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THE ACCUMULATION OF OLIGOSACCHARIDES IN TISSUES AND BODY FLUIDS OF CATS WITH α-MANNOSIDOSIS*

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ABSTRACT

Oligosaccharides were extracted from tissues and body fluids of five kittens with α-mannosidosis, three being from the same litter. The kittens were all of different ages at death and were compared to normal and heterozygote cats. The oligosaccharides were analyzed by high-pressure liquid chromatography after perbenzoylation and were identified by comparison with compounds of known structure. This provided a detailed picture of the distribution of oligosaccharides in each tissue, and a method for quantitation of the total oligosaccharides. With the exception of the youngest animal (death at day 2), the oligosaccharide elution profiles were broadly similar for all tissues and fluids, and were typical of feline α-mannosidosis. In contrast, concentrations of total oligosaccharides diverged widely from one source to another, from a high of 17.3 μmol/g to a low of 0.04 μmol/g. The results are interpreted in the context of glycoprotein catabolism.

INTRODUCTION

α-Mannosidosis is an inborn error of metabolism resulting from a severe deficiency of normal lysosomal α-D-mannosidase activity[1]. This causes the massive accumulation in tissues and body fluids of oligosaccharides derived from the partial

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degradation of *N*-glycoproteins. The deficiency occurs in humans[2], cattle[3], and cats[4,5], and a similar condition can be induced in other species by the ingestion of plants containing swainsonine[6,7]. However, there are remarkable species-differences in the phenotype and rate of progression[3] of the deficiency, and these differences are paralleled by differences in the structures and concentrations of the accumulated oligosaccharides[8]. These differences are particularly striking when human α-mannosidosis is compared with the disease in other species. For this reason, we have undertaken a detailed comparison of the levels and pattern of stored oligosaccharides in a variety of tissues and body fluids from five kittens with α-mannosidosis. There are other important reasons for performing this study. Firstly, the analysis of oligosaccharides in kittens of various ages will eventually allow a correlation of the progression of the condition, in molecular terms, with observed histochemical, skeletal, behavioral, and morphological changes; secondly, it allows a comparison of glycoprotein catabolic pathways in cats and other species[8]; and thirdly, such a study is necessary to provide a scientific basis for the application of oligosaccharide analysis to early diagnosis[9], disease monitoring, and evaluation of therapeutic interventions.

EXPERIMENTAL

Materials and methods. — Kittens were the result of matings of known heterozygotes[9] and were diagnosed by measurement of their acidic α-mannosidase levels[10]. Kittens 56, 57, and 58 were from the same litter; kittens 37 and 60 were from separate litters. Kitten 56 was the only female. For comparison, two normal control cats, Nos. 1 and 2, and one heterozygote cat, No. 30, were processed. The affected animals either died or were euthanized when disease had progressed clinically to a stage where they could no longer be satisfactorily maintained in a humanitarian way; for kitten 56, this was at day 2; for 57, at day 76; for 60, at day 130; for 37, at day 172; and for 58, at day 206. Tissues and body fluids were obtained at autopsy and frozen until use. Extraction of oligosaccharides from tissues and fluids, and preparation of the extracts for high-pressure liquid chromatography (l.c.), were performed as described previously[9], except that all samples were perbenzoylated prior to analysis[11]. L.c. was performed with a model 5020 instrument (Varian Associates, Palo Alto, CA), equipped with a u.v. detector, model ERC 7210 (Erma Optical Co., Japan), and a printer-plotter-integrator, model 3380A (Hewlett–Packard, Avondale, PA). The column used was a Microsorb Short-One 3 μm C-8 reversed phase (Rainin Instruments, Woburn, MA) or a Techsphere-Ultra Short-Chap 3 μm C-8 (H.p.l.c. Technology-Phenomenex, Palos Verdes Estates, CA). The column was eluted with a gradient of acetonitrile–water (80→100% acetonitrile) over 20 min, at a flow rate of 1 to 1.5 mL/min, with detection at 230 nm. Peaks in the chromatographic profile were assigned to specific oligosaccharides by comparison of the elution times with those of oligosaccharides isolated from human α-mannosidosis urine[12,13], bovine α-mannosidosis urine[14], or

swainsonine-induced ovine α-mannosidosis urine[14,15] or, in the case of pentasaccharide $Man_3GlcNAc_2$-Ia*, by comparison with a purified sample of which the structure had been determined by methylation analysis[9]. The structures of the bovine and ovine mannosidosis oligosaccharides were previously determined by a combination of l.c., digestion with *exo-* and *endo*-glycosidases, methylation analysis, and ^{1}H-n.m.r. spectroscopy. When necessary, co-injection with reference oligosaccharides was performed. Peaks in the l.c. elution profile were quantitated by comparison of the integrated area with that of an external standard of raffinose[11], on the basis that absorption at 230 nm is proportional to the number of benzoyl groups in each oligosaccharide. Initially, quantitation was performed by reference to an internal standard of sucrose[9], but later this was found to be unreliable because of the presence of an interfering disaccharide peak in the extracts from some tissues. When the l.c. elution profiles showed unusually poor resolution, or there was visual evidence of protein contamination of the extract, samples were subjected to ultrafiltration with a Centifree Micropartition Unit (Amicon, Danvers, MA).

α-D-Man*p*-(1→6)-β-D-Man*p*-(1→4)-β-D-Glc*p*NAc-(1→4)-D-Glc*p*NAc

$Man_2GlcNAc_2$

α-D-Man*p*-(1→6)-β-D-Man*p*-(1→4)-D-Glc*p*NAc
3
↑
1
α-D-Man*p*

$Man_3GlcNAc$

α-D-Man*p*-(1→6)-β-D-Man*p*-(1→4)-β-D-Glc*p*NAc-(1→4)-D-Glc*p*NAc
3
↑
1
α-D-Man*p*

$Man_3GlcNAc_2$-Ia

α-D-Man*p*-(1→3)-α-D-Man*p*-(1→6)-β-D-Man*p*-(1→4)-β-D-Glc*p*NAc-(1→4)-D-Glc*p*NAc

$Man_3GlcNAc_2$-Ib

α-D-Man*p*-(1→6)-β-D-Man*p*-(1→4)-D-Glc*p*NAc
3
↑
1
α-D-Man*p*-(1→2)-α-D-Man*p*

$Man_4GlcNAc$

*For structures corresponding to the abbreviated formulas, see Chart 1.

α-D-Man*p*-(1→3)-α-D-Man*p*-(1→6)-β-D-Man*p*-(1→4)-β-D-Glc*p*NAc-(1→4)-D-Glc*p*NAc
3
↑
1
α-D-Man*p*

$Man_4GlcNAc_2$

α-D-Man*p*-(1→6)-β-D-Man*p*-(1→4)-D-Glc*p*NAc
3
↑
1
α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-α-D-Man*p*

$Man_5GlcNAc$

α-D-Man*p*-(1→3)-α-D-Man*p*-(1→6)-β-D-Man*p*-(1→4)-β-D-Glc*p*NAc-(1→4)-D-Glc*p*NAc
6 3
↑ ↑
1 1
α-D-Man*p* α-D-Man*p*

$Man_5GlcNAc_2$

Chart 1. Proposed structures of oligosaccharides from feline α-mannosidosis.

RESULTS

Extracts from the tissues and body fluids of all cats showed l.c. peaks corresponding to oligosaccharides. In the samples from the control cats, peaks were usually of very low intensity (picomolar) and without a recognizable pattern. The exceptions were urine samples, which contained oligosaccharides at approximately one tenth the concentration seen in affected animals, but without a recognizable mannosidosis pattern, and a few tissue samples as noted below. In the heterozygote cat (No. 30), picomolar amounts of oligosaccharides having a pattern bearing some resemblance to that of feline mannosidosis were seen in salivary gland, spleen, and thyroid extracts, but levels were too low to be of real significance. The urine of cat 30 contained concentrations of oligosaccharides similar to that of the control cats (30–40 nmol/mL), but only in the sample from cat 30 did the principal peaks in the l.c. elution profile have the same elution times as those from the fluids of affected cats. A few other tissues from the control and heterozygote cats had oligosaccharide levels that were slightly elevated, but still well below those in affected animals, and none showed a mannosidosis pattern. The levels in these tissues were 5 nmol/g for the pancreas of cat 1, 8 nmol/g for the pancreas of cat 2, 11 nmol/g for the pancreas of cat 30, and 37 nmol/g for the thyroid of cat 1. The level in the pancreas of cat 30 was ~50 times lower than that in the pancreas of cat 58, and 350 times lower than that in the pancreas of cat 60. The level in the thyroid of cat 1 was 470 times lower than that in the thyroid of cat 60.

The oligosaccharides extracted from all tissues and body fluids of affected

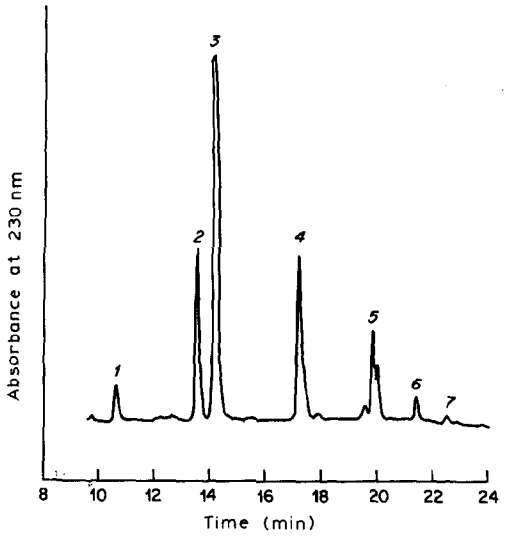

Fig. 1. L.c. of oligosaccharides (83 μg) from pancreas of cat 60, after perbenzoylation (see Methods). The numbers above the peaks designate the fractions as listed in Table I. For the structures corresponding to these formulas (except $Man_6GlcNAc_2$, $Man_7GlcNAc_2$), see Chart 1. When the volume of the injection was larger, peaks for oligosaccharides with 7–9 mannose residues were clearly seen. However, overloading of the column to reveal these minor components compromised the accuracy of the quantitations.

cats showed an l.c. elution pattern characteristic of feline α-mannosidosis[5,9] (see Fig. 1). In every sample, the largest peak corresponded to the pentasaccharide $Man_3GlcNAc_2$-Ia (see Chart 1). This is in sharp contrast to the profiles for bovine α-mannosidosis, where the major peak is $Man_2GlcNAc_2$ (ref. 14), or human α-mannosidosis, where the major peak is $Man_2GlcNAc$ [α-D-Man*p*-(1→3)-β-D-Man*p*-(1→4)-D-Glc*p*NAc] (ref. 13). A second Man_3 compound, $Man_3GlcNAc_2$-Ib (a major component of swainsonine-induced ovine mannosidosis oligosaccharides[6,7]), was eluted 0.3 min later than $Man_3GlcNAc_2$-Ia. That $Man_3GlcNAc_2$-Ib was a very minor component of the feline extracts was shown by the lack of any shoulder on the trailing edge of peak 3 (see Fig. 1). Another difference from the bovine or swainsonine-induced ovine diseases was the occurrence in all extracts of a hexasaccharide, $Man_4GlcNAc_2$, having a different structure (see Chart 1) from the major $Man_4GlcNAc_2$ isomer found in ruminants[14], *i.e.*, α-D-Man*p*-(1→3)-[α-D-Man*p*-(1→6)]-α-D-Man*p*-(1→6)-β-D-Man*p*-(1→4)-β-D-Glc*p*NAc-(1→4)-D-Glc*p*NAc. The feline compound is present as a very minor component in samples from sheep and cattle. The revision of the $Man_4GlcNAc_2$ structure from that reported earlier[5,9] was based on l.c. with columns of superior resolution, after completion of the structural work on the oligosaccharides from swainsonine-induced ovine mannosidosis[14]. Also noted in the l.c. elution profiles of the feline extracts were the high levels of $Man_3GlcNAc$, $Man_4GlcNAc$, and $Man_5GlcNAc$ (for structures, see Chart 1). This was a major difference from the situation in ruminants[8] where only

the pancreas showed very high levels of these particular oligosaccharides. Because these peaks were high (three to eight times larger than those for $Man_4GlcNAc_2$ and $Man_5GlcNAc_2$), they were often not well resolved from the latter, so that they could not be quantitated separately (see Tables I–V).

Some of the most interesting results concerned the pattern and levels of oligosaccharides in the tissues of cats 58 and 60, which were different in age and from different litters. In the case of the kidney and liver, although the l.c. elution profiles of the stored oligosaccharides were virtually identical (see Figs. 2A,B), the levels were different (see Tables IV, V, and Fig. 3). Thus, for cat 58 (the older animal,

TABLE I

OLIGOSACCHARIDE LEVELS[a] IN TISSUES OF CAT 37

Fraction	*Oligosaccharide*	*Brain*	*Kidney*	*Liver*	*Pancreas*
		(μmol/g of tissue)			
1	$Man_2GlcNAc_2$	0.13	0.13	0.11	0.11
2	$Man_3GlcNAc$	0.31	0.17	0.15	0.18
3	$Man_3GlcNAc_2$-Ia + $Man_3GlcNAc_2$-Ib	0.73	1.19	1.42	0.82
4	$Man_4GlcNAc$ + $Man_4GlcNAc_2$	0.18	0.21	0.17	0.18
5	$Man_5GlcNAc$ + $Man_5GlcNAc_2$	0.33	0.66	0.53	0.24
6	$Man_6GlcNAc_2$[b]	0.04	0.07	0.04	0.04
Total[c]		1.79	2.45	2.49	1.87
Fraction 3 as percent of the total		41	48	41	44

[a]Calculated μmol/g of tissue from integrated area of l.c. peak by reference to an external standard (for details, see Experimental section). [b]The structure has not been determined. [c]Derived by addition of the values for all major peaks in l.c. (the fractions listed may or may not comprise all the peaks).

TABLE II

OLIGOSACCHARIDE LEVELS[a] IN TISSUES OF CAT 56

Fraction[b]	*Brain*	*Liver*	*Pancreas*	*Spleen*
	(μmol/g of tissue)			
1	0.12	0.05	0.02	
2	0.31	0.02	0.01	
3	0.84	0.05	0.02	0.02
4	0.20	0.01		
5	0.38			
6	0.05			
Total[a]	2.00	0.13	0.06	0.04
Fraction 3 as percent of the total	42	38	25	55

[a]See footnotes to Table I; values below 0.01 μmol/g are not recorded. [b]See Table I for structural identification.

TABLE III

OLIGOSACCHARIDE LEVELS[a] IN TISSUES OF CAT 57

Fraction[b]	*Brain (μmol/g of tissue)*	*Liver*
1	0.02	0.29
2		0.29
3	0.13	2.48
4	0.02	0.44
5	0.03	0.86
Total[a]	0.2	4.40
Fraction 3 as percent of total	65	56

[a]See footnotes to Table I; values below 0.01 μmol/g are not recorded. [b]See Table I for structural identification.

206 days at death), levels were high in the liver (see comments below) and relatively low in the kidney, but for cat 60 (130 days), levels in both kidney and liver were very high (11.4 and 10.8 μmol/g, respectively). In three kittens from the same litter (56, 57, and 58), the total concentrations in the liver increased with age (see Table VI). This is in sharp contrast to the situation in bovine mannosidosis, where there was little accumulation[3]. For the youngest cat (56; 2 days at death), the pattern of liver oligosaccharides was quite different from that for cats 57, 58, and 60 (see Fig. 2B) in that it showed a much higher ratio of $Man_2GlcNAc_2$ to $Man_3GlcNAc_2$. This was also true for the pancreas (see Fig. 2C) where the total concentration of oligosaccharides was, as in most tissues including the kidney (see above), higher in cat 60 than in cat 58 (see Tables IV, V, and Fig. 3). However, even the level in cat 60 (4.2 μmol/g) was less than that seen in the bovine disease[14] (8.6 μmol/g, see Fig. 3).

In the brain, the most interesting feature was the relative constancy of the levels of total oligosaccharides, (see Tables I–V and Fig. 3), which is in sharp contrast to what is seen in the liver (see above) and to the situation in bovine mannosidosis, where the brain showed cumulative storage with age[3]. For the submandibular gland, the pattern was similar in cats 58 and 60, but levels were very high in cat 60 (see Tables IV, V and Fig. 3). For the latter animal, the level in the thyroid (17.3 μmol/g) was also very high. For other tissues (lymph node, eye lens, and spleen), no particularly surprising results (pattern or levels) were obtained. However, it may be significant that for cat 60 the proportion of $Man_3GlcNAc_2$ was almost always higher than that for cat 58. In body fluids, concentrations in ocular fluid and cerebrospinal fluid were always much lower than in urine, but the patterns were similar. The pattern for urine from cat 60 was remarkable for its low levels of $Man_5GlcNAc$ and $Man_5GlcNAc_2$ relative to $Man_3GlcNAc_2$ (see Table V).

It is necessary to comment briefly on the relationship of the present results to those reported previously[9] for total oligosaccharides in the liver and brain of cats

TABLE IV

OLIGOSACCHARIDE LEVELS[a] IN TISSUES AND BODY FLUIDS OF CAT 58

Fraction[b]	*Brain*	*Kidney*	*Liver*	*Pancreas*[c]	*Salivary*[c] *gland*	*Spleen*[c]	*Urine*[c]	*Ocular fluid*[c]
	(μmol/g of tissue)						*(μmol/mL)*	
1	0.03	0.03	0.26			0.04	0.02	
2	0.05	0.02	0.36	0.06	0.02	0.03	0.03	
3	0.26	0.07	3.90	0.37	0.19	0.44	0.28	0.04
4	0.08	0.01	1.10	0.09	0.06	0.07	0.03	0.02
5	0.04	0.01	1.00	0.09	0.08	0.13	0.04	0.02
6	0.01		0.45	0.02	0.01	0.03	0.01	
7[d]	0.01		0.36	0.01		0.01	0.01	
Total[a]	0.53	0.17	7.90	0.64	0.37	0.75	0.42	0.08
Fraction 3 as percent of the total	49	44	49	58	52	59	67	52

[a]See footnotes to Table I; values below 0.01 μmol per g or mL are not recorded. [b]See Table I for structural identication. [c]These values were determined by use of an internal standard of sucrose, because no interfering peak was present in the l.c. trace (see Experimental section). [d]Fraction 7 corresponds to $Man_7GlcNAc_2$ (structure not determined).

TABLE V

OLIGOSACCHARIDE LEVELS[a] IN TISSUES AND BODY FLUIDS OF CAT 60

Fraction[b]	*Brain*	*Kidney*	*Lens*	*Liver*	*Lymph node*	*Pancreas*	*Salivary gland*	*Spleen*	*Thyroid*	*Urine*	*Ocular fluid*	*Cerebrospinal fluid*
	(μmol/g of tissue)									*(μmol/mL)*		
1	0.38	0.07	0.02	0.04	0.23	0.18	1.20	0.07	0.73	0.04		
2	1.36	1.00	0.02	0.40	0.36	0.84	0.90	0.11	1.10	0.03		0.01
3	4.00	7.30	0.10	6.92	4.64	2.00	7.50	1.71	11.00	0.24		0.03
4	1.25	1.50	0.02	0.70	0.48	0.66	0.80	0.15	1.70	0.08		0.01
5	0.82	1.40		1.12	0.84	0.42	3.00	0.39	2.70	0.02		0.01
6	0.08	0.10		1.56	0.12	0.06	0.30	0.06	0.12			
7[c]	0.04	0.05		0.06		0.02	0.10					
Total[a]	7.90	11.42	0.22	10.85	6.66	4.18	13.80	2.60	17.35	0.42	0.06	0.06
Fraction 3 as percent of the total	51	64	45	64	69	48	54	66	63	57		50

[a]See footnotes to Table I; values below 0.01 μmol per g or mL are not recorded. [b]See Table I for structural identification. [c]Fraction 7 corresponds to $Man_7GlcNAc_2$ (structure not determined).

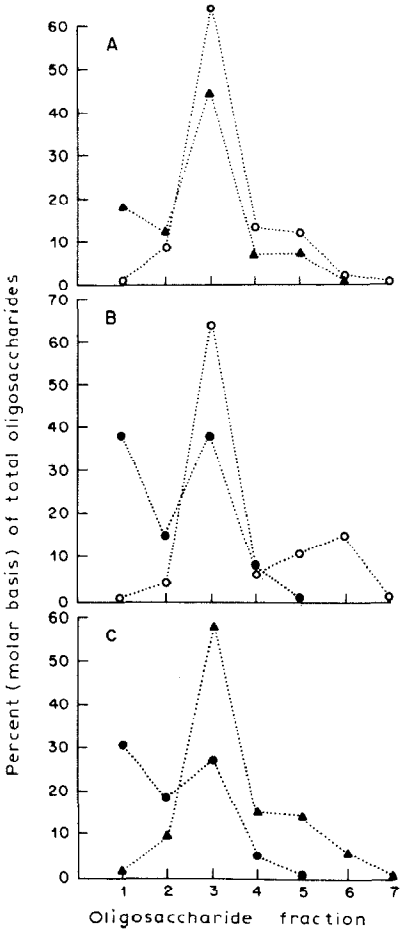

Fig. 2. Pattern of oligosaccharides extracted from kidneys of cats 58 (206 days) and 60 (130 days) (panel A), livers of cats 56 (2 days) and 60 (panel B), and pancreata of cats 56 and 58 (panel C). The percentages were calculated from the concentrations shown in Tables II, IV, and V. The oligosaccharide comprising each fraction is listed in Table I; (▲---▲) cat 58, (○---○) cat 60, and (●---●) cat 56.

57 and 58. For the liver of cat 58, a much lower figure (1.25 *vs.* 7.9 μmol/g) was given in our preliminary communication. This was erroneous, and resulted from a chromatographic artifact. The original study was performed using an internal standard of sucrose for the quantitation of oligosaccharide levels based on peak areas. During the course of the present study, it was observed that an interfering disaccharide of unknown identity, having exactly the same elution time as sucrose, had falsely inflated the area of the sucrose peak, and distorted all the values for oligosaccharide concentration. This interfering peak was not present in blanks and controls, or in most of the other tissue extracts from cat 58, but was present to a minor extent in the brain and kidney, and in some of the extracts from other cats. It was

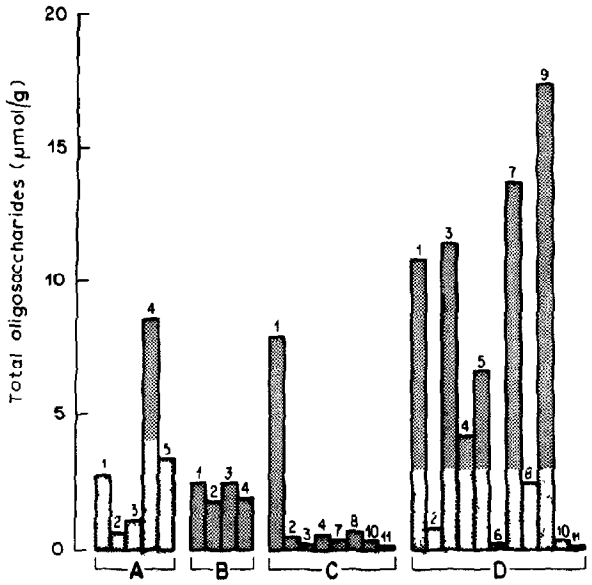

Fig. 3. Total oligosaccharide concentrations (μmol/g tissue) in: (1) liver, (2) brain, (3) kidney, and (4) pancreas from bovine mannosidosis (A; see ref. 14), cat 37 (B), cat 58 (C), and cat 60 (D). Other values are for selected tissues as follows: (5) lymph node, (6) ocular lens, (7) salivary gland, (8) spleen, (9) thyroid, (10) urine (μmol/mL), and (11) ocular fluid (μmol/mL).

not present in the brain and liver of cat 57, so in that case the two sets of values for oligosaccharide concentration are reasonably compatible. Because of the problem with the internal standard, all subsequent analyses were performed with an external standard of raffinose[11] (see Experimental section).

TABLE VI

CONCENTRATIONS OF OLIGOSACCHARIDES AND LEVELS OF TOTAL HEXOSE IN FELINE AND BOVINE MANNOSIDOSIS LIVER EXTRACTS

	Cat No.					*Bovine*
	37	*56*	*57*	*58*	*60*	
	(μmol/g of tissue)					
Oligosaccharide[a]	2.5	0.1	4.4	7.9	10.8	2.7[b]
Hexose[c]		10.0	13.6	10.0	14.4	3.0[d]

[a]Total values were taken from Tables I–V. [b]Value from ref. 14. [c]Measured by the phenol–sulfuric acid procedure (ref. 16) on a portion of the sample employed for perbenzoylation and l.c. [d]Approximate, taken from graph in ref. 3.

DISCUSSION

It is generally agreed that the storage of large quantities of incompletely degraded complex carbohydrate leads to the clinical manifestations of glycoprotein storage diseases[1], but exactly how this occurs is still a matter of speculation. Because the *N*-linked glycoproteins (the class of glycoproteins that are the precursors of mannosidosis oligosaccharides) are preponderant in body fluids and in the surface membranes of cells, where they participate in a spectrum of recognition events involved in growth, development, etc.[17], it is probable that massive oligosaccharide storage leads to aberrations of membrane structure,,synthesis, and turnover. These changes eventually lead to alterations of cellular morphology and function, such as those seen in neurons[4]. Because the conformation of an oligosaccharide, and hence its ability to interact with other membrane components, is strongly dependent on its structure, the structures of the oligosaccharides accumulated in particular cells may affect the extent of storage-induced pathology. Therefore, the observed differences in severity of disease and rate of progression, both between species and between cell types within a species, may be expected to correlate with differences in the pattern and levels of stored oligosaccharides. The factors affecting the extent and pattern of oligosaccharide storage at the molecular, cellular, and tissue or organ level may be summarized as: (a) the level of activity and substrate specificity of lysosomal endo-*N*-acetyl-β-D-glucosaminidase[18] and residual α-D-mannosidase[19]; (b) the types of cells and membranes, structures of secreted and membrane glycoproteins, and their relative rates of lysosomal breakdown; (c) the rate of receptor-mediated endocytosis of extracellular material, involving uptake by the liver[20,21], and by renal tubules[3,22]; and (d) the rate of exocytosis of lysosomal content into the circulation[23].

The results of this study indicate that the kittens examined are handling the problem of oligosaccharide storage in very different ways. The oligosaccharide accumulation in cat 60 is much more severe than for cat 58, especially for the thyroid, salivary gland, and kidney. The high values in the latter suggest an attempt by the renal tubules to sequester oligosaccharides and, thus, lower the storage burden in other tissues[3]. The high values in the thyroid, on the other hand, may not be so surprising in view of the thyroglobulin-synthesizing role of this gland.

These kittens with α-mannosidosis differed significantly in their tissue storage behavior from calves with the same disease[3]. Thus, storage increased in the liver for three cats (56, 57, and 58) from the same litter, but levels in the brain were relatively constant (the opposite was true for the calves). When the corresponding lysosomal α-D-mannosidase activities were determined[24], they were approximately constant in both the brain and the liver, showing that the increased oligosaccharide levels in the liver were the result of accumulation with time, rather than a decreased rate of breakdown. For the pancreas and the spleen, there was also a significant increase in oligosaccharide levels for the 206 day old cat when compared with the 2-day old cat, suggesting that, at least within a litter, there may be a more general

correlation of increased storage with time. (For animals from different litters, the trends are masked by individual variation). Compared to sheep and cattle with induced or genetic α-mannosidosis, the relatively high levels of oligosaccharides having a single, terminal GlcNAc residue ($Man_3GlcNAc$, $Man_4GlcNAc$, and $Man_5GlcNAc$) and the characteristic structures of these compounds[14] suggest the general presence in cat tissues of an endo-*N*-acetyl-β-D-glucosaminidase activity different from that in human tissues[8]. In the ruminants, concentrations of the same oligosaccharides were elevated only in the pancreas.

For the youngest cat (2 days), the l.c. elution profiles of stored oligosaccharides in the pancreas and liver were different from those of all the other cats, showing high levels of $Man_2GlcNAc_2$ relative to $Man_3GlcNAc_2$. This suggests that an α-mannosidase activity with different substrate specificity was active in the very young animal, or that the substrates degraded by neonatal kittens differ from those degraded by older cats, perhaps resembling those of the fetus.

The occurrence of a recognizable mannosidosis pattern in the oligosaccharides extracted from the urine of a heterozygote cat, but the lack of this pattern in the urines of the control cats, may be significant. In earlier work on these cats, low levels of mannosidosis oligosaccharides were observed in extracts of heterozygote placentas from three litters of kittens[9]. Also, characteristic oligosaccharides were found in the allantoic fluid of a goat putatively heterozygous for β-mannosidosis[25].

The structural difference in the $Man_4GlcNAc_2$ oligosaccharides isolated from cats and ruminants has important implications for the pathways of *N*-glycoprotein catabolism in these species. In ruminants, the major pathway from $Man_5GlcNAc_2$ (for structures, see Chart 1) involves initial removal of the inner (1→3)-linked α-D-mannosyl residue, so that $Man_3GlcNAc_2$-Ia cannot be formed from the heptasaccharide, but must arise solely from the degradation of "complex" chains[26]. In cats, however, the initial step is apparently removal of the outer (1→6)-linked α-D-mannosyl residue from $Man_5GlcNAc_2$ to give $Man_4GlcNAc_2$, from which removal of the outer (1→3)-linked residue then yields $Man_3GlcNAc_2$-Ia. The other possibility, removal of the inner (1→3)-linked residue, would have given $Man_3GlcNAc_2$-Ib, but this is ruled out as a major pathway because only trace amounts of $Man_3GlcNAc_2$-Ib were found in all the cat tissues and body fluids. In cats, therefore, in contrast to ruminants, the branched "core" pentasaccharide $Man_3GlcNAc_2$-Ia is a product of both "complex" and "high mannose" chains[26]. Obviously, this also applies to the final product, $Man_2GlcNAc_2$, derived by hydrolysis of the (1→3)-linked α-D-mannosyl residue from $Man_3GlcNAc_2$-Ia. This is in contrast to $Man_4GlcNAc_2$ and $Man_5GlcNAc_2$, which can only arise from the breakdown of "high mannose" chains[26].

REFERENCES

1 A. L. BEAUDET in J. B. STANSBURY, J. B. WYNGAARDEN, D. A. S. FREDERICKSON, J. L. GOLDSTEIN, AND M. S. BROWN (Eds.), *The Metabolic Basis of Inherited Diseases*, 5th edn., McGraw-Hill, New York, 1983, pp. 788–802.

2 J. P. KISTLER, I. T. LOTT, E. H. KOLODNY, R. B. FRIEDMAN, R. NERSASIAN, J. SCHNUR, M. C. MIHM, A. M. DVORAK, AND R. DICKERSIN, *Arch. Neurol.*, 34 (1977) 45–51.
3 R. D. JOLLY, P. M. SLACK, P. J. WINTER, AND C. E. MURPHY, *Aust. J. Exp. Biol. Med. Sci.*, 58 (1980) 421–428.
4 S. U. WALKLEY, W. F. BLAKEMORE, AND D. P. PURPURA, *Acta Neuropathol.*, 53 (1981) 75–79.
5 D. ABRAHAM, P. F. DANIEL, A. DELL, J. OATES, R. SIDEBOTHAM, AND B. G. WINCHESTER, *Biochem. J.*, 233 (1986) 899–904.
6 C. D. WARREN, S. SADEH, P. F. DANIEL, B. BUGGE, L. F. JAMES, AND R. W. JEANLOZ, *FEBS Lett.*, 163 (1983) 99–103.
7 P. F. DANIEL, C. D. WARREN, AND L. F. JAMES, *Biochem. J.*, 221 (1984) 601–607.
8 P. F. DANIEL, C. D. WARREN, B. G. WINCHESTER, AND R. D. JOLLY, *Abstr. Pap. Int. Carbohydr. Symp., XIIIth*, Ithaca, NY, (1986) 328.
9 C. D. WARREN, J. ALROY, B. BUGGE, P. F. DANIEL, S. S. RAGHAVAN, E. H. KOLODNY, J. J. LAMAR, AND R. W. JEANLOZ, *FEBS Lett.*, 195 (1986) 247–252.
10 N. C. PHILLIPS, D. ROBINSON, B. G. WINCHESTER, AND R. D. JOLLY, *Biochem. J.*, 137 (1974) 363–371.
11 P. F. DANIEL, *Methods Enzymol.*, 138 (1987) 94–116.
12 C. D. WARREN, A. S. SCHMIT, AND R. W. JEANLOZ, *Carbohydr. Res.*, 116 (1983) 171–182.
13 N. E. NORDEN, A. LUNDBLAD, S. SVENSSON, P. A. OCKERMAN, AND S. AUTIO, *J. Biol. Chem.*, 248 (1973) 6210–6215.
14 P. F. DANIEL, C. D. WARREN, L. F. JAMES, AND R. D. JOLLY, in A. A. SEAWRIGHT, M. P. HEGARTY, L. F. JAMES, AND R. F. KEELER, (Eds.), *Plant Toxicology*, The Queensland Poisonous Plants Committee, Queensland, Australia, 1985, pp. 290–300.
15 S. SADEH, C. D. WARREN, P. F. DANIEL, B. BUGGE, L. F. JAMES, AND R. W. JEANLOZ, *FEBS Lett.*, 163 (1983) 104–109.
16 G. ASHWELL, *Methods Enzymol.*, 8 (1966) 93–95.
17 R. J. IVATT, *The Biology of Glycoproteins*, Plenum, New York, 1984.
18 M. J. KURANDA AND N. N. ARONSON, JR., *J. Biol. Chem.*, 261 (1986) 5803–5809.
19 B. G. WINCHESTER, *Biochem. Soc. Trans.*, 12 (1984) 522–524.
20 E. F. NEUFELD AND G. ASHWELL, in W. J. LENNARZ (Ed.), *The Biochemistry of Glycoproteins and Proteoglycans*, Plenum, New York, 1980, pp. 241–266.
21 K. SEUBE, V. GROSS, D. HAUSSINGER, T.-A. TRAN-THI, K. DECKER, W. GEROK, AND C. HEINRICH, *Biochem. Biophys. Res. Commun.*, 141 (1986) 949–955.
22 I. T. LOTT AND P. F. DANIEL, *Neurology*, 31 (1981) 1159–1162.
23 P. H. DEBRUYN AND Y. CHO, *Am. J. Anat.*, 177 (1986) 35–41.
24 S. S. RAGHAVAN, G. STUER, L. RIVIÈRE, J. ALROY, AND E. H. KOLODNY, *J. Inher. Metab. Dis.*, 11 (1988) 3–16.
25 C. D. WARREN AND M. Z. JONES, unpublished data.
26 R. KORNFELD AND S. KORNFELD, in W. J. LENNARZ (Ed.), *The Biochemistry of Glycoproteins and Proteoglycans*, Plenum, New York, 1980, pp. 1–34.