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12. Simultaneous pulmonary AV concentration differences were determined for glucose and lactate taken before diving from animals with normal heart rates and cardiac output. The values in micromoles per milliliter for glucose and lactate (in parentheses) were −0.24 (+0.15), −0.28 (+0.08), +0.03 (+0.10), −0.16 (+0.40), 0.0 (0.0), 0.0 (0.0), and −0.16 (−0.10) for seven samples. The average AV difference for glucose was −0.1083 ± 0.1357 (standard deviation), and for lactate it was +0.0900 ± 0.1592. These values are not significantly different from zero (*t*-test, $P > .05$). During bradycardia and 5 minutes of recovery, the values for glucose and lactate (in parentheses) were +0.11 (−0.6), +0.26 (−0.15), +0.37 (0.0), +0.14 (−0.03), +0.08 (−0.11), +0.02 (−0.13), +0.01 (−0.08), +0.05 (−0.10), +0.06 (−0.10), and +0.03 (−0.32) for ten samples. The average AV difference for glucose under these conditions was +0.1130 ± 0.1166, and the average AV difference for lactate was −0.1630 ± 0.1756 ($P < .02$). Under these conditions, therefore, the Weddell seal lung seems to use lactate and produce glucose.
13. Cardiac output in awake seals was about 35 to 40 liter/min; during intense diving bradycardia it dropped to 4 to 8 liter/min. In the dive shown in Fig. 1, cardiac output 25 minutes into the dive was 5 liter/min. In addition, our microsphere experiments established quantitative flow to various tissues and organs before, during, and after diving.
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Mucopolysaccharidosis in a Cat with Arylsulfatase B Deficiency: A Model of Maroteaux-Lamy Syndrome

Abstract. *A Siamese cat that presented clinical signs similar to those seen in humans with mucopolysaccharidoses was studied. The animal excreted increased amounts of polymeric glycosaminoglycans in the urine, consisting almost entirely of dermatan sulfate. Electron microscopy of circulating polymorphonuclear leukocytes revealed the presence of many membrane-bound lamellar inclusion bodies. Sulfate incorporation studies with cultured skin fibroblasts indicated defective glycosaminoglycan degradation. These cells showed a deficiency in arylsulfatase B activity. The disorder appears similar or identical to the Maroteaux-Lamy syndrome described in humans.*

Increasing numbers of inborn errors of metabolism have been described in domestic animals in recent years, many of which have great potential as models of human diseases. Several lysosomal storage diseases, primarily lipid storage disorders, have been reported (*1*), and we have described the occurrence and clinical features of a mucopolysaccharidosis in a Siamese cat (*2*). We now report further evidence that this disease is a lysosomal storage disorder, that the mucopolysaccharide excreted in excess is dermatan sulfate, and that the defect is due to a deficiency in arylsulfatase B and represents an animal model of Maroteaux-Lamy syndrome.

The proposita was first seen at 21 months of age with an acute hindlimb lameness, small stature, a short broadened face with swollen drooping eyelids, corneal clouding, and a history of progressive difficulties with locomotion. Radiographic findings included fusion of cervical and lumbar vertebrae, flaring of the ribs, multiple exostoses, and epiphyseal dysplasia of the long bones. The cat excretes greatly increased amounts of cetylpyridinium chloride (CPC)-precipitable glycosaminoglycans (GAG) in her urine (*2*).

Measurement of the ratio of urinary polymeric GAG to oligosaccharides that contain uronic acid has been shown to be useful in detecting defects in mucopolysaccharide degradation (*3*). This ratio in normal human urine varies between 0.1 and 0.4 and is invariably less than 1. In pooled urine from eight clinically normal cats, the polymeric GAG: oligosaccharide ratio was 0.89 (1.16:1.30 mg of hexuronic acid per 25 ml of urine, respectively); and in the affected cat it was 5.89 (16.70 : 2.83), the absolute amount of polymeric GAG in the affected animal's urine being approximately 14 times greater than that in normal cat urine.

CPC-precipitable GAG's were isolated from the affected cat's urine and from pooled normal cat urine; Tamm-Horsfall glycoprotein was removed by salt precipitation, and the purified GAG was separated by electrophoresis on cellulose acetate (*4*). The primary GAG component from the affected cat migrated with dermatan sulfate (DS) with a small amount of chrondroitin sulfate (CS), and a trace of heparan sulfate (HS) also present, whereas normal cat urine contained primarily CS, with a small amount of DS and a trace of HS (Fig. 1a). Determination of the CS and DS content of similar urines by selective reaction with orcinol (*5*) confirmed the electrophoretic findings. Urine collected from eight clinical-

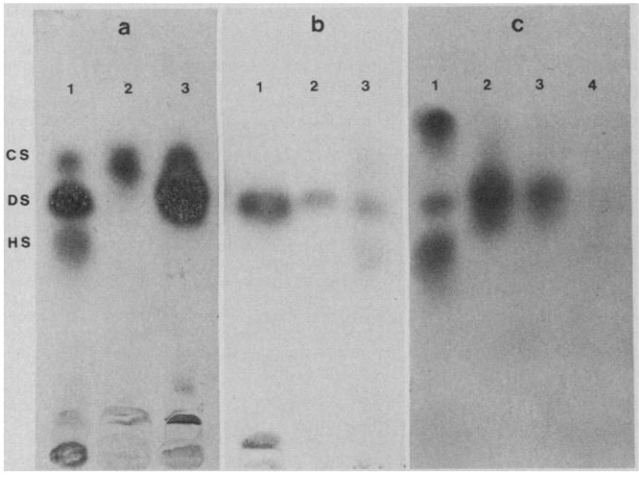


Fig. 1. Electrophoretic separation of urinary glycosaminoglycans (GAG). Separations were performed on strips (5 by 12.7 cm) of cellulose polyacetate (Sepraphore III, Gelman Instrument Co., Ann Arbor, Michigan) using 0.05*M* barium acetate, *p*H 8.0, at 100 volts for 180 minutes. The GAG were visualized with either toluidine blue or alcian blue GZ. (a) Total GAG from urine of a human patient with Hurler's syndrome (1), pooled cat urine (2), and urine from the affected cat (3). (b) The GAG fraction obtained by alkaline copper precipitation from the affected cat's urine (1), DS from pig skin (Sigma Chemical Co., St. Louis, Missouri) (2), total GAG from patient with Hurler's syndrome (3). (c) Mixture of commercially available GAG (1); from top to bottom chondroitin sulfates A and C (*CS*), dermatan sulfates (*DS*), and heparan sulfate (*HS*). Total GAG from the affected cat's urine (2), same, after treatment with chondroitinase AC (3), same, after treatment with chondroitinase ABC (4).

ly normal cats contained 67 μg of chondroitin sulfate and 10 μg of dermatan sulfate per milliliter, whereas urine from the affected animal had 86 μg of chondroitin sulfate and 990 μg of dermatan sulfate per milliliter.

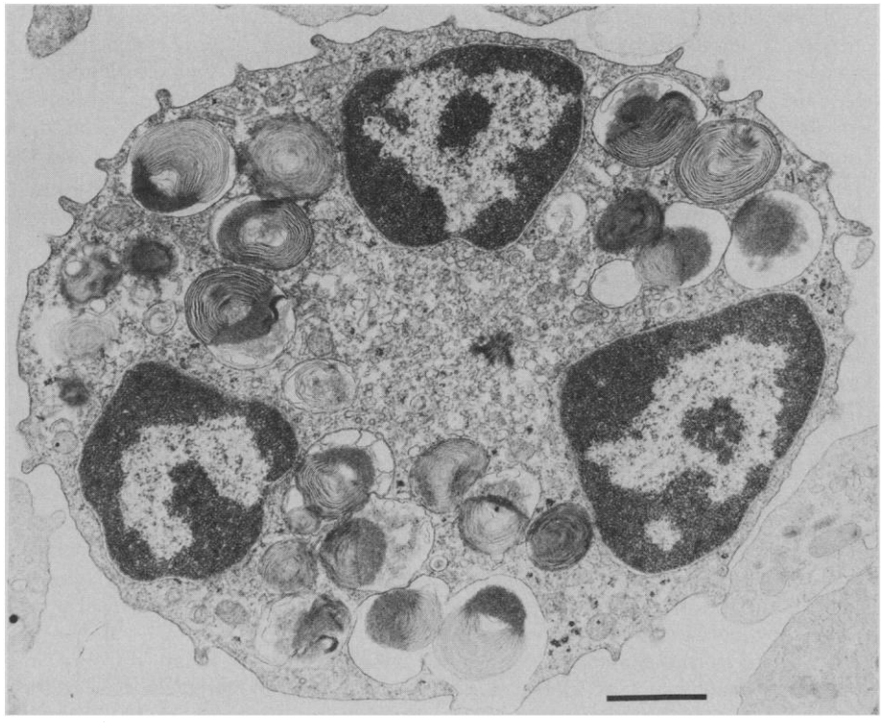
Additional confirmation of the identity of the excess GAG in the affected cat's urine as DS was obtained by other methods. Separation of the CPC-precipitable GAG by chromatography on Dowex-1 Cl^- columns (*6*) resulted in two major peaks being eluted with 1.5 and 2.0*M* NaCl, respectively. Electrophoresis of pooled fractions from this column showed the first major peak to contain primarily DS with some CS and the second peak to contain only DS. The presumed DS could also be isolated from the cat's urine by precipitation with Cu^{2+} (Fig. 1b) with alkaline Benedict's reagent (*7*). Furthermore, digestion of the GAG's with chondroitinase AC resulted in loss of only the CS component, whereas digestion with chondroitinase ABC (*8*) resulted in a loss of the component migrating as DS as well (Fig. 1c). The major unsaturated disaccharide derived from the chondroitinase ABC digestion also cochromatographed with 4,5-glucuronido-acetylgalactosamine-4-sulfate (*8*). These findings are consistent with the major component of the GAG's from the affected cat's urine being DS.

Staining of peripheral blood smears with toluidine blue had initially revealed coarse metachromatic granules in 94 percent of neutrophils and a few lymphocytes, and in large cells resembling histiocytes (*2*). A similar pattern is characteristic of Maroteaux-Lamy syndrome in humans (*9*). Electron microscopy of circulating white blood cells from the cat indicates that most polymorphonuclear leukocytes contain, in a section, 20 to 30 round to oval membrane-bound lamellar inclusions from 0.5 to 1.0 μm in diameter (Fig. 2). While these are similar in appearance to the myelin-like structures found (infrequently) in central nervous system neurons, liver, and lymphocytes of humans with Hurler's and Hunter's syndromes (*10*), we have not found any electron microscopic studies of white cells from Maroteaux-Lamy patients for direct comparison.

The incorporation of $^{35}SO_4$ into mucopolysaccharides of fibroblasts grown from skin biopsies of normal cats and the affected animal was studied (*11*). The intracellular accumulation of $^{35}SO_4$ in normal cat cells reached a maximum at 24 to 48 hours, while cells from the affected animal continued to accumulate $^{35}SO_4$ at 72 hours, when they contained three to five times as much $^{35}SO_4$ per milligram of cell protein as the control cells. The data from a representative experiment are shown in Fig. 3. The differences in incorporation of $^{35}SO_4$ between the normals and the affected cat seen in these experiments are similar to those between fibroblasts from normal humans and fibroblasts from patients with mucopolysaccharidoses, and are consistent with defective degradation of GAG (*11*).

Table 1. Lysosomal enzyme activities in cultured skin fibroblasts from the affected cat and from four normal cats. Enzyme activities are expressed as the mean ± S.D. with the observed range in parentheses (*11*). The number of different preparations studied for each enzyme is indicated.

Enzyme	Activities (nmole/hour per milligram of protein)					
	Affected			Normals		
	N	Mean ± S.D.	Range	*N*	Mean ± S.D.	Range
Arylsulfatase B	8	5.7 ± 6.3	0–17.8	13	52.7 ± 27.0	15.9–91.2
Arylsulfatase A	12	509.9 ± 164.3	270–731	20	268.7 ± 117.6	92–504
β-D-glucuronidase	12	854.1 ± 253.2	505–1378	17	705.3 ± 313.7	264–1191
α-D-mannosidase	12	471 ± 247.4	117–872	17	236.4 ± 149.8	56–538
β-D-acetylglucosaminidase	11	3885 ± 1385	1085–4941	20	1737 ± 1024	533–3784
β-D-galactosidase	10	316.3 ± 58.8	192–382	14	243.5 ± 72.1	162–364



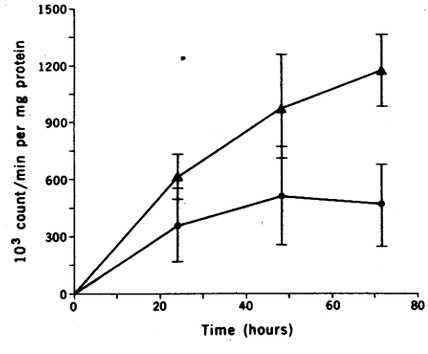


Fig. 2 (left). Electron micrograph of a polymorphonuclear leukocyte from the affected cat which contains many membrane-bound lamellar "myelin-like" inclusions. Lead citrate-uranyl acetate; scale line = 1 μm. Fig. 3 (right). Incorporation of $^{35}SO_4$ by fibroblasts from a normal cat (●—●) and from the affected cat (▲—▲) showing the mean ± 2 S.D. for each time point. Four cultures were used for each point. The cells were incubated in T_{25} flasks with F-12 medium containing 11 × 10^6 counts per minute per milliliter of $^{35}SO_4$, 2 percent fetal calf serum, and 50 μg of aureomycin per milliliter.

We further investigated the defect in degradation by determining the activity of six hydrolytic lysosomal enzymes in fibroblasts from the affected and normal cats (*12*) (Table 1). The activities of five of these enzymes from the affected cat's cells were significantly increased ($P < .02$). The arylsulfatase B activity, however, was significantly lower than that of the controls, averaging 10 percent of the activity observed in normal cells ($P < .01$). Initial determinations have shown that the following enzymes are active in the affected cat: α-L-iduronidase and heparan sulfatase, measured in cultured fibroblasts, and iduronate sulfatase, measured in serum (*13*).

The proposita is the offspring of a mother-son mating. Her pedigree suggests an autosomal recessive mode of inheritance. There were two offspring from a previous mating of the same parents with facial features similar to that of the proposita and these also reportedly experienced progressive difficulty with locomotion. One of these animals died at approximately 1 year of age of undetermined causes and we have been unable to locate the other. The parents, however, are both clinically normal and exhibit normal urinary GAG excretion.

The above results are essentially the same as those in human patients with Maroteaux-Lamy syndrome (*14*). The clinical features of the syndrome closely parallel those of the affected cat, while the patterns of excretion of urinary GAG and the deficiency in arylsulfatase B activity are identical. We therefore suggest that the cat represents a naturally occurring animal model of this syndrome. We are attempting to establish a breeding colony of affected animals for further study of the disease and to make this model available to other investigators.

Note added in proof: Since this manuscript was submitted, we have identified four additional individuals from two different families of Siamese cats with clinical features similar to and biochemical abnormalities identical with those of the affected cat described in this report.

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Postnatal Development of the Human Lateral Geniculate Nucleus: Relationship to a Critical Period for the Visual System

Abstract. *The cross-sectional areas of 31,800 dorsal lateral geniculate nucleus cells were measured in 53 human brains ranging in age from newborn to 40 years. Geniculate cells increase in size rapidly during the first 6 to 12 months of postnatal life, with cells in the parvocellular layers developing faster than cells in the magnocellular layers. At least 2 years are required before all cells have reached their adult size.*

It is generally accepted that there are periods during the development of an organism when it is particularly sensitive to outside influences (*1*). For instance, on the basis of changes in the ocular dominance of cortical cells and in overall visual capabilities, a sensitive or critical period in the development of the visual system has been defined for both the cat (*2*) and the monkey (*3*). In addition, clinical reports and psychophysical studies have suggested that a similar period exists during the development of the human visual system (*4*) sometime during the first 2 years of life; however, the period of susceptibility may continue to some extent until the child is 4 or 5 years old. Although an increasing amount of clinical and psychophysical information about the development of the human visual system is being made available, almost nothing is known about the postnatal growth of human central visual system structures, for example, the dorsal lateral geniculate nucleus (LGN). Such information could be important since other data suggest a close relationship between the time during which the visual system is growing and the time during which it is most susceptible to outside influences (*1*). If such a relationship does exist, it should be possible to define the critical period in the development of the human visual system by first defining the period during which growth occurs. I have found that there are two partially overlapping periods of postnatal cell growth in the human LGN. For cells in the parvocellular layers of the geniculate, there is a period of rapid growth that ends about 6 months after birth. However, cells in the magnocellular layers continue to grow rapidly until one full year after birth and do not reach adult size until the end of the second year. Such a time course of development resembles previous clinical and psychophysical estimates of the critical period in the human.

Brain tissue was obtained from 53 humans ranging in age at the time of their death from newborn to 40 years. All