G_{M1} gangliosidosis (Type 1) in a cat

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A kitten with clinical and morphological symptoms of a neurovisceral lysosomal-storage disease has been shown to have a marked deficiency of acidic β -D-galactosidase in the brain, kidney and spleen. Chromatography on concanavalin A–Sephacrose and inhibition studies with 2,5-dihydroxymethyl-3,4-dihydropyrrolidine, a selective inhibitor of the neutral broad-specificity β -D-galactosidase, have shown that the residual β -D-galactosidase at pH 4.0 in the tissues of the affected cat is due to the neutral β -D-galactosidase and that there is a complete deficiency of the acidic (lysosomal) β -D-galactosidase. There is marked accumulation in all tissues and excretion in the urine of neutral oligosaccharides. Analysis of these oligosaccharides by fast-atom-bombardment mass spectrometry and g.l.c. suggests that they arise from the incomplete catabolism of *N*-glycans of glycoproteins. The ganglioside content of all the tissues is elevated, and it has been shown by t.l.c. that the concentration of a ganglioside fraction with a mobility similar to that of G_{M1} ganglioside is particularly increased. There is also some evidence of accumulation of glycosaminoglycans in the brain. The clinical symptoms, the complete deficiency of acidic β -D-galactosidase and the storage products in visceral organs all suggest that this is a case of feline G_{M1}-type gangliosidosis comparable with the severe infantile (Type 1) form of the disease in humans.

INTRODUCTION

G_{M1} gangliosidosis is an inherited lysosomal neurovisceral storage disorder resulting from a deficiency of the enzyme acidic β -D-galactosidase (Okada & O'Brien, 1968). The enzymic defect leads to the accumulation of the ganglioside G_{M1} (Landing *et al.*, 1964) and water-soluble galactose-containing glycoconjugates (Suzuki *et al.*, 1969; Wolfe *et al.*, 1974). The disorder occurs in cats (Handa & Yamakada, 1971; Blakemore, 1972), cattle (Donnelly *et al.*, 1972) and dogs (Read *et al.*, 1976), as well as in humans. Several clinical variants of the human disease have been described which differ in time of onset of symptoms, involvement of visceral organs or skeletal tissue and in the degree of neuronal and mental deterioration (O'Brien, 1978). These multiple phenotypes have been related to different mutations leading to different properties of the enzyme (O'Brien, 1982), but further investigation is required to understand the nature of β -D-galactosidase deficiency at a molecular level.

Feline G_{M1} gangliosidosis has been reported both in Siamese cats (Handa & Yamakada, 1971; Baker *et al.*, 1976) and in domestic short-haired cats (Blakemore, 1972; Barnes *et al.*, 1981). The disease in Siamese and domestic short-haired kittens, which has been extensively investigated biochemically and clinically (Holmes & O'Brien, 1978*a, b*; Barnes *et al.*, 1981), has been likened to juvenile G_{M1} (Type 2) gangliosidosis in children, on the basis of morphology, clinical signs and biochemistry

(Holmes & O'Brien, 1978*a*; Dorling, 1984; Barnes *et al.*, 1981).

Here we have investigated the biochemistry of a domestic short-haired kitten with G_{M1} gangliosidosis. This animal, like the other reported cases in mixed-breed cats (Murray *et al.*, 1977), was apparently suffering from a more severe disorder than the Siamese cats, with visceral involvement, hepatomegaly, facial deformity and marked vacuolation of all neurons, hepatocytes, lymph nodes, endothelial cells and fibroblasts. Typical membranous lamellar bodies were observed in the vacuolated neurons by electron microscopy. These clinical signs (W. F. Blakemore & A. C. Palmer, unpublished work) together with the biochemical evidence presented here suggest a feline form of the disorder comparable with infantile G_{M1} (Type 1) gangliosidosis in children.

MATERIALS AND METHODS

Tissues

Samples of the brain, liver, kidney and spleen of a 5½-month-old male domestic kitten diagnosed biochemically and clinically as suffering from G_{M1} gangliosidosis were removed at post mortem and stored at –20 °C. The corresponding organs of an age- and sex-matched control kitten were also stored frozen. Homogenates (20%, w/v) were prepared in distilled water in a Potter–Elvehjem homogenizer and then centrifuged at 18000 rev./min

Abbreviations used: DMDP, 2,5-dihydroxymethyl-3,4-dihydropyrrolidine; f.a.b.m.s., fast-atom-bombardment mass spectrometry; Hex, hexose; HexNAc, *N*-acetylhexosamine.

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(37 000 g) in an MSE high-speed centrifuge for 30 min at 4 °C.

Neutral oligosaccharides were isolated from these supernatants by a modification of the procedure of Daniel *et al.* (1981). After precipitation of proteins with chilled trichloroacetic acid (10%, w/v, final concn.) the homogenate was centrifuged at 18 000 rev./min in an MSE high-speed centrifuge for 30 min at 4 °C. The supernatant was extracted with 5 vol. of diethyl ether to remove lipids and passed through a mixed-bed resin (Duolite MB6113; BDH Chemicals, Poole, Dorset, U.K.), which was eluted with water. The eluate was freeze-dried and redissolved in a smaller volume of water for analysis.

Urinary oligosaccharides

Urine samples from the control and affected kittens were stored at -20 °C until required. Samples of urine (1–5 ml) were passed through a column of mixed-bed resin (Duolite MB6113; BDH) to remove charged material before freeze-drying. The dried material was redissolved in deionized water for analysis.

Enzyme assays

Glycosidase activities were assayed by using the appropriate fluorogenic 4-methylumbelliferyl glycoside (Koch-Light) with a substrate concentration of 0.5 mM in the McIlvaine (1921) phosphate/citrate buffer system as described previously (Burditt *et al.*, 1980). The pH-dependence of β -D-galactosidase was also measured by using the McIlvaine phosphate/citrate buffer system over the pH range 3.7–7.0 and a Tris buffer system over the pH range 7.7–8.9, with a substrate concentration of 0.5 mM. In each case 1 unit of enzyme activity is defined as the amount that transforms 1 μ mol of substrate/min under the conditions specified.

Inhibition studies using DMDP

DMDP, which has been isolated from *Lonchocarpus sericeus* (Evans *et al.*, 1985), was kindly provided by Dr. L. Fellows (Jodrell Laboratory, Royal Botanical Gardens, Kew, Surrey, U.K.). It was used at a concentration of 1 mM.

Affinity chromatography on concanavalin A–Sephrose

This was carried out as described previously (Phillips *et al.*, 1976). Fractions (1 ml) were collected and the material binding to concanavalin A was eluted with 0.5 M-methyl α -D-mannopyranoside.

Protein determination

Protein was measured by the Bradford (1976) method, with bovine serum albumin as a standard.

Analysis of neutral oligosaccharides

1. Determination of total carbohydrate. The urine samples and tissue extracts were assayed for total reducing sugar by the phenol/H₂SO₄ method (Dubois *et al.*, 1956), with galactose as a standard over the range 0–100 μ g.

2. T.l.c. T.l.c. of neutral oligosaccharides in urine samples and tissue extracts was carried out on plastic-backed silica-gel plates (F1500/LS254, Schleicher

und Schüll G.m.b.H., Dassel, Germany) using propan-1-ol/acetic acid/water (3:3:2, by vol.) as the solvent. After chromatography the plates were dried and neutral oligosaccharides were detected with orcinol [0.2% (w/v) in H₂SO₄ (5%, w/v, in methanol)]. A hydrolysate of dextran was used as an aid to size estimation.

Preparative t.l.c. was carried out under the same conditions, except that the sample was applied in bands across the plate and the chromatogram was run twice in the same direction.

3. G.l.c. Samples (50 μ l) of deionized urine were analysed by g.l.c. as described previously (Burditt *et al.*, 1980; Abraham *et al.*, 1984).

4. Analysis of oligosaccharides by f.a.b.m.s. Neutral oligosaccharides were isolated from deionized extracts of the affected cat tissues by preparative t.l.c. for analysis by f.a.b.m.s. Permethylated and acetylated of the samples, operation of the f.a.b. mass spectrometer and recording of the spectra were all carried out as described previously (Abraham *et al.*, 1984).

Analysis of gangliosides

1. Extraction of gangliosides. Tissue (1 g) was homogenized in 7 ml of methanol. After addition of 14 ml of chloroform, homogenization was repeated. The homogenate was filtered and the residue extracted with 10 ml of chloroform/methanol (2:1, v/v). The residue was further extracted with 5 ml of chloroform/methanol (1:2, v/v). The combined extracts were adjusted to a chloroform/methanol ratio of 2:1 (v/v). This combined organic extract containing total lipid was partitioned with 5.5 ml of 0.1 M-KCl (Folch, 1957). The mixture was shaken vigorously and left at 4 °C overnight. The upper aqueous phase containing gangliosides was removed and washed three times with a theoretical lower phase made by shaking 1 vol of chloroform/methanol (2:1, v/v) with 0.2 vol. of 0.1 M-KCl. The ganglioside-containing aqueous phase was freeze-dried and redissolved in 10 ml of chloroform/methanol/water (10:5:1, by vol.). After filtering to remove insoluble proteins, it was concentrated by evaporation.

2. Sialic acid determination. Total sialic acid in the ganglioside-containing fraction was determined by the thiobarbiturate method (Aminoff, 1961).

3. T.l.c. of gangliosides. The ganglioside-containing fractions were analysed by t.l.c. on plates identical with those used for analysis of neutral oligosaccharides. The solvent used was chloroform/methanol/water (60:3:8, by vol.) and the gangliosides were detected by spraying with resorcinol reagent [9.3 M-HCl containing resorcinol (0.2%) and 0.25 mM-CuSO₄] and heating for 15 min at 120 °C.

Determination of glycosaminoglycans. Glycosaminoglycans in aqueous tissue extracts were determined by the cetylpyridinium chloride turbidity test of Pennock (1976), with keratan sulphate (Sigma) as a standard. Values are given as units per g of tissue, where 1 C.P.C. unit is equivalent to the absorbance given by a solution containing 10 mg of keratan sulphate/litre.

Table 1. Glycosidase activities in the tissues of the diseased kitten and control

Enzymic activities were measured in supernatants from tissue homogenates (20%, w/v) at pH 4, except for β -D-glucosidase and α -L-fucosidase at pH 5.5, by using the appropriate 4-methylumbelliferyl fluorogenic substrate.

Tissue	Substrate...	Glycosidase activity (munits/mg of protein)									
		β -D-Glc		α -D-Glc		β -D-Gal		α -L-Fuc		β -N-Acetyl-D-Gal	β -N-Acetyl-D-Glc
		pH 4	pH 5								
Liver	Control	13.21	17.55	2.16	1.43	0.11	5.01	5.60	17.86	1.33	1.04
	Affected	1.40	5.70	6.20	4.80	0.01	28.10	18.10	64.20	1.70	0.47
Brain	Control	0.10	0.12	0.60	0.34	0.52	2.03	2.48	7.0	0.53	0.16
	Affected	0.60	0.40	1.60	3.30	0.04	3.20	6.10	14.5	3.10	0.10
Kidney	Control	21.75	22.0	3.12	3.70	0.53	11.54	33.62	111.2	1.19	4.35
	Affected	9.48	59.5	3.28	6.16	0.14	19.41	29.70	115.8	1.25	4.47
Spleen	Control	0.34	0.15	0.58	0.74	0.80	3.27	6.88	44.97	1.09	0.17
	Affected	0.39	0.12	2.23	5.60	0.01	10.70	26.70	77.80	4.20	0.15

RESULTS

Lysosomal-enzyme activities

The enzymic activities of eleven different glycosidases were measured in the liver, brain, kidney and spleen of the affected cat and in the corresponding tissues of a control cat (Table 1). There was a marked deficiency of β -D-galactosidase in all tissues of the affected cat. The proportions of residual acidic β -D-galactosidase were 1–10% in the liver, brain and spleen and 26% in the kidney. The activities of the other glycosidases were in general elevated up to 8-fold in the affected cat. However, β -D-mannosidase and α -L-arabinosidase were unaltered or lower by about 50% in the affected cat, and the β -D-glucosidase activity was elevated in some tissues and markedly decreased in others. A non-lysosomal β -D-galactosidase that is active towards both 4-methylumbelliferyl β -D-galactoside and 4-methylumbelliferyl β -D-glucoside substrates over a broad pH range is present in cat liver (Holmes & O'Brien, 1978a). As this activity may account for all or a part of the residual β -D-galactosidase activity and contribute to the β -D-glucosidase activity in affected cat tissues, further experiments were carried out to clarify the deficiency of acidic β -D-galactosidase.

pH-dependence of β -D-galactosidase in normal and affected cat liver

The activity-pH profile for β -D-galactosidase in liver of the normal cat (Fig. 1) has a pH optimum at pH 7.7 and a marked shoulder at pH 4.5–5.5. This profile indicates the presence of more than one enzymic component, i.e. an acidic form and a neutral form. In the affected cat liver the shoulder in the activity-pH profile is far less pronounced than in the normal cat liver, suggesting a deficiency of the acidic form of β -D-galactosidase. The level of the neutral β -D-galactosidase was also lower in the affected cat liver than in the control liver, but this was not the case in other affected tissues. In particular the neutral activity in the affected kidney was 2-fold higher than in the control kidney.

Fig. 1. pH-dependence of β -D-galactosidase in livers of normal and affected cats

●, Control; ○, affected cat.

Fig. 2. Chromatography on concanavalin A-Sepharose of β -D-galactosidase in livers of normal (a) and affected (b) cats

β -D-Galactosidase was assayed at pH 5 (Δ) and at pH 6 ($\blacktriangle$). The arrow indicates commencement of elution with 0.5 M-methyl α -D-mannopyranoside.

Chromatography on concanavalin A-Sepharose of cat liver β -D-galactosidase

Holmes & O'Brien (1978a) had shown that cat liver lysosomal acidic β -D-galactosidase binds to concanavalin A, whereas the broad-specificity β -D-galactosidase does not bind. Therefore extracts of the normal and affected cat livers were analysed by chromatography on concanavalin A-Sepharose to investigate whether the residual β -D-galactosidase activity in the affected cat liver was due to the acidic lysosomal form of the enzyme or to the neutral non-lysosomal form. Fig. 2(a) shows the elution profile for control cat liver extract. The acidic β -D-galactosidase activity assayed at pH 4 was associated with a discrete bound peak (II), whereas the neutral β -D-galactosidase activity appeared as a major unbound peak (I) with a little activity at pH 4.0. Acidic lysosomal β -D-galactosidase activity was totally absent from the

affected cat liver (Fig. 2b), and the neutral β -D-galactosidase activity was present as an unbound peak (I) in amounts comparable with those present in the normal cat liver. The activity-pH profile for the control acidic β -D-galactosidase that bound to the concanavalin A had a pH optimum of 4 and covered the pH range 3.7 (10% maximum activity) to 6.5 (10% maximum activity). This corresponds to the pH range over which there is a marked decrease of activity in an extract of the affected liver. A deficiency of concanavalin A-binding acidic β -D-galactosidase was found in the other tissues of the affected cat. These observations suggest that the acidic β -D-galactosidase is absent from the tissues of the affected cat and that the neutral β -D-galactosidase accounts for the residual activity at pH 4.0 in these tissues.

Inhibition by DMDP of different β -D-galactosidases

DMDP, a β -D-glucosidase inhibitor, inhibits the broad-specificity β -D-galactosidase/ β -D-glucosidase activity, but not the true lysosomal β -D-galactosidase in mammalian tissues (Cenci di Bello *et al.*, 1985). Therefore it was used to investigate the nature of the residual acidic β -D-galactosidase in the tissues of the affected cat. The neutral β -D-galactosidase that did not bind to concanavalin A (peak I) from both the normal and the affected cat livers had greater β -D-glucosidase than β -D-galactosidase activity and was inhibited by 60–65% by DMDP. In contrast, the unbound true acidic β -D-galactosidase in the normal liver (peak II) had no β -D-glucosidase activity and was uninhibited by DMDP. The residual acidic β -D-galactosidase in the affected cat liver was inhibited by 50% by DMDP at pH 4, whereas the acidic β -D-galactosidase in the normal liver was only inhibited by 16%. This is consistent with the residual β -D-galactosidase activity in the affected liver being attributable predominantly to the neutral β -D-galactosidase/ β -D-glucosidase and the activity at pH 4.0 in the normal liver being a mixture of the two types of β -D-galactosidase.

Thus chromatography on concanavalin A-Sepharose and inhibition by DMDP suggest that the residual acidic β -D-galactosidase in the tissues of the affected cat is due to the broad-specificity β -D-galactosidase. As this activity is structurally and genetically distinct from the lysosomal β -D-galactosidase, its concentration will vary independently. This explains the different proportions of residual β -D-galactosidase at pH 4.0 in the different tissues of the affected cat, e.g. the apparently high residual activity in the kidney and the levels of β -D-glucosidase in these tissues. The 2-fold reduction in α -L-arabinosidase activity in the affected cat can be accounted for by a similar reduction of the broad-specificity β -D-galactosidase, which also possesses α -L-arabinosidase activity. This decrease probably reflects normal variation among cats.

It is concluded that there is a complete deficiency of lysosomal β -D-galactosidase in the affected cat. The secondary hyperactivity of the other lysosomal glycosidases is characteristic of a lysosomal-storage disease.

Analysis of storage products

(a) **Carbohydrate content in affected and control tissues.** The total carbohydrate content of crude aqueous extracts (20%, w/v) of normal and affected tissues was measured by the phenol/ H_2SO_4 method (Table 2). The affected brain, kidney and spleen all had a markedly elevated carbohydrate content, expressed as equivalent weight of galactose as determined from a standard curve for

Table 2. Storage products in G_{M1}-gangliosidosis cat and control cat tissues and urine

The amounts of carbohydrate are expressed in equivalent weights of galactose as determined from a standard curve for galactose.

Tissue	Carbohydrate content (mg/g wet wt.)	Sialic acid (μg/g wet wt.)	Glycosaminoglycan (C.P.C. units/g wet wt.)
Liver			
Control	11.2	16	375
Affected	13.3	40	440
Brain			
Control	0.65	300	80
Affected	2.2	504	197
Kidney			
Control	2.2	6	35
Affected	3.7	12	37
Spleen			
Control	3.2	7	170
Affected	6.8	13	74
Urine (mg/ml)			
Control	1.5	—	—
Affected	8.1	—	—

galactose. The value for affected liver was not as greatly elevated as in other tissues; however, the observed hepatomegaly in the affected cat meant that there was a major overall increase in hepatic carbohydrate content. These data indicate an accumulation of carbohydrate as a result of the β -D-galactosidase deficiency.

(b) T.l.c. of neutral-oligosaccharide storage products.

T.l.c. of neutral oligosaccharides showed the presence of at least four such storage products in all four affected tissues and urine, but not in the controls (Fig. 3). The four main components, (1), (2), (3) and (4) in order of increasing size, were present in all affected tissues studied and in the urine. Densitometric scanning of the t.l.c. plate showed that these four components were present in the approximate proportions 1:6:2:2. Component (1) had a mobility on t.l.c. similar to that of a hexasaccharide in the dextran hydrolysate, and components (2), (3) and (4) had mobilities similar to those of oligosaccharides of 9, 11 and 13 hexose units respectively. Smaller amounts of larger oligosaccharides were also clearly present in the urine and tissues of the affected cat.

(c) G.l.c. analysis of oligosaccharide storage products.

Neutral carbohydrate from whole urine of the affected cat was derivatized by methanolysis, re-*N*-acetylation and trimethylsilylation and analysed by g.l.c. Galactose, mannose and *N*-acetylglucosamine were identified as the constituent sugars by comparison with authentic standards. No other carbohydrate-containing peaks were detected in the g.l.c. elution profile. It was concluded that the neutral oligosaccharides detected by t.l.c. contained only galactose, mannose and *N*-acetylglucosamine.

(d) Analysis of neutral-oligosaccharide storage products by f.a.b.m.s.

The four main neutral oligosaccharides in the urine of the affected cat were prepared by preparative t.l.c. Samples (1) and (2) were peracetylated and produced good f.a.b.m.s. spectra. Sample (1) yielded a molecular ion at *m/z* 1828 corresponding to the composition Hex₃(HexNAc)₃ (*M_r* 1113). In addition, a minor signal was present corresponding to

Fig. 3. T.l.c. of neutral oligosaccharides in tissues and urines of normal and affected cats

T.l.c. of samples from urines and tissues and standards were carried out on silica gel with propan-1-ol/acetic acid/water (3:3:2, by vol.) as the solvent. Oligosaccharides were detected with orcinol/H₂SO₄. Across: 1 and 12, dextran hydrolysate; 2, affected cat brain; 3, control cat brain; 4, affected cat spleen; 5, control cat spleen; 6, affected cat kidney; 7, control cat kidney; 8, affected cat liver; 9, control cat liver; 10, affected cat urine; 11, control cat urine.

Fig. 4. Proposed structures for oligosaccharide storage products in feline- G_{M1} -gangliosidosis urine

Hex₄(HexNAc)₃. Sample (2) yielded data consistent with the composition Hex₅(HexNAc)₄ (M_r 1640). All four samples were also permethylated and the molecular ions obtained for samples (1) and (2) confirmed the peracetylation data. The molecular ions obtained for samples (3) and (4) corresponded to the compositions Hex₆(HexNAc)₅ (M_r 2005) and Hex₇(HexNAc)₆ (M_r 2370) respectively.

These results confirm that fucose and sialic acids are absent from these compounds and suggest that chitobiose is probably present at the reducing terminal of the four main components. On the basis of results of f.a.b.m.s. analysis, composition analysis by g.l.c. and on the results of other studies on canine (Warner & O'Brien, 1982) and feline G_{M1} gangliosidosis (Holmes & O'Brien, 1978b) the structures shown in Fig. 4 are proposed for the main oligosaccharide storage products (1), (2) (3) and (4) and the minor components associated with product (1).

(e) **Determination of gangliosides and glycosaminoglycan in affected and control tissues.** Total sialic acid determination was carried out on the ganglioside-containing fractions of affected and control cat tissues after the Folch partition method of extraction (Table 2). The total sialic acid content was elevated by 70–150% in the affected tissues, and t.l.c. of normal and affected brain and liver samples demonstrated an increased proportion of one ganglioside compared with the controls. This

component had a mobility corresponding to G_{M1} ganglioside. These data are consistent with an accumulation of G_{M1} ganglioside in the affected animal.

There was more glycosaminoglycan in the brain (+140%), liver and kidney (+6%) of the affected cat than in the corresponding control tissues, but less in the spleen. These results indicate that there is accumulation of glycosaminoglycans, probably keratan sulphate, owing to the deficiency of acidic β -D-galactosidase, in some of the tissues of the affected cat.

DISCUSSION

The biochemical characteristics of human G_{M1} gangliosidosis are a deficiency of acidic β -D-galactosidase and the accumulation of G_{M1} ganglioside and galactose-containing oligosaccharides in the tissues. The acidic β -D-galactosidase deficiency and the accumulation of such storage products in the kitten under investigation, together with the clinical symptoms, justify diagnosis of feline G_{M1} gangliosidosis.

The enzymic defect affects the lysosomal acidic β -D-galactosidase, but not the neutral β -D-galactosidase. Concanavalin A-Sepharose chromatography and inhibition by DMDP have shown that the residual β -D-galactosidase activity in the affected cat was in fact attributable to the neutral form of the enzyme and that there is a striking deficiency of acidic β -D-galactosidase

in the affected cat. In contrast with this case, approx. 10% residual acidic β -D-galactosidase activity, which was structurally and antigenically different from the normal enzyme, was found in the Siamese cats with G_{M1} gangliosidosis (Holmes & O'Brien, 1978a). Therefore there appears to be a more severe enzymic deficiency in the propositus than in the Siamese cats. The postulated structures of the oligosaccharide storage products found in the affected animal are consistent with the incomplete catabolism of glycoproteins as a result of a deficiency of β -D-galactosidase. The structures proposed for compounds (1) and (2) have the same composition as the storage products in feline G_{M1} gangliosidosis in Siamese kittens (Holmes & O'Brien, 1978b) and are identical with those proposed for the storage products in canine G_{M1} gangliosidosis (Warner & O'Brien, 1982). The structures are also similar to compounds isolated from human-GM₁-gangliosidosis liver and urine, except that the latter terminate in a single *N*-acetylglucosamine residue at the reducing end. This difference between storage products in feline and human G_{M1} gangliosidosis may occur because the endoglucosaminidase enzyme present in humans is not active in cats. In humans this enzyme hydrolyses the core chitobiosidic linkage, resulting in an oligosaccharide with a single *N*-acetylglucosamine residue at its reducing terminus and a glycopeptide containing an *N*-acetylglucosamine-asparagine unit. The glycopeptide is hydrolysed by an aspartamidohydrolase and the oligosaccharide chain is degraded stepwise by exoglycosidases. Thus, in the cat, the action of the aspartamidohydrolase would give rise to an oligosaccharide with two *N*-acetylglucosamine residues at the reducing terminus. This scheme has been proposed previously for cats (Burditt *et al.*, 1980) and for dogs (Warner & O'Brien, 1982; Abraham *et al.*, 1984). However endo- β -D-*N*-acetylglucosaminidase activity has been shown to be present in pig and rat (Nishigaki *et al.*, 1974; Pierce *et al.*, 1980) and humans (Overdijk *et al.*, 1981), although not perhaps in lysosomes (Pierce *et al.*, 1979), thus indicating that there may be significant differences in glycoconjugate catabolism between closely related mammalian species.

The increased storage in liver of a ganglioside, with a similar mobility to G_{M1} ganglioside, and the elevated sialic acid content of the ganglioside fraction of all the tissues of the affected cat are also indicative of G_{M1} gangliosidosis. The presence of lipid storage products in the viscera suggests that feline G_{M1} gangliosidosis more closely resembles the infantile (Type I) form of human G_{M1} gangliosidosis than the juvenile (Type II) form, in which there is little visceral storage of lipids. However the increase in liver G_{M1} ganglioside in the affected cat is not as high as that observed in the human infantile form of the disease. However, an unspecified increase in hepatic G_{M1} ganglioside was also found in two domestic short-haired kittens diagnosed with G_{M1} gangliosidosis Type II. The presence of larger oligosaccharides, as has been found in Type I but not in Type II human G_{M1} gangliosidosis, (Yamashita *et al.*, 1981) also suggests that the cat disorder studied here more closely resembles the human infantile disease. Although there appears to be an increase in glycosaminoglycan in some of the tissues, particularly the brain, it is not clear whether the deficiency of acidic β -D-galactosidase leads to extensive accumulation of keratan sulphate-derived material.

The clinical, morphological and biochemical features of the acidic β -D-galactosidase deficiency in this cat are

more similar to those associated with infantile G_{M1} gangliosidosis (Type I) than juvenile (Type II) in humans, although not identical. As the previously well studied form of G_{M1} gangliosidosis in Siamese cats was analogous to the juvenile form of the disease, the present case is a valuable addition to the list of animal models of lysosomal-storage diseases.

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