The catabolism of mammalian glycoproteins

Comparison of the storage products in bovine, feline and human mannosidosis

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Analysis of the neutral urinary oligosaccharides in bovine, feline and human mannosidosis by thin-layer and gel-permeation chromatography has shown that the patterns of stored oligosaccharides in the three species are different. In bovine and feline mannosidosis the most abundant urinary oligosaccharide is also the most abundant in the tissues of each species. The predominant oligosaccharides were purified by a combination of gel-filtration, ion-exchange and thin-layer chromatography and shown to contain only mannose and *N*-acetylglucosamine by g.l.c. and g.l.c.–mass spectrometry. The probable composition and size of each oligosaccharide were predicted from its chromatographic properties, sugar composition and the known structure of asparagine-linked oligosaccharides. The bovine and feline oligosaccharides belonged to a homologous series of general composition $\text{Man}_n(\text{GlcNAc})_2$, whereas the human oligosaccharides belong to a different series, $\text{Man}_n\text{GlcNAc}$. These structures suggest that lysosomal endohexosaminidase is not present in bovine and feline tissues. The predominant feline storage product, $\text{Man}_3(\text{GlcNAc})_2$, was the expected storage product from the catabolism of complex asparagine-linked glycans. In contrast, the predominant bovine oligosaccharide, $\text{Man}_2(\text{GlcNAc})_2$, probably lacks one of the α -linked mannose residues in the core region. A similar situation occurs in human mannosidosis. It is predicted that in these species either that the residual mutant α -D-mannosidase retains activity towards one of the core α -linked mannose residues or that another form of lysosomal α -D-mannosidase that is unaffected in these disorders occurs. It is concluded that the differences in storage products are due to differences in the catabolic pathways of glycoproteins among the species.

Mannosidosis is the lysosomal storage disease that results from a genetic deficiency of lysosomal α -D-mannosidase (EC 3.2.1.24). It is characterized by excretion in the urine and accumulation in the tissues of mannose-rich oligosaccharides. It has been described in humans, Angus cattle and cats (Jolly *et al.*, 1981; Vandevlede *et al.*, 1982). The mixtures of urinary oligosaccharides excreted in the three species with mannosidosis are different, reflecting either differences in glycoprotein structure or differences in catabolism (Burditt *et al.*, 1980). The structures of over 20 urinary oligosaccharides excreted in human mannosidosis have been established (Nordén *et al.*, 1973a, 1974; Strecker *et al.*, 1976; Yamashita *et al.*, 1980; Egge *et al.*, 1982). All have the general structure $(\text{Man})_{2-9}\text{GlcNAc}$. The storage products in

bovine mannosidosis are not well characterized, but the structure of one urinary oligosaccharide (Lundblad *et al.*, 1975) and the composition of two brain oligosaccharides (Nordén *et al.*, 1973b) have been published. They are different from those found in human mannosidosis. The only information about the feline storage products is that they contain only mannose and *N*-acetylglucosamine (Burditt *et al.*, 1980). In the present paper we compare the pattern of stored oligosaccharides in mannosidosis in the urines of the three species and in the bovine and feline tissues. The probable compositions of the main storage products are deduced by a combination of gel filtration, t.l.c. and g.l.c. and the differences in catabolism of glycoproteins in the species are discussed.

Materials and methods

Tissues

Samples of the brain, kidney and liver of a 9-month-old female domestic kitten diagnosed as suffering from mannosidosis were stored at -20°C after euthanasia (Burditt *et al.*, 1980). Samples of the brain, kidney, lymph nodes, liver and spleen from a 3-week-old Angus calf that had been diagnosed by histopathology and biochemically as suffering from mannosidosis were also stored at -20°C . Neutral oligosaccharides were extracted from these tissues by a modification of the procedure of Daniel *et al.* (1981). Homogenates (20%, w/v) were prepared in distilled water in a Potter-Elvehjem homogenizer and then sonicated for 3×15 s at 20 kHz in an MSE Ultrasonic disintegrator. After precipitation of proteins with chilled trichloroacetic acid (10%, w/v, final concentration) the homogenate was centrifuged for 30 min at 4°C in the 8×50 ml angle rotor of an MSE High-Speed 18 centrifuge at 18000 rev./min (37000 g). 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 from cases of bovine, feline and human mannosidosis were stored at -20°C until required. Samples of urine (1–5 ml) were passed through a column of mixed-bed resin (Duolite MB6113) before freeze-drying. The dried material was reconstituted in deionized water for analysis. Authentic samples of two human urinary oligosaccharides from a patient with mannosidosis were kindly provided by Dr. G. Strecker (Laboratoire de Chimie Biologique, Lille, France): $\text{Man}(\alpha 1-3)\text{Man}(\beta 1-4)\text{GlcNAc}(\text{M}_2\text{G})$ and $\text{Man}(\alpha 1-2)\text{Man}(\alpha 1-3)\text{Man}(\beta 1-4)\text{GlcNAc}(\text{M}_3\text{G})$.

Determination of carbohydrate in tissues and urines

The carbohydrate in urines and extracts of tissues was determined by the phenol/ H_2SO_4 method (Dubois *et al.*, 1956). A standard curve for mannose was used over the range 0–500 μg .

T.l.c.

T.l.c. of the neutral oligosaccharides in urines and tissue extracts was carried out on plastic-backed silica gel plates (F1500/LS254; Schleicher & Schüll G.m.b.H., Dassel, Germany) using n-propanol/water (8:3, v/v) as the solvent (Friedman *et al.*, 1978). After chromatography the plates were dried and carbohydrates were detected with orcinol [0.2% (w/v) in H_2SO_4 (5%, w/v, in methanol)]. A hydrolysate of dextran (Grade C; BDH Chemicals)

was prepared by heating dextran (20 mg) in 0.5 M- H_2SO_4 (4 ml) for 80 min at 100°C . The hydrolysate was neutralized with solid BaCO_3 , centrifuged and the supernatant concentrated to 0.5 ml by rotary evaporation before storage at -20°C .

Preparative t.l.c. was carried out under the same conditions, except that the partially purified oligosaccharides were applied in bands across the plates. Oligosaccharides were located by spraying guide strips cut from the edges and middle of the plate with orcinol. Single bands were scraped off the plate and extracted twice with water. The eluted oligosaccharides were freeze-dried. The purity of the eluted oligosaccharides was checked by repeating t.l.c.

Gel-filtration chromatography

Gel-filtration chromatography was carried out using a column (1.0 cm $\times$ 100 cm) of Bio-Gel P4 (–400 mesh; Bio-Rad Laboratories, Watford, Herts., U.K.) at room temperature. Samples were applied in deionized water and the column was eluted at 8 ml/h with deionized water. The eluate was monitored continuously at 520 nm for carbohydrate by reaction with orcinol (1%, w/v) in aq. H_2SO_4 (80%, v/v) using a modified Technicon Autoanalyzer I colorimetric system. The column was calibrated by chromatography of a series of isomaltose oligosaccharides (dextran hydrolysate), mannose, *N*-acetylglucosamine and the two authentic human urinary oligosaccharides. The elution volume of the *N*-acetylglucosamine was determined by using Tetrazolium Blue to detect the sugar (Mopper & Degens, 1972). For preparative work, fractions (1 ml) were collected and the presence of carbohydrate was detected by the phenol/ H_2SO_4 method.

G.l.c. and g.l.c.–mass spectrometry

Samples of the purified oligosaccharides or standard monosaccharides (10–100 nmol) were prepared for g.l.c. and g.l.c.–mass spectrometry, either as described previously (Burditt *et al.*, 1980) or by the method of Chaplin (1982). In the latter procedure, neutralization of the methanolysed monosaccharides by Ag_2CO_3 is replaced by addition of t-butanol. G.l.c. and g.l.c.–mass spectrometry were performed on the same apparatus as described previously (Burditt *et al.*, 1980).

Results

Comparison of storage products by t.l.c.

There is a distinctive pattern of neutral urinary oligosaccharides in mannosidosis for each species (Fig. 1a). This has been noted previously (Burditt *et al.*, 1980). At least eight oligosaccharides were resolved in the bovine and feline urines. The predominant oligosaccharide in the feline urine (F_{II})

Fig. 1. T.l.c. of neutral oligosaccharides in urines and tissues of bovine, feline and human mannosidosis

T.l.c. of samples from urines and tissues and standards was carried out on silica gel with n-propanol/water (8:3, v/v) as the solvent. Oligosaccharides were detected with orcinol/ H_2SO_4 . (a) (1) and (10) Dextran hydrolysate; (2) feline liver; (3) feline brain; (4) feline kidney; (5) feline urine; (6) bovine urine; (7) human urine; (8) $Man(\alpha 1-3)Man(\beta 1-4)GlcNAc$; (9) $Man(\alpha 1-2)Man(\alpha 1-3)Man(\beta 1-4)GlcNAc$. (b) (1) Dextran hydrolysate; (2) bovine urine; (3) bovine liver; (4) bovine kidney; (5) bovine lymph nodes; (6) bovine brain; (7) bovine spleen.

had the same mobility as one of the most abundant oligosaccharides (B_{III}) in the bovine urine. All the other components in the feline urine corresponded to components in the bovine urine, suggesting that the bovine and feline storage products were very similar. None of the major components in the human urine corresponded to those in the bovine and feline urines or to the dextran hydrolysate. The major component in the human urine had the same mobility as the authentic sample of $Man(\alpha 1-3)Man(\beta 1-4)GlcNAc$. Another spot corresponded to the authentic sample of $Man(\alpha 1-2)Man(\alpha 1-3)Man(\beta 1-4)GlcNAc$. The values of relative mobility (R_m) for the bovine oligosaccharides were plotted against the degree of polymerization based on B_{II} and B_{III} being a tetramer and pentamer respectively (Fig. 2). A straight line was obtained, suggesting that the oligosaccharides belong to a homologous series. The

Fig. 2. Analysis of t.l.c.

The R_F value for each oligosaccharide was measured and $R_m \{=\log[(1-R_F)/R_F]\}$ was calculated and plotted against the degree of polymerization.

R_m values for the feline oligosaccharides also fitted on the line. In contrast, the R_m values for the predominant human tri- and tetra-saccharides of known structure did not fit on the line, suggesting that the human oligosaccharides were different from the bovine and feline oligosaccharides. Both sets of oligosaccharides had different values of R_m from the dextran hydrolysate.

Neutral oligosaccharides were also extracted from the tissues of the kitten and of a calf with mannosidosis. It can be seen that the same oligosaccharide predominates in the feline urine, liver, brain and kidney (Fig. 1a). However, the relative proportions of the other components were different in the liver from those in the other tissues and in the urine. Quantitatively the liver contained most storage product (Table 1). Similarly, the most abundant oligosaccharide in the bovine urine, B_{III} , was also the most abundant oligosaccharide in the liver, spleen, brain, kidney and lymph nodes of the calf with mannosidosis (Fig. 1b). The pattern of stored oligosaccharides was very similar in all the tissues. The concentration of neutral sugars was lowest in the brain of the calf with mannosidosis.

Analysis of the urinary oligosaccharides by gel-permeation chromatography

To obtain an estimate of the size of the stored oligosaccharides, samples of the deionized urines from the three species with mannosidosis were

Table 1. Carbohydrate in mannosidosis tissues and urines

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

	Carbohydrate content					
	Urines (mg/ml of urine)	Tissues (mg/g wet wt.)				
		Brain	Kidney	Liver	Lymph nodes	Spleen
Bovine	0.24	0.08	0.43	0.85	0.28	0.85
Feline	1.98	0.17	0.31	0.82	—	—
Human	0.37	—	—	—	—	—

analysed by gel filtration on Bio-Gel P4. The column was calibrated with a partial hydrolysate of dextran, which contains glucose and a series of isomaltose homologues with degrees of polymerization from 2 to 12. *N*-acetylglucosamine (G), mannose (M) and the authentic human urinary oligosaccharides in mannosidosis, $\text{Man}(\alpha 1-3)\text{Man}(\beta 1-4)\text{GlcNAc}$ (M_2G) and $\text{Man}(\alpha 1-2)\text{Man}(\alpha 1-3)\text{Man}(\beta 1-4)\text{GlcNAc}$ (M_3G), were also run as standards. The elution volumes of these standards are indicated by their degree of polymerization or by their abbreviation (Fig. 3). Chromatography of oligosaccharides of known structure on suitably calibrated columns has shown that the elution of a neutral oligosaccharide reflects the sum of the contributions of the constituent monomers to the chromatographic properties (Yamashita *et al.*, 1977; Hounsell *et al.*, 1981; R. Sidebotham, unpublished work). It has been found that the addition of a mannosyl unit to a monosaccharide or oligosaccharide causes the elution volume to decrease by the equivalent of one hexosyl unit. In contrast, addition of a 2-acetamido-2-deoxyhexosyl unit alters the elution volume by the equivalent of two hexosyl units and addition of a 2-acetamido-2-deoxyhexose residue (i.e. at the reducing terminal) by approx. 2.5 hexosyl units. Thus any oligosaccharide eluted in a volume corresponding to an integer and a half number of hexosyl units will have *N*-acetylglucosamine at the reducing terminal. This information has been used to predict possible compositions of the oligosaccharides in the mannosidosis urines from their elution volumes expressed as hexosyl units (Table 2).

All the urines contained some monosaccharide and di- and tri-hexosyl material, but the major oligosaccharides were eluted in volumes corresponding to half-hexosyl units, implying that there was an *N*-acetylglucosamine residue at the reducing terminal. The elution volume of the main human oligosaccharide was the same as that of the authentic trisaccharide, $\text{Man}(\alpha 1-3)\text{Man}(\beta 1-$

Fig. 3. Gel-filtration chromatography on Bio-Gel P4 of neutral urinary and tissue oligosaccharides

The mixtures of neutral oligosaccharides were fractionated on a column (1 cm × 100 cm) of Bio-Gel P-4, which was eluted with water (8 ml/h). Carbohydrates were detected with orcinol/ H_2SO_4 using a Technicon Autoanalyzer. The elution volumes of the standards are indicated at the top of the Figure: dextran hydrolysate of degrees of polymerization 1–12; M, mannose; G, *N*-acetylglucosamine; M_2G , $\text{Man}(\alpha 1-3)\text{Man}(\beta 1-4)\text{GlcNAc}$; M_3G , $\text{Man}(\alpha 1-2)\text{Man}(\alpha 1-3)\text{Man}(\beta 1-4)\text{GlcNAc}$. The fractions pooled for purification of oligosaccharides are shown.

3) GlcNAc . Other major peaks corresponded to the authentic tetrasaccharide $\text{Man}(\alpha 1-2)\text{Man}(\alpha 1-3)\text{Man}(\beta 1-4)\text{GlcNAc}$ and to an oligosaccharide

Table 2. *Possible composition of oligosaccharides*
Abbreviation used: N.D., not determined. Values in parentheses indicate numbers of determinations.

Oligosaccharide	Gel filtration		Man/GlcNAc ratio from g.l.c.	Probable composition
	Elution volume (hexosyl units)	Possible compositions		
Human urine				
Authentic samples	4½	Man ₂ GlcNAc	1.9 : 1 (2)	Man ₂ GlcNAc
	5½	Man ₃ GlcNAc or Man(GlcNAc) ₂	3.1 : 1 (2)	Man ₃ GlcNAc
Bovine urine				
B _{II}	6½	Man ₄ GlcNAc or Man ₂ (GlcNAc) ₂	0.9 : 1 (2)	Man ₂ (GlcNAc) ₂
B _{III}	7½	Man ₅ GlcNAc, Man ₃ (GlcNAc) ₂ or Man(GlcNAc) ₃	1.57 : 1 (2)	Man ₃ (GlcNAc) ₂
B _{IV}	8½*	Man ₆ GlcNAc, Man ₄ (GlcNAc) ₂ or Man ₂ (GlcNAc) ₃	1.9 : 1 (1)	Man ₄ (GlcNAc) ₂
B _V	9½	Man ₇ GlcNAc, Man ₅ (GlcNAc) ₂ , Man ₃ (GlcNAc) ₃ or Man(GlcNAc) ₄	N.D.	Man ₅ (GlcNAc) ₂
Feline urine				
F _{II}	7½	Man ₃ GlcNAc, Man ₃ (GlcNAc) ₂ or Man(GlcNAc) ₃	1.5 : 1 (4)	Man ₃ (GlcNAc) ₂
Feline liver				
F _{II}	7½	Man ₃ GlcNAc, Man ₃ (GlcNAc) ₂ or Man(GlcNAc) ₃	1.65 : 1 (1)	Man ₃ (GlcNAc) ₂

* Not fully resolved, but shown to be present by t.l.c. of pooled fractions.

equivalent to 6½ hexosyl units, which is probably (Man)₄GlcNAc. These conclusions are consistent with the published abundances and structures of the main urinary oligosaccharides in human mannosidosis (Nordén *et al.*, 1973a, 1974; Strecker *et al.*, 1976; Yamashita *et al.*, 1980).

The elution profiles for the urinary oligosaccharides in bovine and feline mannosidosis confirm that the main storage products in these two species are different from those in humans. It has been shown previously that the bovine and feline storage products contain only mannose and *N*-acetylglucosamine (Nordén *et al.*, 1973b; Lundblad *et al.*, 1975; Burditt *et al.*, 1980). As the elution volumes of the main components correspond to half-hexosyl units it can be deduced that all the oligosaccharides have an *N*-acetylhexosamine residue in the reducing terminal position. The compositions compatible with the elution volumes of the main bovine (B_{II}, B_{III} and B_V) and feline (F_{II}) oligosaccharides are given in Table 2. Thus the bovine and feline oligosaccharides could either be higher homologues of the human series or a different series of composition (Man)_{*n*}(GlcNAc)₂. The difference in mobility on t.l.c. suggests the latter.

The elution profile for the neutral oligosaccharides in the feline mannosidosis liver shows that the main tissue component is probably identical with the main feline urinary component (F_{II}).

Determination of composition of oligosaccharides by g.l.c. and g.l.c.-mass spectrometry

The material in the main peaks obtained by chromatography on Bio-Gel P4 of the neutral oligosaccharides in feline liver and urine and bovine urine was pooled and concentrated. The fractions pooled are indicated in Fig. 3. The oligosaccharides

were then purified further by preparative t.l.c. The main peak from both feline liver and feline urine was homogeneous and corresponded to the component F_{II} in the analytical t.l.c. (Fig. 1). The fractions from the main bovine peak contained oligosaccharides B_{II}, B_{III}, B_{IV} and B_V. After chromatography on the silica gel thin layer they were obtained as single bands. Derivatives of the purified oligosaccharides were formed as described in the Materials and methods section and analysed by g.l.c. and g.l.c.-mass spectrometry. Only mannose and *N*-acetylglucosamine were detected in the hydrolysates by g.l.c. The identity of the monosaccharides was confirmed by g.l.c.-mass spectrometry. The ratio of mannose to *N*-acetylglucosamine was calculated for the purified oligosaccharides and the two authentic human urinary oligosaccharides (Table 2). The probable composition of each oligosaccharide was predicted from these ratios and the size of the oligosaccharide according to gel filtration. The homology observed by t.l.c. was also taken into account. The results for the two human oligosaccharides of known structure show that this procedure is valid. It is concluded that the oligosaccharides that accumulate in bovine and feline mannosidosis have the general formula (Man)_{*n*}(GlcNAc)₂, whereas the human oligosaccharides belong to a series of composition (Man)_{*n*}GlcNAc.

Discussion

The mannose-rich oligosaccharides that accumulate in mannosidosis result from the incomplete catabolism of the high-mannose and complex types of asparagine-linked oligosaccharides of glycopro-

teins. The structures of the human urinary oligosaccharides in mannosidosis all correspond to known fragments of glycoproteins or processing intermediates in their biosynthetic pathway (for a review of structures and their biosynthesis, see Kornfeld, 1982). All have a single *N*-acetylglucosamine, at the reducing terminal, and from one to eight α -linked mannosyl units at non-reducing positions (Yamashita *et al.*, 1980). This suggests that an endohexosaminidase that cleaves the chitobiosidic linkage in the common core region is involved in the lysosomal catabolism of human glycoproteins. Endohexosaminidase activity towards both types of oligosaccharides has been described in human tissues (Overdijk *et al.*, 1981) and rat and pig tissues (Nishigaki *et al.*, 1974), but it does not appear to be located in the lysosomes (Pierce *et al.*, 1979, 1980). An aspartamidohydrolase, capable of catalysing the hydrolysis of the linkage between the terminal *N*-acetylglucosamine and asparagine, is also present in human (Pollitt *et al.*, 1968) and other mammalian tissues (Makino *et al.*, 1966). It is probably located in the lysosomes, but whether it acts before or subsequent to the endohexosaminidase has not been established. The preponderant human urinary oligosaccharide has the structure $\text{Man}(\alpha 1-3)\text{Man}(\beta 1-4)\text{GlcNAc}$. The expected storage product from the catabolism of complex oligosaccharides would be the core tetrasaccharide $\text{Man}(\alpha 1-3)[\text{Man}(\alpha 1-6)]\text{Man}(\beta 1-4)\text{GlcNAc}$. The present analysis of the storage products in the urine and tissues of cases of bovine and feline mannosidosis provides more information about the lysosomal catabolism of glycoproteins and offers a possible explanation of the anomalous predominant storage product in human mannosidosis.

The patterns of neutral urinary oligosaccharides in bovine, feline and human mannosidosis are different. These patterns and the predominant storage products are characteristic of the species, as very similar patterns were found in the tissues and urine of an affected calf or kitten (Fig. 1). Some variation in the relative proportions of the oligosaccharides between organs was observed, but the predominant oligosaccharide was the same for all tissues from a species. In contrast, in human mannosidosis the tetra- and penta-saccharides Man_3 - and Man_4 -GlcNAc are more abundant in gingiva than is the trisaccharide $\text{Man}_2\text{GlcNAc}$ (Daniel *et al.*, 1981). The trisaccharide is, however, the most abundant storage oligosaccharide in human mannosidosis fibroblasts (B. Winchester, unpublished work). The oligosaccharides stored in bovine and feline mannosidosis belonged to the same homologous series, but were different from those stored in human mannosidosis.

These differences in the storage oligosaccharides among the species were confirmed by gel-filtration

chromatography (Fig. 3). This technique also permitted predictions about the size and composition of the oligosaccharides to be made (Table 2). The main human urinary oligosaccharide had the same elution volume as an authentic sample of the trisaccharide $\text{Man}(\alpha 1-3)\text{Man}(\beta 1-4)\text{GlcNAc}$. Other human components corresponded to elution volumes for oligosaccharides of the general composition $\text{Man}_n\text{GlcNAc}$. This is in agreement with the known structures of the urinary oligosaccharides in human mannosidosis. The elution volumes of the main bovine and feline oligosaccharides were consistent with oligosaccharides with compositions of either $(\text{Man})_n\text{GlcNAc}$ or $(\text{Man})_n(\text{GlcNAc})_2$. However, the mobilities of these oligosaccharides in t.l.c. suggested that they were not members of the series $(\text{Man})_n\text{GlcNAc}$ and their size and composition suggest that they contain two rather than one *N*-acetylglucosamine molecules. Thus the probable composition of the predominant feline oligosaccharide is $\text{Man}_3(\text{GlcNAc})_2$. This would be the expected storage product from the lysosomal catabolism of complex asparagine-linked glycans in glycoproteins if lysosomal α -D-mannosidase was deficient and there was no endohexosaminidase activity. The larger oligosaccharides present in urine and the tissues would be the corresponding catabolic products of high-mannose glycans. T.l.c. indicates that they belong to a homologous series of general composition $\text{Man}_n(\text{GlcNAc})_2$. Bands corresponding to a series from $\text{Man}_4(\text{GlcNAc})_2$ to $\text{Man}_9(\text{GlcNAc})_2$ can be seen in Fig. 1. This would be consistent with our present knowledge of the structure of high-mannose glycans and processing intermediates in the post-translational modification of mammalian glycoproteins. The preponderance of $\text{Man}_3(\text{GlcNAc})_2$, the product of the catabolism of complex glycans, indicates that such glycans are more abundant than the high-mannose type in feline tissue glycoproteins. Similarly, oligosaccharides with mobilities consistent with compositions of $\text{Man}_2(\text{GlcNAc})_2$ to $\text{Man}_9(\text{GlcNAc})_2$ can be seen in the bovine mannosidosis urine, again suggesting that endohexosaminidase is absent in this species. It is interesting that the oligosaccharide $\text{Man}_2(\text{GlcNAc})_2$, rather than $\text{Man}_3\text{GlcNAc}$, is the most abundant oligosaccharide in bovine mannosidosis. The trisaccharide $\text{Man}_2\text{GlcNAc}$ rather than $\text{Man}_3\text{GlcNAc}$ is the predominant human urinary oligosaccharide. Perhaps in these species the residual mutant α -D-mannosidase retains activity towards one of the α -mannosidic linkages of the core oligosaccharide, $\alpha 1-6$ in the human case. Alternatively, another lysosomal α -D-mannosidase unaffected in these disorders could catalyse the hydrolysis of a core α -mannosidic linkage.

It has been claimed previously that the most abundant urinary oligosaccharide in bovine manno-

sidosis has the structure α -D-Manp(1-6) β -D-Manp-(1-4) β -D-GlcNAcp(1-4) β -D-GlcNAcp(1-4)GlcNAc (Lundblad *et al.*, 1975). It is not possible to tell whether this oligosaccharide corresponds to our oligosaccharide B_{II} or B_{III} because a different isolation procedure was used. However, although this structure is not compatible with current ideas about the structure of mammalian glycoproteins, it does reinforce our conclusion that endohexosaminidase activity is absent from, or insufficient in, bovine tissues. Endohexosaminidase activity may also be absent from caprine tissues, as the main storage product in caprine tissue β -mannosidosis is the trisaccharide Man(β 1-4)GlcNAc(β 1-4)GlcNAc with an intact chitobiosidic linkage at the reducing terminal (Jones & Laine, 1981). Two different tetrasaccharides isolated from the brain of a calf with mannosidosis and also present in urine contain two mannose and two *N*-acetylglucosamine residues (Nordén *et al.*, 1973*b*). We have found that the oligosaccharide Man₂(GlcNAc)₂ is abundant in the bovine brain.

It has been shown by other investigators that the separation of storage oligosaccharides by size and solubility yields fractions containing compositional isomers (Yamashita *et al.*, 1980; Egge *et al.*, 1982). This would also be expected with our methods. However, this does not obviate our conclusions about the presence or absence of endohexosaminidase activity, because any *N*-acetylglucosamine not in the core region will be removed by the β -*N*-acetylglucosaminidase present in those tissues (Phillips *et al.*, 1974; Burditt *et al.*, 1980).

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