 \pm 0.015$ ) was significantly greater ( $P < 0.05$ ) than that of its littermate, transplanted dog ( $0.027 \pm 0.003$ ).

## Discussion

Since the first attempt at treatment of Hurler syndrome in children by bone marrow transplantation, the concern whether intellectual improvement would be realized in this disease (or other

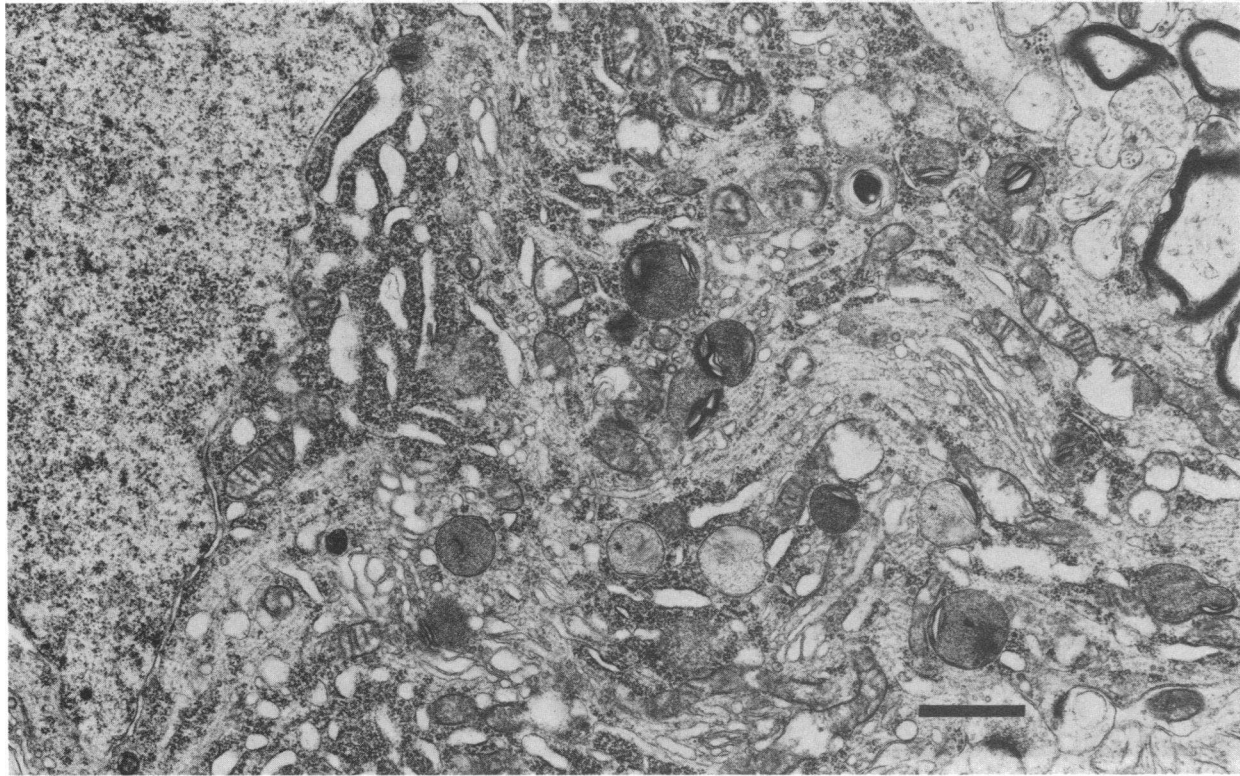


*Figure 2.* A normal-appearing perithelial cell and capillary containing an erythrocyte from the cerebral cortex of an MPS I dog 9 mo after receiving a bone marrow transplant from an unaffected littermate. The perithelial cell cytoplasm is devoid of enlarged lysosomes, indicating that storage of GAG is not present. The neuropil surrounding the capillary contains normal-appearing dendrites with mitochondria. Bar, 1  $\mu$ m.

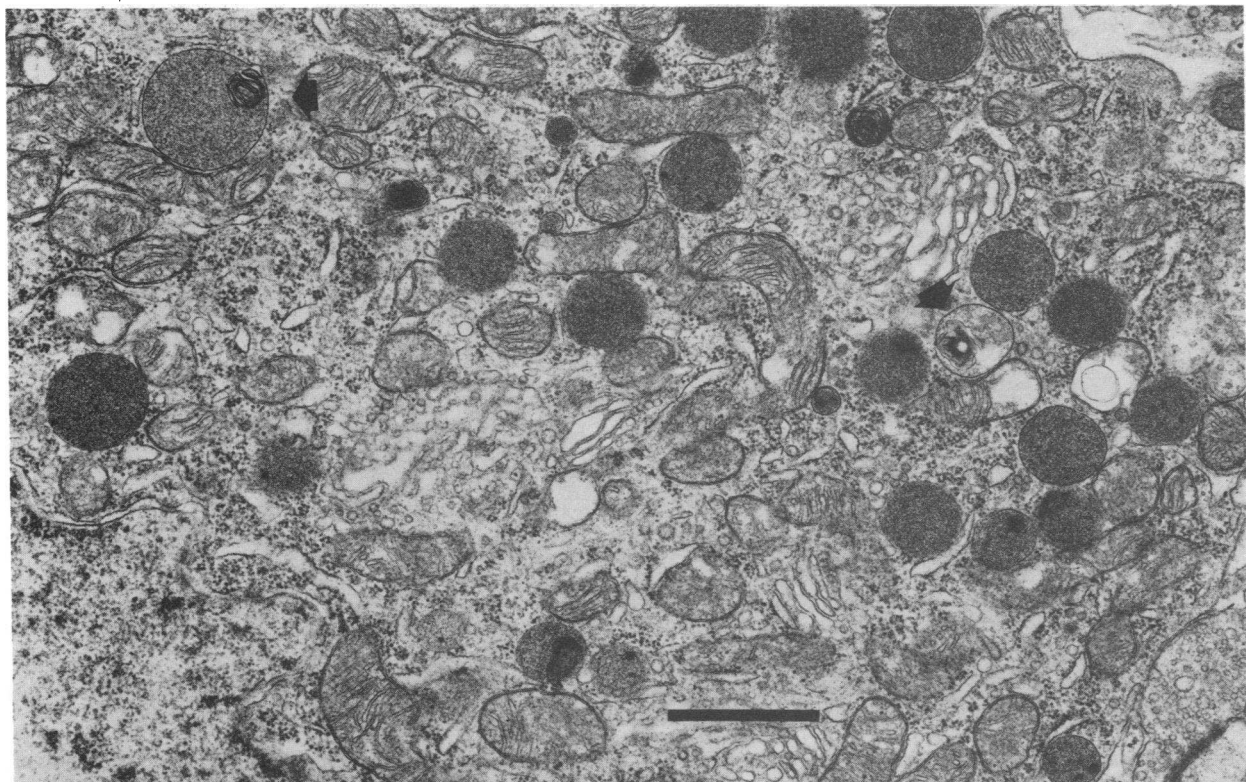


*Figure 3.* Perithelial cell with marked lysosomal distension from the cerebral cortex of an affected, nontransplanted MPS I dog (control to the dog in Fig. 1). Grossly dilated lysosomes containing granular material are indicative of marked storage of GAG. Bar, 1  $\mu$ m.

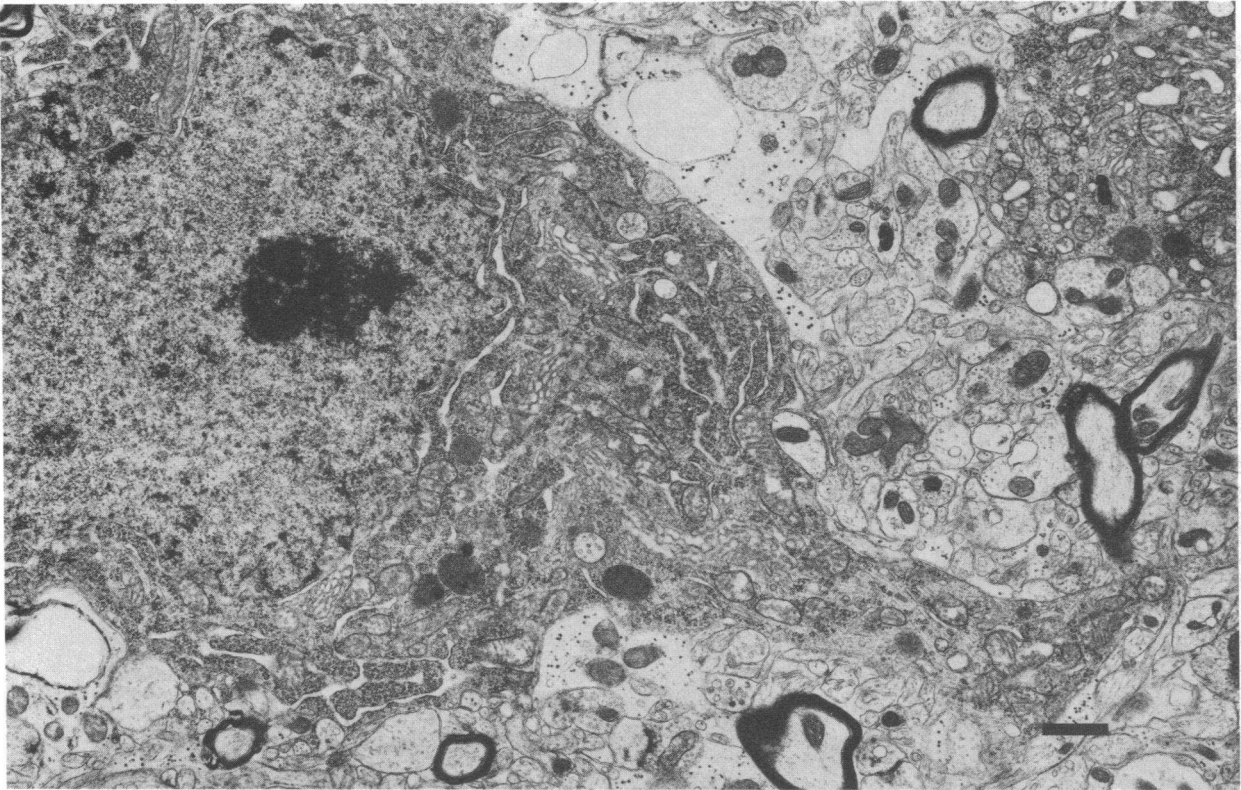




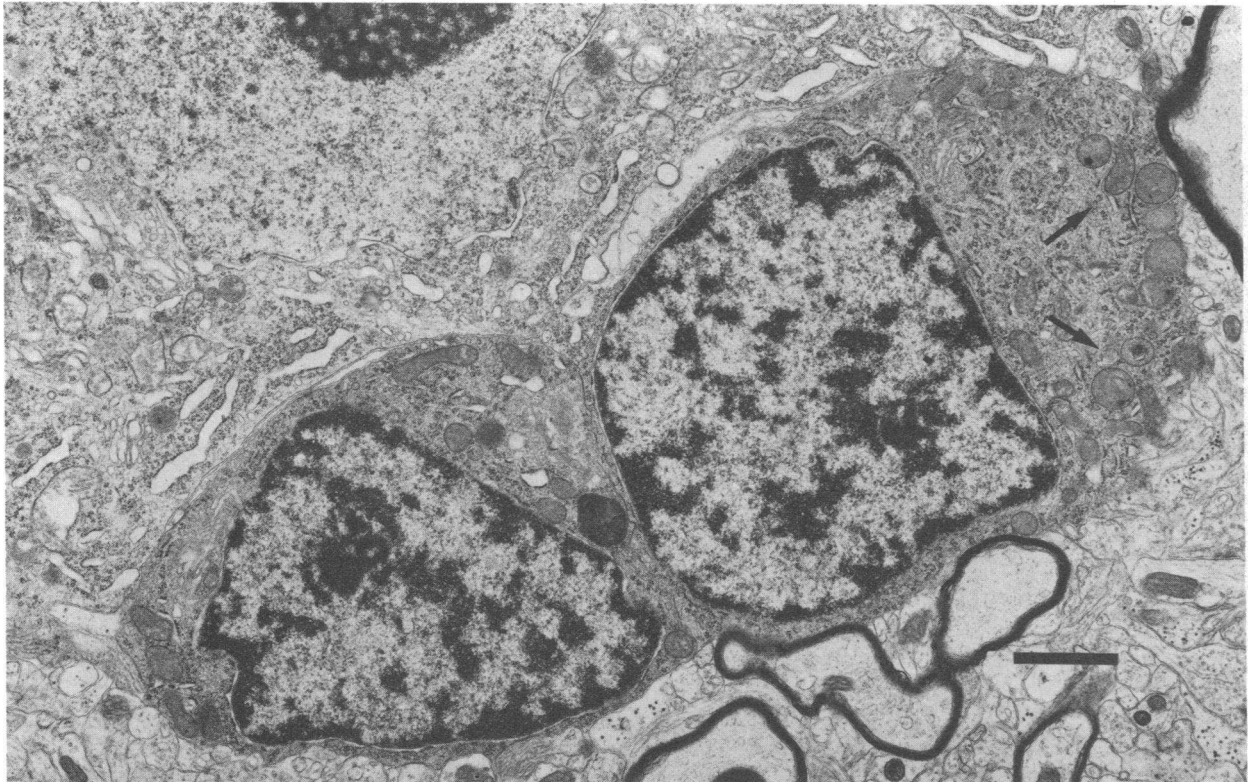
*Figure 4.* Cerebral neuron (nucleus on the left) from a nontransplanted, 14-mo-old MPS I dog. Early zebra body formation within multiple lysosomes, as well as increased number and size of lysosomes, is evident. The lysosomes contain variable amounts of granular storage material, which has not been lost during tissue processing. Bar, 1  $\mu$ m.



*Figure 5.* Cerebral neuron cytoplasm from another nontransplanted MPS I dog at 8 mo of age. The degree of lysosomal change is similar to that in Fig. 4 except that early zebra body formation is only present in two lysosomes (arrows). Bar, 1  $\mu$ m.

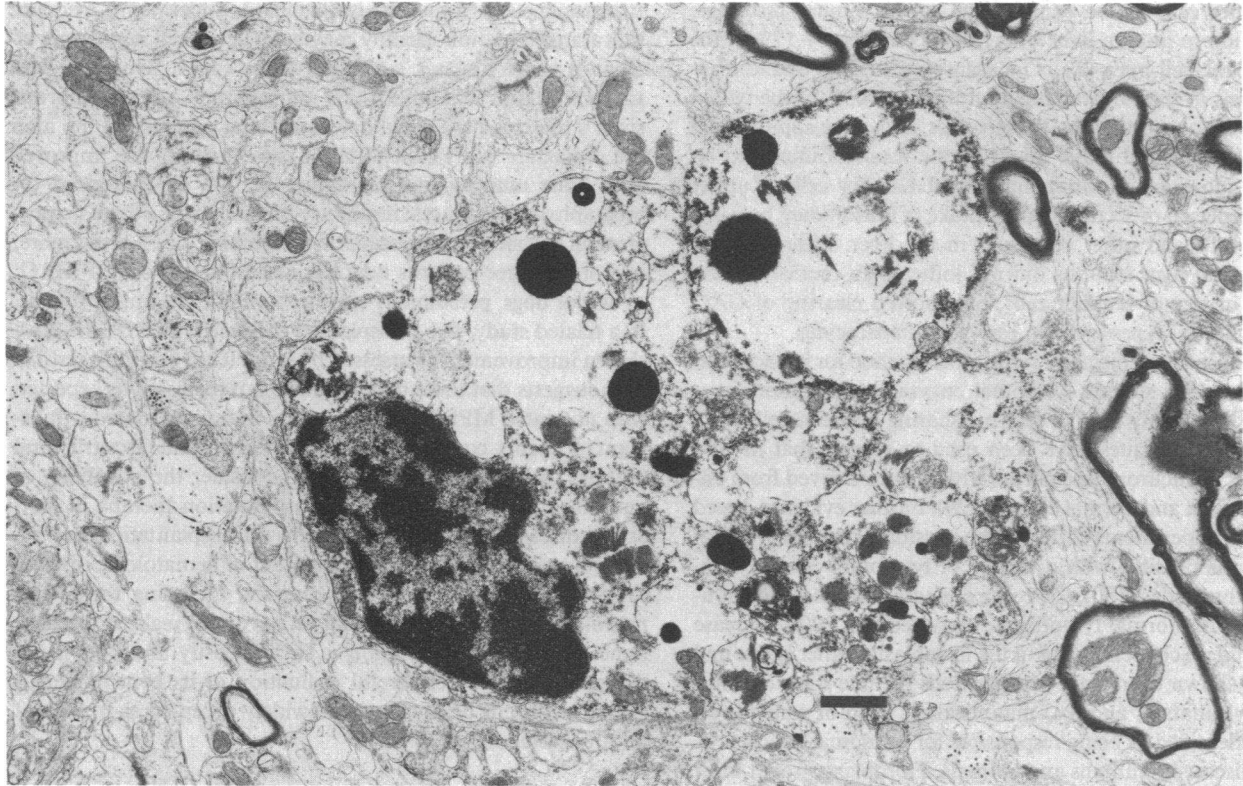


**Figure 6.** Cerebral neuron from an MPS I dog given a bone marrow transplant at 5 mo of age. The number and appearance of lysosomes is normal in this tissue biopsied 9 mo posttransplantation. Bar, 1  $\mu$ m.



**Figure 7.** Two neuroglial cells in close approximation to a neuron in the cerebral cortex of another transplanted MPS I dog. The glial cell on the right has more lysosomes (*arrows*) than the other neuroglial cell or the neuron, but none appears to be accumulating large amounts of

GAG as did similar cells in the nontransplanted dogs. The patterns of rough endoplasmic reticulum in the neuroglial cells suggests that the cell on the left is a microglial cell and on the right is an oligodendrocyte. Bar, 1  $\mu$ m.



**Figure 8.** Macrophage, perhaps of microglial cell origin, with marked lysosomal distension and disruption within the cerebral cortex of a nontransplanted MPS I dog. No such cells were found 9 mo after transplantation (14 mo of age) in any of the treated dogs. Bar, 1  $\mu$ m.

neurodegenerative disorders) has been repeatedly expressed (20–22). Whereas the canine model of MPS I has afforded the opportunity to examine biochemical and ultrastructural changes in the CNS after transplantation, unfortunately there is no way of assessing mental development in dogs. Nonetheless, findings in dogs may provide information relevant to the human situation regarding the ability of transplanted cells or enzyme to cross the blood–brain barrier and affect some degree of metabolic correction within the CNS.

Compared with donor levels, the activity of recipient brain iduronidase was quite small, although consistently detectable. The concentrations found in transplant recipients (0.8–7.4% of paired donor samples) are similar to the single report of 4% normal iduronidase in brain tissue of a child that died 15 mo after receiving a marrow transplant for MPS I (2). Decreased CNS GAG storage in the treated dogs indicates that the iduronidase activity detected is not entirely the result of blood cells within the biopsied tissue. The finding that even small amounts of enzyme can be beneficial is compatible with the theory of Conzelmann and Sandhoff (23) who proposed that small levels of residual enzyme activity are likely responsible for the marked neurologic differences between human MPS IH and MPS IS phenotypes.

Electron microscopic examination of cells in the CNS is important in identifying which cells are affected by the enzyme present. In untreated human and canine MPS I, accumulation of DS and HS occurs within neurons, glial cells, and cells surrounding blood vessels (12, 24, 25). While GAG storage can be identified in various CNS cells in MPS I, the exact defect leading to mental retardation is not known. That iduronidase deficiency

without CNS GAG accumulation (MPS IS) results in no loss of mental capabilities implies that disruption of neuronal function related to GAG accumulation may in some way be involved. The development of hydrocephalus and meningeal thickening may also be important factors involved with mental retardation. Therefore, therapy that reduces these nonneuronal conditions might be beneficial regardless of whether the effect on neuronal function is direct or indirect.

The concentration and composition of sulfated GAG in the brain and liver of normal dogs and humans are very similar (12, 26). In canine MPS I, glial and neuronal accumulation of GAG, with demonstrable ultrastructural changes of those cells' lysosomes, occurs after cells surrounding small and large blood vessels are markedly distended. The magnitude of white and gray matter GAG accumulation in the canine disease is similar to that in human MPS IH (15). Neuronal storage of GAG within increased numbers of lysosomes is demonstrable in the untreated MPS I dogs at 14 mo of age, however, the progression of storage and lesions in transplanted dogs' neurons was comparatively much less. In two recipients surviving 13 and 17 mo, posttransplantation neuronal changes are not evident, while the third recipient subjectively has very mild neuronal changes compared with its control littermate. Significant reductions in lysosomal storage of GAG in brain tissue, and the reduction in ultrastructural changes in perivascular cells, which are located within the brain parenchyma, thus suggest that enzyme from transplanted cells can lead to changes across the blood–brain barrier. The degree to which reduced storage of GAG in any location in the CNS would correlate with reduction in intellectual deterioration is unknown.



Kupffer cells in the canine liver have a similar histologic distribution to human liver where they comprise ~ 17% of the total hepatic cell mass (27). Our transplanted dogs had iduronidase levels in their livers ranging from 26 to 45% of the paired donor liver concentrations. These values, above the approximate normal Kupffer cell mass, might be the result of increased lysosomal iduronidase within individual Kupffer cells, Kupffer cell hyperplasia, or secretion and uptake of iduronidase between Kupffer cells and other cell types in the liver. Histologic examination of liver sections did not indicate an increase in the number or size of Kupffer cells, but showed clearing of GAG stores in all cell types present, especially hepatocytes.

Without immunologic or some other method for intracellular localization of a specific lysosomal enzyme, one cannot document with certainty in vivo enzyme sharing between cells. Histochemical techniques have been used to suggest that in vivo sharing of  $\beta$ -glucuronidase occurs within mice derived from enzyme-deficient and normal cells (28, 29). Further evidence, based on in vitro experiments supports the possibility that such a phenomenon is possible with a variety of lysosomal enzymes, including iduronidase (30). Co-culture of iduronidase-deficient fibroblasts with normal lymphocytes resulted in a 290% increase in iduronidase concentration in the fibroblasts. Other lysosomal enzymes shown to transfer between cells include  $\beta$ -hexosaminidase, arylsulfatase, and  $\alpha$ -D-mannosidase, each of which is an enzyme identified as being responsible for lysosomal deficiency-storage disease in humans and animals. Fibroblasts grown from patients deficient in  $\alpha$ -D-mannosidase have been shown to benefit from co-culture with normal lymphocytes, from which enzyme can be transferred and result in decreased oligosaccharide storage in the fibroblasts (31). Human hexosaminidase has been shown to cross the blood-brain barrier after osmotic modification in rats and to then localize in a subcellular organelle in the rat's brain (32).

Whereas it is generally accepted that bone marrow origin cells are responsible for continued replacement of macrophages in tissues such as lung and liver (33, 34), the origin and turnover of CNS phagocytes is less well understood. There is evidence that murine brain contains a resident population of phagocytic cells that are of bone marrow origin (35, 36). To what extent there is postembryologic renewal of cells within the CNS from the marrow is not well established. It has been shown, however, that circumstances such as injury or inflammation (possibly radiation) will cause blood-borne cells to enter the CNS in significant numbers (37). More relevant to the bone marrow transplantation situation are the findings of Ting et al. (38), who showed that murine radiation-induced chimeras have cells containing Ia antigens of donor origin within their brains. This study concluded the Ia<sup>+</sup> brain cells turnover and are replaced by cells of bone marrow origin. If similar human and canine cells exist, and are capable of entering the CNS of radiation induced bone marrow chimeras, the potential for some degree of CNS improvement exists in neurodegenerative disorders. Donor cells containing a deficient enzyme may cause decreased lysosomal storage in recipient cells either by transfer of enzyme or by creating a concentration gradient of the storage product, essentially causing transfer of substrate.

Despite failures to affect nervous system improvement after bone marrow transplantation in several storage diseases (metachromatic leukodystrophy [39], adrenoleukodystrophy [8], murine Krabbe's disease [10], murine Niemann-Pick disease [40]), as well as lack of CNS improvement in a chimeric calf with

mannosidosis (41), the case is not closed. Encouraging evidence that transplantation therapy may have beneficial neurologic effects in some diseases continues to be found. One boy with metachromatic leukodystrophy transplanted with his normal sister's marrow has shown developmental progress 33 mo after the procedure (7). Three MPS IH patients have also improved after bone marrow transplantation in that computerized tomography and magnetic resonance imaging have shown no progression of characteristic lesions in their brains (42). Furthermore, one of the three patients now has normal levels of CSF GAG, as do our dogs, presumably another sign of CNS improvement. In a related study, five transplanted children with MPS IH have shown improvement as assessed by serial Ruth Griffith's testing. This suggests that these children have not regressed as expected with untreated MPS IH (43). Findings in human marrow transplant recipients, coupled with the biochemical and ultrastructural findings in dogs, provide reasons to consider the possibility of neurologic improvement after transplantation therapy in metabolic disease. Whereas bone marrow transplantation has become much more routine for treatment of hematologic disease, the full potential in metabolic diseases, including those with neurologic involvement, may not yet have been realized. Because bone marrow transplantation is such a costly and potentially dangerous procedure, careful evaluation of its benefits versus risks must occur before it can be widely accepted as treatment for any metabolic disease.

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