Tissue distribution of glycosphingolipids in a case of Fabry's disease

JAMES M. SCHIBANOFF, SHIGEHIKO KAMOSHITA,* and JOHN S. O'BRIEN‡

Department of Pathology, University of Southern California, School of Medicine, Los Angeles, California 90033, and the Department of Pathology, Children's Hospital, Los Angeles, California 90033

ABSTRACT A survey was made of the glycolipid composition of various tissues, including liver, spleen, kidney (cortex and medulla), lymph node, pancreas, prostate gland, heart muscle, thenar muscle, gastrointestinal smooth muscle, frontal cerebral cortex, anterior thalamus, brain stem, a peripheral autonomic ganglion, and renal arterial intima and media, from a patient who died with Fabry's disease. The tissues had been fixed in formalin for 3 yr. Analytical data on trihexosyl ceramide from heart muscle and pancreas indicate a structure identical to trihexosyl ceramide from kidney: galactosylgalactosylglucosyl ceramide. Fatty acid compositions of trihexosyl ceramide and dihexosyl ceramide revealed a wide range of fatty acids, with 16:0, 18:0, 20:0, 22:0, 24:0, and 24:1 predominating. These glycolipids comprised 10–41% of the total lipid in the formalin-fixed organs studied. Trihexosyl ceramide predominated in all tissues and was the only glycolipid found in muscle tissues, lymph node, and arterial tissues. Dihexosyl ceramide was found in kidney, pancreas, liver, spleen, and cerebral tissues. The accumulation of trihexosyl ceramide in cardiac muscle and arterial tissues may account in part for the cardiovascular complications so prominent in Fabry's disease.

SUPPLEMENTARY KEY WORDS trihexosyl ceramide · dihexosyl ceramide · organs · muscular tissue · nervous tissue

FABRY'S DISEASE (angiokeratoma corporis diffusum) is an inborn error of glycolipid metabolism transmitted in an X-linked recessive manner (1). Hemizygous males have a characteristic angiokeratomatous rash as well as peripheral burning pain, renal dysfunction, and ischemic coronary heart disease. The average age of males at death is 41 yr and the cause of death is usually chronic renal failure or cardiovascular disease (2).

Sweeley and Klionsky (3) demonstrated that two glycolipids accumulate in the kidney in Fabry's disease; the first, a trihexosyl ceramide (galactosylgalactosylglucosyl ceramide) is stored in approximately threefold higher proportions than the second, a dihexosyl ceramide (galactosylgalactosylceramide). Recently, Brady, Gal, Bradley, Martensson, Warshaw, and Laster (4) demonstrated that tissues of patients with Fabry's disease are deficient in a hydrolytic enzyme which cleaves the terminal galactose from trihexosyl ceramide. Fabry's disease appears to be a manifestation of a glycolipid hydrolase deficiency.

Birefringent lipid material accumulates in the cytoplasm of various cells, especially glomerular epithelial cells, vascular epithelial cells, smooth and striated muscle cells, and neurons of the autonomic nervous system (1). It is not known whether the two glycolipids that accumulate in the kidney are stored in all affected tissues. Bagdade, Parker, Ways, Morgan, Lagunoff, and Eidelman (5) have recently demonstrated the accumulation of trihexosyl ceramide, but not dihexosyl ceramide, in lung, and Loeb, Jonniaux, Tondeur, Danis, Gregoire, and Wolff (6) report a similar finding in liver. The present report is concerned with the distribution of both glycolipids in the central nervous system, liver, spleen,

Abbreviations: C–M, chloroform–methanol. Fatty acids are designated by number of carbons: number of double bonds.

* Present address: Department of Pediatrics, Tokyo University, School of Medicine, Tokyo, Japan.

‡ Present address: Department of Neurosciences, University of California at San Diego, School of Medicine, La Jolla, California 92037.

kidney, pancreas, lymph node, heart muscle, striated muscle, smooth muscle, prostate gland, vascular epithelium, and a peripheral autonomic ganglion from a patient who died with Fabry's disease.

Case History

The patient was a 37 yr old Caucasian male with a past history of episodic anginal chest pain of 6 yr duration and chronic renal disease of 10 yr duration with progressive renal failure. He also complained of easy fatigability and of episodic burning pain in his extremities. The patient was an only child and the family history was noncontributory. Peripheral neuropathy typical of Fabry's disease was evident. At age 37 yr, the patient died 2 days after an acute anterior myocardial infarction.

Pathological Examination

Pathological examination revealed a large recent myocardial infarct in the left ventricular wall as a result of a left anterior coronary artery thrombosis. Microscopic examination of the heart revealed fine sudanophilic, periodic acid–Schiff-positive granules in myocardial and vascular epithelial cells. Tubular and glomerular epithelial cells in the kidney were ballooned with cytoplasmic vacuolar material which, on frozen sections, stained intensely with the periodic acid–Schiff reagent and Sudan Black B.

Neurolipidosis was observed almost exclusively in the neurons of the autonomic nervous system, namely, the myenteric plexus, the substantia nigra, the anterior thalamus, and certain areas of the hypothalamus. Myelin of the internal capsule, cerebral peduncle, and other minor tracts was normal in appearance.

METHODS

Tissues had been preserved for 3 yr in formalin prior to analysis. These included liver, spleen, kidney (cortex and papilla), lymph node, pancreas, prostate gland, heart muscle, striated muscle (thenar muscle from the foot), smooth muscle from the media and intima of the gastrointestinal tract (jejunum), frontal cerebral cortex, anterior thalamus, brain stem at the junction of the pons and midbrain, and a peripheral autonomic ganglion overlying and renal artery. Intima and media from the renal artery were dissected and analyzed separately; no claim is made for complete separation of each.

Lipid Analysis

Each specimen was extracted with chloroform–methanol (C-M) 2:1 as described previously (7). An aliquot of the total lipid extract was chromatographed on plates coated with Silica Gel H in chloroform–methanol-water 65:25:4 to give the total lipid pattern of each tissue. Lipids were made visible by charring with sulfuric acid–dichromate (8); glycolipids were selectively visualized by means of the anthrone reagent (9). Glycosphingolipids were isolated from the total lipid extract of each tissue by chromatography on Florisil and silicic acid columns (10). The total lipid extract was applied to a Florisil column in chloroform and eluted with chloroform (cholesterol and neutral lipids), C–M 9:1 (cerebroside and free fatty acids), and C–M 2:1 followed by methanol (dihexosyl ceramide and trihexosyl ceramide).

Fractions containing the glycosyl ceramides were pooled and chromatographed on thin-layer plates to reveal the glycolipid distribution in each tissue; the mixture was also applied to silicic acid columns and dihexosyl ceramide was eluted with C–M 9:1, trihexosyl ceramide with C–M 7:3. In some instances the glycosyl ceramides were purified by preparative thin-layer chromatography as a band on Silica Gel G in C–M–water 65:25:4. The two glycolipids were detected as white bands after the plate had been sprayed with water and were eluted from the adsorbent with methanol. Prior to carbohydrate or fatty acid analysis, each lipid was purified by thin-layer chromatography until it gave a single spot.

After purification the glycosyl ceramides were analyzed as follows. Hexose content was determined by the anthrone method (9). Glucose and galactose analyses were performed by gas–liquid chromatography of the fully acetylated hexoses (10). For fatty acid analyses, lipids were hydrolyzed in 2 N HCl and the fatty acids were isolated by chromatography on silicic acid columns (11). After methylation, the fatty acid composition was determined by gas–liquid chromatography on a 3% Apiezon L column (11, 12). Although dihexosyl ceramide contained hydroxy fatty acids (trihexosyl ceramide contained none), no attempt was made to isolate and quantify these. Fatty acids were identified by their carbon numbers (13) by comparison with available standards. Quantitative results with standards agreed with 7% for minor constituents (less than 5% of the total mixture) and 4% for major constituents (more than 10% of the total mixture).

RESULTS

Analytical data on trihexosyl ceramide (Table 1) isolated from heart muscle and pancreas indicate a structure consistent with that reported by Sweeley and Klionsky (3) for this lipid isolated from Fabry's kidney. The sugar content, the ratios of galactose to glucose, and the fatty acid content were close to theoretical for galactosyl-galactosylglucosyl ceramide. Dihexosyl ceramide isolated from pancreas and kidney gave analytical results close to those expected for galactosylgalactosyl ceramide

TABLE 1 ANALYTICAL DATA ON GLYCOLIPIDS IN FABRY'S DISEASE

	Trihexosyl Ceramide			Dihexosyl Ceramide		
	Heart	Pancreas	Theor.	Kidney	Pancreas	Theor.
Hexose content*	48%	43%	(46%)	36%	38%	(36%)
Galactose:Glucose	2.1	1.7	(2.0)	All Galactose		
Fatty acid content†	24%	22%	(26%)	27%	30%	(28%)

* Anthrone analysis.
† Weighed after isolation by column chromatography.

with regard to hexose content, absence of glucose, and fatty acid content (Table 1). The composition of unsubstituted fatty acids of trihexosyl ceramide (Table 2) from heart and pancreas and dihexosyl ceramide from kidney was qualitatively similar to that reported by Sweeley and Klionsky (3) for trihexosyl ceramide from kidney. A wide range of fatty acids from 14 to 24 carbons was present and polyunsaturated fatty acids were not detected. The major fatty acids were 16:0, 18:0, 20:0, 22:0, 24:0, and 24:1. However, the proportions of these fatty acids differed somewhat from tissue to tissue.

The concentration of glycosyl ceramides in the lipids from each organ and tissue (Table 3) was obtained by weighing both glycolipids eluted from Florisil columns and calculating glycolipid content as a percentage of the total lipid. We have determined (S. Okada and J. S. O'Brien, unpublished) the total percentage of the two glycolipids in lipids of human tissue that had not been formalinized. These values were: brain, 0.1%; liver, less than 0.1%; spleen, 0.4%; and kidney, 0.6%. The ratio of trihexosyl ceramide to dihexosyl ceramide is 3:1 to 5:1 in each tissue. The concentrations in fresh muscle tissue are lower than that in liver and have not been accurately defined. Heslinga and Deierkauf (14) have shown that formalin treatment does not greatly alter the concentrations of other glycosyl ceramides (cerebroside and cerebroside sulfate) in brain. If we assume, then, that formalin does not greatly alter the concentrations of dihexosyl and trihexosyl ceramides in the tissues studied here, and that the total lipid concentrations of the tissues from the patient are close to normal, the magnitude of the glycosyl ceramide accumulation in the patient's tissues is 30-to 300-fold normal. These numbers must be taken as rough approximations until glycolipids are quantified on unfixed tissues in Fabry's disease. However, they do indicate that the magnitude of the glycolipid accumulation is very large.

In each tissue the glycosphingolipids constituted between 10 and 41% of the total lipid (Table 3). All types of muscle tissue accumulated large amounts of glycolipid, especially arterial media, striated muscle, and smooth muscle from the prostate gland. Autonomic ganglion contained especially high concentrations of trihexosyl ceramide.

TABLE 2 FATTY ACID COMPOSITIONS OF GLYCOLIPIDS

	Trihexosyl Ceramide		Dihexosyl Ceramide, Kidney
	Pancreas	Heart	
14:0	0.8	0.5	1.5
15:0	1.1	tr	2.0
16:1	1.4	0.8	2.7
16:0	9.0	4.1	16.0
17:0	0.3	tr	0.5
18:1	1.7	1.9	13.1
18:0	3.3	15.9	8.3
19:0	0.3	0.7	
20:1	0.2	tr	0.4
20:0	5.9	10.2	6.1
22:1	15.7	1.0	3.9
22:0	17.2	24.5	18.8
23:0	5.1	9.3	2.0
24:1	14.6	20.8	9.5
24:0	23.4	10.3	15.4

Each fatty acid is expressed as a percentage of the total unsubstituted fatty acids of each lipid. Hydroxy fatty acids, present in dihexosyl ceramide only, were not analyzed.

TABLE 3 GLYCOLIPID CONTENT OF TISSUE LIPIDS

Organs		Muscular Tissue		Nervous Tissue	
Spleen	13	Heart	25	Cortical gray matter	10
Liver	20	Striated muscle (foot)	35	Thalamus	20
Kidney	24	Smooth muscle (jejunum)	30	Brain stem	22
Pancreas	31	Renal artery	38	Autonomic ganglion	41
Lymph Node	15	Prostate	38		

All values are the sum of both glycolipids expressed as a percentage of the total lipid in each tissue.

A chromatographic study of the glycolipids from each organ (Figs. 1–3) revealed that trihexosyl ceramide was present in all tissues analyzed. Kidney contained both glycosyl ceramides, trihexosyl ceramide being present at levels visually estimated to be 3- to 4-fold higher than dihexosyl ceramide. The proportions of these two glycolipids seemed (Fig. 1) to be the same in renal outer cortex and in medullary tissue dissected from the renal pelvis. This finding suggests no preferential storage of either glycolipid in a glomerulus-rich (cortex) or a tubule-rich (papilla) region. Pancreas also contained dihexosyl ceramide in readily detectable amounts. A relatively high proportion of the dihexosyl ceramide that contains hydroxy acids (slower-moving dihexosyl ceramide spot in Fig. 1) was found in pancreatic tissue. Spleen and liver lipids contained only small amounts of dihexosyl ceramide, trihexosyl ceramide being the most important glycolipid. Lymph node contained almost

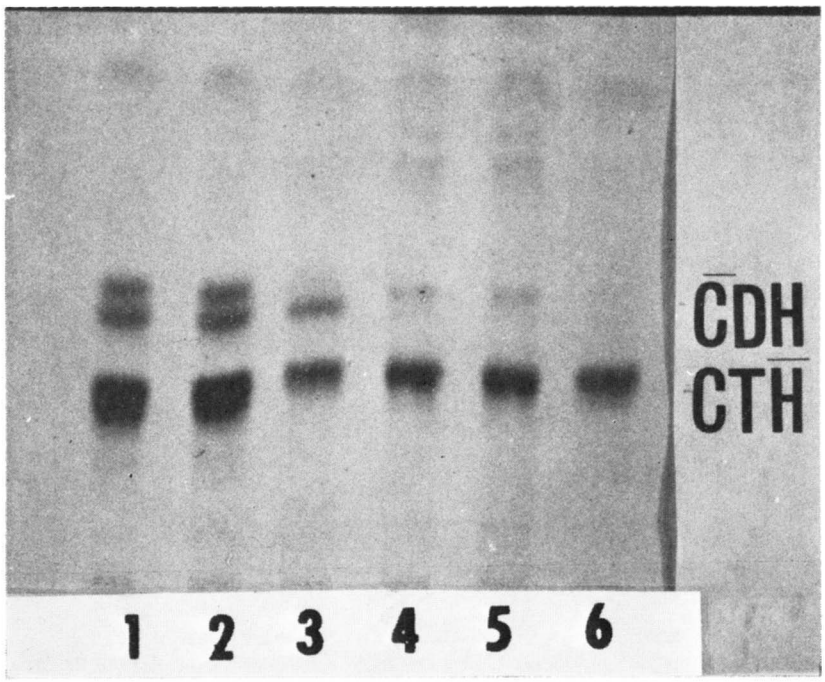

FIG. 1. Thin-layer chromatography of glycosphingolipids isolated from (*1*) renal cortex, (*2*) renal medulla, (*3*) pancreas, (*4*) spleen, (*5*) liver, and (*6*) lymph node. Major spot is trihexosyl ceramide (CTH); faster-moving double spot is dihexosyl ceramide (CDH).

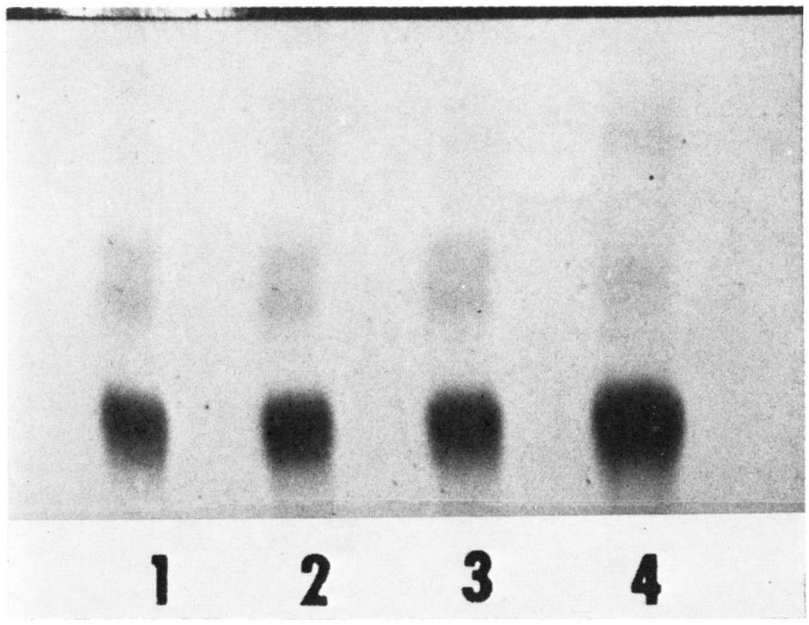

FIG. 2. Thin-layer chromatography of glycosphingolipids isolated from (*1*) frontal cortical gray matter, (*2*) anterior thalamus, (*3*) brain stem, and (*4*) a peripheral autonomic ganglion overlying the renal artery.

exclusively trihexosyl ceramide, dihexosyl ceramide being detected in only trace amounts.

Cerebral tissue contained both glycosyl ceramides, with trihexosyl ceramide predominating (Fig. 2). The central nervous tissues (frontal cortex, thalamus, and brain stem) appeared to contain higher proportions of dihexosyl ceramide than the peripheral autonomic ganglion.

The muscular and vascular epithelial tissues were strikingly similar in glycolipid composition regardless of location (Fig. 3). Cardiac muscle, gastrointestinal smooth muscle, arterial intima and media, striated

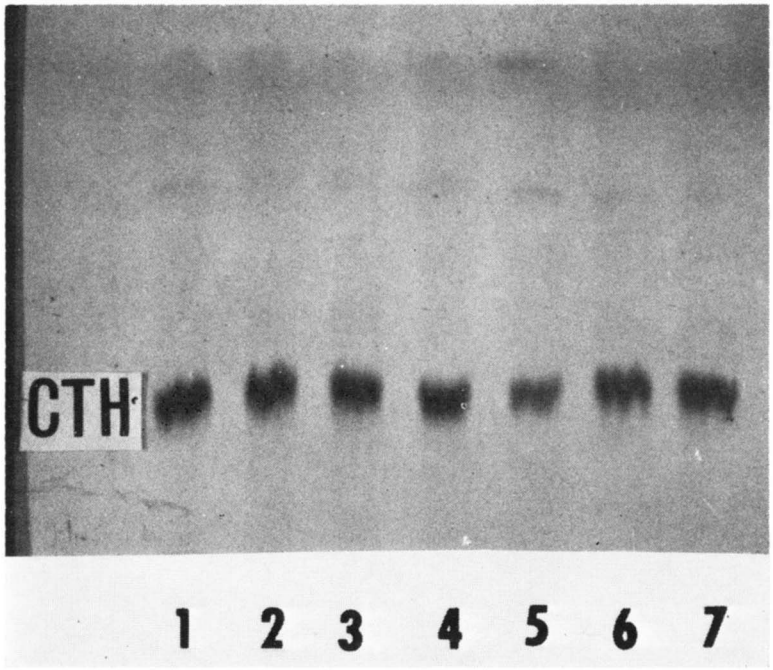

FIG. 3. Thin-layer chromatography of glycosphingolipids isolated from (*1*) striated muscle (foot), (*2*) heart muscle, (*3*) smooth muscle (media of jejunum), (*4*) intima of jejunum, (*5*) prostate gland, (*6*) renal arterial media, and (*7*) renal arterial intima.

muscle, and prostatic smooth muscle all contained trihexosyl ceramide as the major glycolipid. Dihexosyl ceramide was occasionally detected but in trace amounts only.

DISCUSSION

This study demonstrates that the major glycolipid that accumulates in a variety of organs and tissues in Fabry's disease is trihexosyl ceramide. Analytical data on trihexosyl ceramide from heart muscle and pancreas suggest that it is identical with trihexosyl ceramide from kidney. Of particular interest is the fact that trihexosyl ceramide accumulates in the muscular tissues examined, including cardiac muscle, smooth muscle, and striated muscle. The accumulation of a sphingolipid in muscles is unique among the sphingolipidoses.

The pathogenesis of Fabry's disease may be explained in part on the basis of the distribution of trihexosyl ceramide. Arterial epithelial cells are especially involved in the cytoplasmic lipidosis (unpublished observations). The increase in the size and number of these cells leads to intimal hyperplasia of the medium-sized vessels. It seems likely that intimal hypertrophy of coronary and cerebral vessels, resulting from vascular epithelial lipidosis, predisposes towards coronary and cerebral thrombosis, ischemia, and infarctions.

The accumulation of trihexosyl ceramide in muscle fibers might also result in muscular weakness in Fabry's disease. Leder and Bosworth (15) reported a 27 yr old patient with Fabry's disease who died after mitral commisurotomy due to ventricular fibrillation. They speculate that the sudden increased ventricular load following mitral commissurotomy may have been too great a burden for the weakened myocardial muscle cells. One wonders also whether the cramps, malaise, and easy fatigability of patients with Fabry's disease may be related in part to the large accumulations of glycolipid in striated muscle fibers throughout the body.

The analytical data also confirm what has been noted pathologically, namely, that the autonomic nuclei in the brain stem and peripheral autonomic ganglia are especially involved in glycolipid storage (16). The glycolipid concentration is higher (Table 2) in tissues rich in autonomic neurons (thalamus, brain stem, and an autonornic ganglion) than in gray matter of the cerebral cortex.

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