Hepatic Storage of Oligosaccharides and Glycolipids in a Cat Affected with G_{M1} Gangliosidosis

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(Received 20 April 1978)

1. Glycopeptides and glycolipids were isolated from normal cat liver and liver from a cat affected with G_{M1} gangliosidosis. 2. Bio-Gel P-6 chromatography of the crude glycopeptide fractions demonstrated three major peaks of hexose-containing compounds that were greatly increased in the mutant liver sample; these peaks contained oligosaccharides that comprised over 2% of the liver wet weight. 3. Two of the major pathological oligosaccharides, GP5 and GP6, were purified by chromatography on charcoal/Celite and Sephadex G-25. Oligosaccharides GP5 and GP6 had apparent mol.wts. of 1800 ± 200 and 1350 ± 200 respectively, and contained galactose, mannose and *N*-acetylglucosamine in molar proportions of 2.0:3.1:4.1 (GP5) and 1.0:2.2:2.7 (GP6). Periodate oxidation studies demonstrated the presence of galactose in a non-reducing terminal position. 4. The neutral glycolipid fraction from the mutant cat liver has a 1.3-fold increase in hexose content accompanied by an increased concentration of asialo-(ganglioside G_{M1}). 5. There was a 2-fold increase of gangliosides in the mutant cat liver compared with normal cats. Ganglioside G_{M1} and a compound tentatively identified as *N*-glycolloyl-(ganglioside G_{M1}) were the major glycolipids accumulated.

G_{M1}* gangliosidosis is an inherited lysosomal storage disorder resulting from a deficiency of the enzyme acid β -D-galactosidase (O'Brien, 1978a). A prominent feature of the disease is the accumulation of ganglioside G_{M1} in the brain and visceral tissues of affected individuals (Landing *et al.*, 1964; Suzuki *et al.*, 1969; Wolfe *et al.*, 1970; Lowden *et al.*, 1973). Several classes of non-lipid storage compounds have also been isolated from a number of human patients. These include partially degraded keratan sulphates (Suzuki *et al.*, 1969; Tsay & Dawson, 1973; Calatroni, 1974) and oligosaccharides or glycopeptides that appear to derive from cellular glycoproteins (Brunngraber *et al.*, 1976; Calatroni, 1974; Wolfe *et al.*, 1974).

G_{M1} gangliosidosis occurs in cats as well as humans (Handa & Yamakawa, 1971; Blakemore, 1972; Farrell *et al.*, 1973; Cheetham *et al.*, 1974). Among the many similarities between the human and cat disorders are the near-total deficiency of acid β -galactosidase and histological evidence for the accumulation of storage products in the brain and viscera of affected cats (Baker *et al.*, 1976). Although hepatic ganglioside storage has been demonstrated in the cat disorder (Handa & Yamakawa, 1971; Blakemore, 1972; Baker *et al.*, 1976), a comprehensive examination of the storage material in visceral tissues has not been reported. The present study was

undertaken to further investigate the extent and nature of the biochemical pathology in liver tissue from a cat with G_{M1} gangliosidosis.

Experimental

Materials

Liver tissue from normal control cats and a cat affected with G_{M1} gangliosidosis were provided by Dr. H. J. Baker, University of Alabama, Birmingham, AL, U.S.A. The human G_{M1} gangliosidosis storage oligosaccharide (fraction 4) was a gift from Dr. L. S. Wolfe, McGill University, Montreal, Canada. Papain, bovine brain gangliosides, activated charcoal and monosaccharide standards were from Sigma Chemical Co., St. Louis, MO, U.S.A. Bio-Gel P-6 was from Bio-Rad Laboratories, Richmond, CA, U.S.A. Sephadex G-25 and Blue Dextran 2000 were obtained from Pharmacia, Uppsala, Sweden. Dimethylchlorosilane, hexamethyldisilazane, heptafluorobutyric anhydride and sialylation-grade pyridine were from Pierce Chemical Co., Rockford, IL, U.S.A. Ultrafiltration cells and UM-2 membranes were products of Amicon Corp., Lexington, MA, U.S.A. Pre-coated analytical t.l.c. plates of silica gel 60 were from E.M. Laboratories, Elmsford, NY, U.S.A. Ceramide dihexoside was obtained from Miles Laboratories, Elkhart, IN, U.S.A. Ceramide monohexoside and ceramide tri-

* The nomenclature of Svennerholm (1963) is used for gangliosides.

hexoside were purified from tissues obtained after autopsy of patients with Gaucher's and Fabry's disease respectively. Asialo-(ganglioside G_{M1}) was prepared by mild acid hydrolysis of purified bovine brain ganglioside G_{M1} .

Methods

Initial fractionation of liver tissue. Glycopeptide, upper-phase lipid, lower-phase lipid and glycosaminoglycan fractions were prepared from 2 g samples of liver from mutant and normal cats as previously described (Brunngraber *et al.*, 1971). Briefly, the method involved: (a) homogenization of tissue in 19 vol. of chloroform/methanol (2:1, v/v) followed by re-homogenization of the tissue residue in 10 vol. of chloroform/methanol (1:2, v/v); (b) partitioning of the combined organic extracts (total-lipid fraction) with an aq. KCl solution to prepare upper- and lower-phase-lipid fractions; (c) extensive digestion of the lipid-free tissue residue with papain; and (d) precipitation of glycosaminoglycans from the proteolytic digest (glycopeptide fraction) with cetylpyridinium chloride. The original procedure was modified in the desalting of the proteolytic digest that, in this study, was accomplished in an ultrafiltration cell over a UM-2 membrane. The upper-phase-lipid fractions were dialysed against water at 4°C and freeze-dried. The lower-phase-lipid fractions were saponified (Abramson *et al.*, 1965) before analysis. Protein determinations were by the method of Lowry *et al.* (1951), with bovine serum albumin as a standard. Total hexosamine was determined by the method of Levvy & McAllen (1959), with *N*-acetylglucosamine as standard, after hydrolysis of the samples in 4M-HCl for 6 h at 100°C.

Fractionation of the glycopeptides. The liver glycopeptides from one mutant animal and three normal animals were fractionated by gel filtration on a bed (2.0cm×87cm) of Bio-Gel P-6 at 23°C. Samples (3.5ml) were applied and eluted with water at a flow rate of 32ml/h and 4.0ml fractions were collected. The fractions were assayed for total hexose by the phenol/ H_2SO_4 method (Dubois *et al.*, 1956), with galactose as standard. Amino groups were determined with the trinitrobenzenesulphonic acid assay (McKelvy & Lee, 1969) relative to a glycine standard. Various column fractions were analysed for carbohydrate by g.l.c. as described below.

Glycopeptide fractions were analysed by ascending t.l.c. on plates (10cm×20cm) of silica-gel 60 by using the solvent system propan-1-ol/acetic acid/water (3:3:2, by vol.) (running time 12–16h). A square of filter paper was clamped to the top 2cm of the plate to permit extended development. After drying, the plates were stained for sugar-containing compounds by spraying with orcinol (1% in 50% H_2SO_4) and heating for 10min at 100°C.

The three major peaks of carbohydrate-containing material resolved by Bio-Gel chromatography of the mutant glycopeptide fraction were further purified by chromatography on charcoal/Celite (Spiro, 1966). Samples were applied in water (1mg of hexose/ml bed vol.) and eluted stepwise with increasing concentrations of ethanol in water at 23°C. The eluted fractions were decreased in volume in a rotary evaporator and analysed for hexose by the phenol/ H_2SO_4 assay. The fractions were analysed by t.l.c. as described above and those that appeared homogeneous were used for further purification.

Peak fractions from the charcoal columns (compounds GP5 and GP6) were next chromatographed at 23°C on a column (2.0cm×87cm) of Sephadex G-25 (medium mesh) with 0.1M-acetic acid as the eluent at a flow rate of 24ml/h. The column was calibrated with carbohydrate standards of various molecular weights (Bhatti & Clamp, 1968). The peak fractions from this column were pooled and used for the final analyses of compounds GP5 and GP6.

Analysis of the glycopeptides. The carbohydrate compositions of crude and purified fractions were determined by g.l.c. analysis of the trimethylsilyl derivatives of the monosaccharide methyl glycosides, which were prepared and chromatographed as described by Clamp *et al.* (1971), except for the following modifications. (a) Methanolysis was carried out in methanolic 1.5M-HCl (0.5ml) at 80°C for 12h in vials with Teflon-lined screw caps; (b) the methanolysates were neutralized with 0.15ml of anhydrous pyridine and acetylated for 1h at 23°C by adding 0.10ml of acetic anhydride; and (c) the samples were thoroughly dried in a vacuum desiccator over phosphoric oxide and silylated by using 'silylating agent a' (Clamp *et al.*, 1971). Mannitol was included in the samples as the internal standard. Analyses were carried out on a Nuclear-Chicago Selecta System 5000 gas-liquid chromatograph equipped with a flame-ionization detector and a column (2mm×2m) of 3% SE-30 on 100/120 Gas-Chrom Q (Applied Science Laboratories, State College, PA, U.S.A.).

Periodate oxidation of the purified compounds was carried out by the method of Tsay *et al.* (1976). The procedure was followed by the addition of the mannitol standard, methanolysis and g.l.c. analysis of the surviving sugars as described above.

Amino acids were analysed by g.l.c. as *N*(*O*)-heptafluorobutyrate of their isopentyl esters (Zanetta & Vincendon, 1973) after hydrolysis in 6M-HCl for 24h at 110°C under N_2 . Pípecolic acid served as the internal standard.

Analysis of the lipid fractions. The lipid fractions were examined by t.l.c. on plates (10cm×20cm) of silica gel 60. Chloroform/methanol/water (35:15:2,

by vol.) was used for the lower-phase lipids; propan-1-ol/water (7:3, v/v) (system 1), chloroform/methanol/2.5M-NH₃ (60:40:9, by vol.) (system 2) and butan-1-ol/pyridine/water (3:2:1, by vol.) (system 3) were used for the upper-phase lipids. The glycolipids were detected with either the α -naphthol or resorcinol spray reagents (Esselman *et al.*, 1972). The glycolipids from cat liver were tentatively identified by their co-migration with standard compounds. Total lipid-bound sialic acid was determined by the method of Svennerholm (1957). Neutral hexose was determined on sample zones scraped from t.l.c. plates by the anthrone method of Rouser *et al.* (1970).

The carbohydrate compositions of several mutant-liver upper-phase glycolipids were determined by g.l.c. analysis. After separation by t.l.c. in system 2, the glycolipids were located with I₂ vapour and by staining a sample on the edge of the plate with α -naphthol. Regions were scraped from the plate and the glycolipids were eluted with chloroform/methanol (1:1, v/v). After filtering the eluents through a plug of glass wool, mannitol was added, and the samples were thoroughly dried. Methanolysis, conversion into derivatives and g.l.c. were carried out as described for the glycopeptides, except that the methanolysates were extracted with three 1 ml portions of hexane before neutralization.

Results

Gel filtration of the crude glycopeptide fractions

The total hexosamine content of the mutant-glycopeptide fraction was 10–20-fold higher than the controls. An accompanying increase in total hexose was apparently obscured by the large amount of glycogen in all of the samples. Bio-Gel P-6 chromatography of the normal glycopeptides gave two main peaks of hexose, corresponding to the

elution volumes of the Blue Dextran and galactose standards. In between these peaks were several minor peaks of hexose-containing compounds. The elution profile of the mutant glycopeptides resembled the normal controls in that both the high- and low-molecular-weight peaks were present. However, the majority of the hexose-containing compounds in the sample from mutant liver were eluted in the intermediate fractions, where massive increases were evident (Fig. 1).

The majority of the mutant compounds were eluted as three peaks between fractions 45 and 60. In addition, several minor hexose-containing fractions were eluted earlier, indicating the presence of several additional higher-molecular-weight species. The major peaks of glycopeptide were separated from the bulk of the peptide material that eluted in fractions 24–46 and 58–75. Carbohydrate analysis of selected fractions from the column run of mutant glycopeptides (Table 1) showed that the material that eluted in the void volume was probably a mixture of glycoproteins or large glycopeptides and glycogen. The compounds of intermediate molecular weight (fractions 42, 48, 50, 51, 55 and 56) were composed primarily of galactose, mannose and *N*-acetylglucosamine. The mutant-glycopeptide fractions were pooled as indicated (Fig. 1). On the basis of phenol/H₂SO₄ determinations, pools 4, 5 and 6 contained 8.5, 13.5 and 9.2 mg of hexose respectively. Analysis of the three pools by t.l.c. showed that each was composed of a major and one or more minor constituents (Fig. 2). These pools were used for further purification and analysis. The glycopeptide fractions from the controls were not investigated further.

Fractionation of pooled fractions 4–6

Charcoal/Celite chromatography of pooled fractions 4–6 resulted in 80% recoveries of hexose in all cases. About 70% of the hexose recovered from

Table 1. Carbohydrate composition of fractions from mutant liver after Bio-Gel P-6 chromatography

Samples (100 μ l) of selected fractions from the mutant glycopeptide fractionation (Fig. 1) were analysed for carbohydrate by g.l.c. as described under 'Methods'. Fraction volume was 4.0 ml. Abbreviations: tr, trace; —, not detected.

Fraction	Sugar composition (μ mol/fraction)						Sialic acid
	Man	Gal	GlcNAc	GalNAc	Glu	Fuc	
27	1.02	3.63	2.36	1.24	5.80	tr	0.63
42	1.14	2.15	1.99	tr	0.25	tr	—
48	5.35	4.54	4.95	—	0.27	—	—
50	6.93	4.88	5.43	—	0.29	—	—
51	8.33	5.41	7.70	—	0.21	—	—
55	4.40	2.41	2.07	—	0.22	—	—
56	3.78	2.13	3.77	—	0.29	—	—
65	—	—	—	—	1.40	—	—
70	—	—	—	—	5.51	—	—

Fig. 1. Bio-Gel P-6 chromatography of glycopeptide fractions from normal and mutant livers

The glycopeptide fractions were chromatographed as described under 'Methods'. Samples of the column fractions were assayed for hexose by the phenol/ H_2SO_4 method and free amino groups by the trinitrobenzenesulphonic acid method. The results are expressed relative to galactose and glycine standards respectively. Symbols: ●, hexose, mutant; ○, hexose, normal; ----, amino groups, mutant. The amino-group data for the normal sample were deleted for clarity; however, the normal and mutant-liver profiles are virtually superimposable. Asterisks denote the individual mutant-liver fractions that were analysed for carbohydrate by g.l.c. (Table 1). The mutant-liver column fractions were then combined into pools as indicated by the numbered bars at the top of the Figure.

Fig. 2. T.l.c. analysis of glycopeptide pools 4, 5 and 6 and purified compounds GP5 and GP6

Samples of the concentrated Bio-Gel pools (Fig. 1) or the final purified compounds (Fig. 4) were chromatographed for 12h on silica gel 60 with propan-1-ol/acetic acid/water (3:3:2, by vol.) as solvent. The plates were stained for carbohydrate with orcinol. Lane (1), pool 4 (10 μg of hexose); lane (2), pool 5 (15 μg); lane (3), pool 6 (20 μg); lane (4), compound GP5 (5 μg); lane (5), compound GP6 (5 μg).

pool 4 eluted in the 25% ethanol fraction (Fig. 3a). This fraction was not homogeneous by t.l.c. as it contained the same two constituents found in unfractionated pool 4 (Fig. 2, lane 1). This mixture, designated compound GP4, was not purified further, but was analysed for amino acids and carbohydrates to determine the general properties of the constituents. About 60% of the hexose recovered after charcoal/Celite chromatography of pool 5 eluted in the 15% and 20% ethanol fractions (Fig. 3b), both of which contained the same single component by t.l.c. These two fractions were combined and designated compound GP5. About 55% of the hexose recovered from pool 6 eluted in the 15% ethanol fraction (Fig. 3c), which also contained a single component by t.l.c.; this fraction was designated compound GP6. It was estimated that compound GP5 comprised 70% of the total hexose in Bio-Gel pool 5, whereas compound GP6 comprised 65% of the total hexose in Bio-Gel pool 6. The minor constituents of pools 5 and 6 were recovered as mixtures in other fractions of the respective charcoal/Celite columns and were not studied further.

Sephadex G-25 chromatography of compounds GP5 and GP6 gave single sharp peaks for both

Fig. 3. Charcoal/Celite chromatography of pools 4, 5 and 6

The three major pathological peaks of hexose from Bio-Gel chromatography of the mutant glycopeptide fraction (Fig. 1) were adsorbed on charcoal/Celite columns and eluted stepwise with increasing concentrations of ethanol in water as described under 'Methods'. The volume of each ethanol fraction was 40 times the column volume. The eluted fractions were concentrated and total hexose was determined by the phenol/ H_2SO_4 method relative to a galactose standard. The bars indicate those fractions used for further studies. (a) Fractionation of 1.1 mg of hexose from pool 4; (b) fractionation of 3.8 mg of hexose from pool 5; (c) fractionation of 4 mg of hexose from pool 6.

compounds, indicating that each was homogeneous with respect to molecular weight (Fig. 4). Calibration of the molecular weights with respect to carbohydrate standards gave values of 1800 and 1350 for compounds GP5 and GP6 respectively. The apparent molecular weights of the pool 4 compounds were estimated to be 2100 on the basis of their elution volumes from the initial Bio-Gel column. The peak fractions from each Sephadex G-25 column were pooled and used for the final analyses. T.l.c. of the purified compounds (Fig. 2, lanes 4 and 5), followed by scanning densitometry of the plate, showed that 95% of the stained material in compound GP6 and 99% of the stained material in compound GP5 were associated with single orcinol-positive bands.

Characterization of compounds GP4, GP5 and GP6

Amino acid analysis. Amino acid analysis of hydrolysates of compounds GP4, GP5 and GP6 demonstrated an absence of amino acid constituents. Thus, although these compounds were isolated from a proteolytic digest of mutant liver, they apparently accumulated as oligosaccharides rather than as glycoproteins or glycopeptides.

Carbohydrate analysis. Galactose, mannose and *N*-acetylglucosamine were the only sugar constituents detected in methanolysates of oligosaccharides GP4, GP5 and GP6. The composition and the observed molecular weights of oligosaccharides GP5 and GP6 (Table 2) were consistent with the former being an oligosaccharide with nine sugar moieties, and the latter an oligosaccharide containing six sugars. The calculated molecular weights were in agreement with those observed on gel filtration.

Periodate oxidation. The galactose residues of oligosaccharides GP5 and GP6 (and the two

components of oligosaccharides GP4) were completely destroyed by periodate treatment, indicating that this sugar occupied a non-reducing terminal position in these oligosaccharides.

The partial destruction of *N*-acetylglucosamine in oligosaccharide GP5 indicated the presence of either terminal or internal C-6-substituted residues. In oligosaccharide GP5, 67% of the mannose residues were oxidized. The negligible quantity of erythritol among the oxidation products indicated that the oxidized mannose residues were probably single substituted at either C-2 or C-6 or di-substituted (C-2 and C-6). The surviving mannose was apparently di-substituted.

In oligosaccharide GP6, about 40% of the *N*-acetylglucosamine and 90% of the mannose residues were destroyed after periodate oxidation. The oxidation products were similar to those found for oligosaccharide GP5. Thus oligosaccharide GP6 also appeared to contain terminal or internal C-6-linked *N*-acetylglucosamine moieties as well as mannose residues that were unsubstituted at C-3 or C-4.

Glycolipid analysis. Lower phase. Ceramide monohexoside was the major neutral glycolipid in normal cat liver, comprising about 70% of the total lipid-bound hexose. The mutant-liver lower phase contained a normal amount of ceramide monohexoside, but had a 1.3-fold increase in neutral lipid-bound hexose compared with the controls. This difference was primarily due to the presence of a compound that reacted with α -naphthol but not with resorcinol and had the chromatographic mobility of asialo-(ganglioside $\text{G}_{\text{M}1}$) (Fig. 5a).

Upper phase. The mutant-liver upper phase had a 2-fold increase in total sialic acid compared with the controls (normal, 0.25–0.35 $\mu\text{mol/g}$ wet wt. of tissue;

Fig. 4. Sephadex G-25 chromatography of compounds GP5 and GP6

The major charcoal/Celite fractions from pools 5 and 6 (compounds GP5 and GP6) were each chromatographed on a calibrated column of Sephadex G-25 as described under 'Methods'. Samples (50 μ l) of column fractions were assayed for hexose by the phenol/ H_2SO_4 method and the results are plotted as A_{490} . Fraction volume was 1.9 ml. Symbols: $\bullet$, compound GP5; $\circ$, compound GP6. The column was standardized (Bhatti & Clamp, 1968) with the following compounds (mol.wt.): galactose, 180; sucrose, 342; stachyose, 667; human G_{M1} gangliosidosis storage oligosaccharide, 1800 ± 200 . The apparent molecular weights of compounds GP5 and GP6 were 1800 ± 200 and 1350 ± 200 respectively. The bars indicate the fractions that were pooled for final analysis.

mutant, $0.60 \mu\text{mol/g}$ wet wt. of tissue). Analysis of normal and mutant-liver samples by t.l.c. in solvent system 1 demonstrated that the upper phase of the latter contained an increased proportion of a compound that co-migrated with bovine brain ganglioside G_{M1} . In solvent system 2, the mutant-liver upper phase was resolved into at least seven resorcinol-positive bands that appeared increased relative to the

controls (Fig. 5b). The four most prominent components in the mutant sample migrated as two doublet bands. One doublet had the mobility of bovine brain ganglioside G_{M1} and the second had a mobility intermediate between those of bovine brain gangliosides G_{D1a} and G_{D1b} . None of the mutant-liver oligosaccharides migrated from the origin in solvent system 2. Examination of the upper phases in solvent system 3 revealed two major bands with increased glycolipid in the mutant-liver sample. One had the mobility of the ganglioside G_{M1} standard and the other had a lower mobility that was distinct from those of the disialo standards.

Carbohydrate analysis of the mutant-liver upper phase glycolipids after separation by t.l.c. in solvent system 2 indicated that all four of the major species contained glucose, galactose, *N*-acetylglucosamine and sialic acid in the approximate molar proportions (1:2:1:1) expected for monosialogangliosides of the ganglio-*N*-tetraoside series. The major constituents of the material remaining at the origin in this system were mannose, galactose, *N*-acetylglucosamine and glucose, indicating that the non-migrating material was probably related to the mutant-liver oligosaccharides. The minor bands in the mutant-liver sample were not analysed.

Discussion

The present results demonstrate significant hepatic accumulations of galactose-containing macromolecules in a cat with G_{M1} gangliosidosis. Both glycolipid and glycopeptide fractions from the mutant liver contain compounds that were not detected in normal tissue samples of comparable size.

Although a compound identified as asialo-(ganglioside G_{M1}) was stored in mutant liver, the most impressive evidence for glycolipid pathology was found in the ganglioside fraction. Analysis by t.l.c. showed that the major pathological gangliosides migrated as 1, 2 or 4 bands depending on the solvent system used. These glycolipids had the same carbohydrate composition as ganglioside G_{M1} . One likely possibility is that the faster- and slower-migrating doublet bands are ganglioside G_{M1} containing *N*-acetylneuraminic acid and *N*-glycolylneuraminic acid respectively. The *N*-glycolyl derivative migrates close to ganglioside G_{D1a} in solvent system 2 (Fong *et al.*, 1976), as we observed for the more polar doublet. Extra-neural gangliosides of certain species, including the cat, have been shown to contain *N*-acetyl and *N*-glycolyl sialic acids (Handa & Handa, 1965; Wiegandt, 1971). The diversity of fatty acid compositions of extra-neural gangliosides (Puro & Huttunen, 1969) and previous reports of the effects of fatty acid and long-chain base composition on the chromatographic properties of ceramide polyhexos-

Table 2. Carbohydrate analysis and periodate oxidation of oligosaccharides GP5 and GP6

Oligosaccharides GP5 and GP6 were purified by Bio-Gel, charcoal/Celite and Sephadex G-25 column chromatographies. Samples of the purified compounds were analysed for carbohydrate by g.l.c. before and after periodate oxidation as described under 'Methods'. Abbreviation: —, not detected.

Sugar composition in 50 μ l sample of:

Constituent	Oligosaccharide GP5				Oligosaccharide GP6			
	Molar ratio	Native (nmol)	Oxidized (nmol)	Percentage oxidized	Molar ratio	Native (nmol)	Oxidized (nmol)	Percentage oxidized
Man	3.1	126.5	41.1	67	2.2	179.9	20.0	89
Gal	2.0	81.2	—	100	1.0	82.7	—	100
GlcNAc	4.1	167.5	142.2	15	2.7	221.9	136.0	39
Amino acids	<0.02				<0.02			
Molecular weight								
Calculated	1680				1140			
Observed	1800 $\pm$ 200				1350 $\pm$ 200

Fig. 5. T.l.c. analysis of lipid fractions from normal and mutant liver

Lipid fractions from the mutant and a representative control liver were examined by t.l.c. on silica gel 60, along with reference standards. In each case the normal and mutant-liver samples were derived from equivalent amounts of tissue protein. The small square to the right of a band indicates a positive reaction to the detection reagent. (a) Neutral glycolipid fractions. Lane (1), standards: A, glucosylceramide; B, lactosylceramide; C, ceramide trihexoside; D, asialo-(ganglioside G_{M1}); lane (2), normal liver saponified lower phase; lane (3), mutant-liver saponified lower phase. The solvent system was chloroform/methanol/water (35:15:2, by vol.); the spots were detected by spraying with α -naphthol. (b) Acidic glycolipid fractions. Lane (1), standards: A, ganglioside G_{M2} ; B, ganglioside G_{M1} ; C, ganglioside G_{D1a} ; D, ganglioside G_{D1b} ; E, ganglioside G_T ; lanes (2) and (5), normal liver upper phase; lanes (3) and (6), mutant-liver upper phase; lanes (4) and (7) standards ganglioside G_{M1} and ganglioside G_{D1a} . Lanes (1)–(4) were detected with α -naphthol and lanes (5)–(7) with resorcinol. The solvent system was chloroform/methanol/2.5M- NH_3 (60:40:9, by vol.).

ides (Hakomori, 1966; Wiegandt & Bucking, 1970) could explain the double bands observed for compounds. The limited amount of mutant tissue available for analysis in the present study did not permit conclusive identification of the accumulated glycolipids and these structural proposals remain tentative.

The extent of oligosaccharide storage in the mutant liver was particularly striking. The three major abnormal peaks of material from Bio-Gel chromato-

graphy contained neutral hexose equivalent to about 2% of the tissue wet weight. The presence of higher-molecular-weight hexose-containing compounds (pools 2 and 3 from Bio-Gel chromatography) and the heterogeneity of the three major pools is an indication of the wide variety of galactose-containing compounds that accumulate as a result of the enzyme defect. All of the pathological oligosaccharides have the same major sugar constituents, but they appear to differ both in size and structure.

Oligosaccharide storage occurs in human metabolic disorders due to deficiencies of β -galactosidase (Wolfe *et al.*, 1974), α -fucosidase (Tsay *et al.*, 1976) and sialidase (Strecker *et al.*, 1977; O'Brien, 1978b). In each instance the pathological oligosaccharides have similar internal structures, but differ in their chain lengths and non-reducing termini, depending on which hydrolase activity is deficient. The structures of some of these human storage compounds suggest that they are derived from the asparagine-linked oligosaccharide chains of naturally occurring glycoproteins, such as erythrocyte stromal glycoprotein and the immunoglobulins (Wolfe & Ng Ying Kin, 1976). By analogy, the storage oligosaccharides in cat liver may have similar origins.

The two major storage oligosaccharides in the cat appear to be similar to compounds isolated from human G_{M1} gangliosidosis liver and urine (Wolfe & Ng Ying Kin, 1976) with respect to carbohydrate composition and molecular weight. However, the oligosaccharides from cat contain a higher ratio of *N*-acetylglucosamine to galactose as well as periodate-sensitive *N*-acetylglucosamine residues. Further, both oligosaccharides GP5 and GP6 were distinct from the major human storage oligosaccharide on t.l.c. (E. W. Holmes & J. S. O'Brien, unpublished work). Perhaps the differences between the cat and human compounds reflect species differences in the oligosaccharide structures of the glycoproteins from which they originate.

The compositional and periodate oxidation data suggest that the hexasaccharide GP6 may have the structure:

with an additional *N*-acetylglucosamine linked to either of the two mannose residues at C-2 or C-6. This tentative structure has a terminal β -linked galactose as indicated by the periodate studies, in keeping with the β -D-galactosidase deficiency in the mutant animal. The two periodate-sensitive mannose residues would give rise to glycerol on oxidation (unsubstituted at C-3 and C-4) as observed. Since 40% of the *N*-acetylglucosamine residues were periodate-sensitive, this suggests that one of the three is in a terminal position. The oligosaccharide GP5 might represent an elaboration of this structure containing an additional ($\leftarrow\beta 1\text{Gal})\text{GlcNAc1}\rightarrow 2\text{Man}$ linked (at C-3 or C-4) to either of the mono-substituted mannose residues, protecting it from oxidation. Linkage of this trisaccharide to the internal mannose of the proposed structure would form a branched trimannosyl core similar to that found in the human storage oligosaccharides (Wolfe *et al.*, 1974; Tsay *et al.*, 1976). The structural heterogeneity indicated by the incomplete oxidation of mannose residues in oligosaccharide GP6 and the partial

oxidation of *N*-acetylglucosamine residues in oligosaccharide GP5, as well as the possibility of minor compositional heterogeneity in both compounds, require that additional structural studies be carried out to elucidate the precise structures.

Recent studies of human G_{M1} gangliosidosis (Tsay & Dawson, 1973; Calatroni, 1974; Wolfe & Ng Ying Kin, 1976) suggest that keratan sulphate is not stored in the viscera of patients; however, the storage of ketatan sulphate-related glycopeptides has been reported in some cases (Tsay & Dawson, 1973; Calatroni, 1974), but not in others (Wolfe & Ng Ying Kin, 1976). Initial studies of the glycosaminoglycan fractions from normal and mutant cat liver revealed no major differences, but, because the method of tissue fractionation did not ensure the quantitative recovery of keratan sulphate, we cannot rule out the possibility of hepatic keratan sulphate storage in the mutant cat. In fact, some of the carbohydrate-containing compounds in the void volume after Bio-Gel chromatography of the mutant-liver glycopeptides may be keratan sulphates that were not precipitated by cetylpyridinium chloride. Bio-Gel chromatography of the mutant-liver glycopeptides indicated that keratan sulphate-related glycopeptides (Tsay & Dawson, 1973) are not among the major hepatic storage compounds in mutant cats. Further analysis of the storage compounds from affected cats and humans may resolve the conflict over this aspect of the biochemical pathology in G_{M1} gangliosidosis.

The similarities in the biochemical pathology of cat and human G_{M1} gangliosidosis is further evidence for the suitability of the cat as a model system for the study of the clinical, biochemical and genetic aspects of this rare storage disorder. The cat model may also be useful in the development of techniques for therapeutic intervention in the lysosomal storage disorders. A decrease in the tissue storage or urinary excretion of the pathological oligosaccharides described in this report would seem to offer a sensitive method for assessing the success of various kinds of corrective therapy.

This work was supported by Sloan Foundation to E. W. H. and National Institutes of Health grant NS 08682 to J. S. O'B.

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