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Glycolipids of the Spinal Cord, Sciatic Nerve, and Systemic Organs of the Twitcher Mouse

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Abstract. The glycolipid composition of the spinal cord, sciatic nerve, kidney, liver and lung of twitcher mice, neurological mutants caused by a genetic deficiency of galactosylceramidase activity, was analyzed during development. No abnormal increase of galactosylceramide or sulfatide was detected in the spinal cord and the sciatic nerve at any age. Galactosylceramide and sulfatide in the whole tissue were in fact decreased in the spinal cord toward later stages of the disease. These findings were qualitatively similar but quantitatively milder than those in the genetically and enzymatically equivalent human disease, globoid cell leukodystrophy (Krabbe disease). Lactosylceramide was abnormally increased in both the spinal cord and the sciatic nerve, but glucosylceramide was increased only in the sciatic nerve. In contrast, there was a massive accumulation of galactosylceramide in the systemic organs. The hydroxy-fatty acid-containing (HFA-) galactosylceramide was predominant in all organs. In the kidney at 42 days, HFA-galactosylceramide was 50-fold and the non-hydroxy-fatty acid-containing (NFA-) galactosylceramide 5-fold higher than the normal levels. Although the absolute amount of galactosylceramide in the liver and lung was much smaller, it was also increased greatly in the twitcher mouse. Lactosylceramide was slightly to moderately increased in the systemic organs but there was no abnormality in glucosylceramide. The analytical findings in the twitcher kidney were in sharp contrast to those in the human disease, in which no abnormal accumulation of galactosylceramide occurs. The abnormal accumulation of the substrate for the deficient enzyme is more consistent with the concept of "lysosomal storage disease". Even though the underlying genetic defect is the same, its consequences show clear species differences.

Key Words: Glycolipids; Kidney; Sciatic nerve; Spinal cord; Twitcher mouse.

INTRODUCTION

Globoid cell leukodystrophy is known in man, sheep, cat, dog and mouse (1, 2). The disease in all species exhibits similar clinical and pathological manifestations. Except for the disease in the cat, which was not studied enzymatically (3), the underlying genetic cause of globoid cell leukodystrophy in all species is a deficiency of galactosylceramidase activity [EC 3.2.1.46]. Because of the constraints of rarity and ethical considerations in studying human patients, the genetically authentic disease in other mammalian species can serve as an invaluable model for studies of globoid cell leukodystrophy. The canine model was utilized extensively for this purpose (1). The more recently discovered murine mutant, the twitcher, however, is finding a wider acceptance as an experimental tool because of its rapid reproduction and ease of maintenance (4). The use of clipped tail for determination of the genetic

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status almost immediately after birth further contributes to efficient experimental designs and colony maintenance (5).

The clinical and neuropathological features of the twitcher mutant are fundamentally the same as those in the human disease (6), as expected from the same genetic cause (7). Nevertheless, some significant differences have been noted between human and murine globoid cell leukodystrophy. The central nervous system (CNS) involvement is milder while the peripheral nervous system (PNS) involvement is much more severe in the murine disease (6, 8). The lipid composition of the twitcher brain during development was consistent with this relatively mild CNS manifestation (9). More recently, abnormal inclusions were found in the lymph nodes and kidney of affected mice in contrast to the apparently normal histological appearance in human patients (10). This morphological difference was reflected in the enormous accumulation of galactosylceramide in the kidney of older affected mice (11, 12) in contrast to no accumulation in human patients (13). The finding in the twitcher came as a surprise because we had considered that the lack of abnormal galactosylceramide accumulation was a unique feature of globoid cell leukodystrophy in general. At least for the kidney, it may be unique only for the human disease. It was clear that more careful characterization of the murine model was required in order for experimental results obtained on this model to be interpreted properly. In this report we describe the results of analytical studies of the spinal cord, sciatic nerve, kidney, liver and lung of the twitcher mouse. A preliminary report has appeared in abstract form (14).

MATERIALS AND METHODS

Animals

A colony of the twitcher mutant is maintained in the animal facility of this institution. The genetic status of each animal—normal, carrier and affected—was determined soon after birth by galactosylceramidase assays on clipped tails (5). All mice were kept with their mothers until weaning. The diagnosis of affected mice was readily confirmed by clinical disease after 25 days. After 30 days, the standard laboratory animal chow was soaked with tap water and was placed on the floor of the cage to facilitate feeding by affected mice. It was still difficult to maintain affected mice beyond 45 days. At 16, 25 and 42 days, the animals were weighed and killed by decapitation. The spinal cord, liver, bilateral sciatic nerves, kidneys and lungs, were carefully dissected out, free of the surrounding connective tissues and were kept frozen in air-tight containers until analysis. The liver specimens were analyzed for 25 and 42 days, and the lung specimens only for 42 days.

Analytical Procedures

The spinal cords from two animals were pooled for each 16-day-old sample. The spinal cords from the older animals were analyzed individually, except for one 25-day-old twitcher sample for which two spinal cords were pooled. Bilateral sciatic nerves from two mice (i.e. four nerves) were pooled for 16- and 25-day samples, while bilateral nerves from single mice were combined for the 42-day samples. At all ages, bilateral kidneys and lungs of individual mice were combined as a sample. For all of the 16-day and 25-day kidney specimens, male animals were used for analysis, while female and male samples were separately analyzed at 42 days. Liver samples were not pooled but analyzed individually. Samples of the same organs from the same ages were processed together simultaneously.

The tissues were weighed and extracted with 19 volumes (vol) of chloroform–methanol (2:1, v/v) (15). Sciatic nerves from 16- and 25-day-old mice were first homogenized in 0.35 ml of water with a motor-driven all-glass Potter-Elvehjem homogenizer, the protein content determined on an aliquot (16), and then the remainder extracted with 19 vol of chloroform–methanol (2:1, v/v). The major tissue constituents were fractionated into the chloroform–

methanol-insoluble and soluble fractions. The latter was further partitioned into the upper water-soluble and the lower chloroform-soluble fractions (17). These three major fractions were dried and weighed, and their sum was taken as the dry weight of the tissue. Due to the generally small amounts of the available materials, the lower chloroform-soluble fraction was designated as the "total lipid" fraction and was used for subsequent analysis without elimination of small amounts of the protein initially soluble in the chloroform–methanol mixture.

The total lipid fraction was dissolved in a known volume of chloroform–methanol (2:1, v/v), and the total lipid phosphorus (18, 19) was determined. The total phospholipid was estimated by an assumption that the average molecular weight of phospholipids was 775. The mercuric chloride-base treatment was according to Abramson et al (20). After the treatment, which eliminated most of the glycerophospholipids, the remaining lipid was subjected to silicic acid column chromatography according to Norton and Autilio (21). The first chloroform–methanol (98:2, v/v) fraction was discarded. Then the glycolipids of interest were eluted with chloroform–methanol (80:20, v/v). This procedure eliminated the bulk of cholesterol in the first fraction, left sphingomyelin on the column, and improved the thin-layer chromatographic (TLC) resolution of the eluted glycolipids in the subsequent analyses.

Identification and quantitative determination of cholesterol and glycolipids were by thin-layer chromatography and densitometry. High-performance silica gel 60 plates from E. Merck (purchased from American Scientific Products, Rochester, NY) were used with or without borate impregnation. The borate-impregnated plates were prepared by suspending the plate in a chromatographic chamber for 20 hours over a mixture of methanol and 4% aqueous sodium tetraborate decahydrate (3:1, v/v). They were activated at 120° for 60 minutes and stored in a desiccator until used. Although we do not know the exact mechanism by which borate impregnation occurs, exposure of the thin-layer plate to the vapor phase only was far superior to direct contact of the plate with the liquid phase, either by spraying or by immersion and ascending development, in yielding uniform impregnation and better resolution of glycolipids. At least two and usually more of the following four TLC systems were used for identification of glycolipids by co-migration with authentic standards: I. total untreated lipid on a plain TLC plate in the solvent system of chloroform–methanol–water (65:25:4, by volume); II. mercuric chloride–base-treated lipid on a plain plate in the same solvent system as in I; III. mercuric chloride–base-treated lipid on a plain plate in chloroform–methanol–conc ammonia (65:25:5, by volume); and IV. same as in III except the borate-impregnated plate was used.

Quantitation of individual glycolipids was done by the following TLC systems depending on the amounts available and on the optimal separation of the respective compounds from other interfering materials. System I was used for determination of cholesterol in all samples, and galactosylceramide and sulfatide in 42-day-old spinal cord and sciatic nerve samples. System III was used for renal sulfatide and renal lactosylceramide of all ages. System IV was used for determination of all glycolipids in all other samples. The lipid samples were dissolved in chloroform–methanol (2:1, v/v) at 5 mg/ml (untreated lipid) or, when the sample had been subjected to the mercuric chloride- saponification-silicic acid chromatography treatment, at 10–20 mg equivalent of untreated lipid/ml for the spinal cord and sciatic nerve samples, and 50 mg equivalent/ml for the renal and pulmonary samples. For saponified hepatic lipid, the concentration was 100–200 mg equivalent/ml. Up to 10 μl of the lipid sample was spotted on the TLC plate as a 5-mm streak. During the procedure, the sample tubes were kept in an ice-bath and great care was exercised to minimize evaporation of the solvent. Two different amounts of each sample from the same age group, and at least three different amounts of standard glycolipids were always applied to a single plate and analyzed together to correct for possible plate-to-plate variations. The spots were visualized by the cupric acetate spray and heating (22). Quantitation was done with a Shimadzu CS-910 dual wave-length TLC scanner equipped with a CR-1B data processor. Densitometry was carried out at 350 nm with the reflectance linear scanning mode with the appropriate channel of the linearizer. In rare instances when the density of cholesterol spots was too high, the wave-length of 550 nm was used. The amounts of standards on the plate were chosen so that the sample density did not

fall outside the range of the standards. The amount of the sample was calculated against the standards on the same plate. The standard for galactosylceramide was a mixture of hydroxy–fatty acid-containing (HFA-) and nonhydroxy–fatty acid-containing (NFA-) compounds and an assumption was made that their absorbance for unit-weight was the same.

Commercial sources of the standards were as follows: cholesterol and sulfatide (Applied Science Labs, State College, PA), glucosylceramide (Sigma Chemical Co., St. Louis, MO), lactosyl–stearoyl–dihydrosphingosine (Miles Labs, Elkhart, IN), and galactosylceramide (either Sigma Chemical Co., or Research Products International, Mount Prospect, IL).

RESULTS

Major Tissue Constituents

Spinal Cord and Sciatic Nerve (Tables 1 & 2): Despite the precaution taken to prevent drying of tissues during storage, there was evidence of loss of water in some samples. Sciatic nerves began dehydrating even while being dissected. We therefore considered the wet weight unreliable. In addition, the very small amounts available for the 16- and 25-day-old sciatic nerve samples made the determination of dry weight less reliable than those for other specimens. The protein content was used for those samples as a supplementary basis for interpretation of the analytical results.

The size and dry weight of the twitcher spinal cords were less than normal at 42 days. At 42 days, the proportion of "total lipid" was also reduced and that of the chloroform–methanol-insoluble residue increased. Because of the decrease in dry weight, the lipid content of the whole spinal cord in the 42-day-old twitcher mice was approximately one-third of normal. The proportion of free cholesterol and total phospholipids was unaltered on the basis of total lipid even at 42 days. These major constituents in twitcher spinal cord did not show any abnormalities at 16 and 25

TABLE 1
Major Constituents of Spinal Cord

Age and animal	Dry weight* (mg)	C-M insoluble residue	"Total lipid"	Upper phase solid	Cholesterol (free)	Phospholipid (total)
		(% of dry weight)			(% of "total lipid")	
16 Days						
Control	4.1	45.4	39.4	15.2	21.3	52.6
Control	4.9	48.3	40.4	11.3	21.8	61.0
Twitcher	4.1	47.1	37.2	15.7	22.6	53.6
Twitcher	3.6	47.6	38.8	13.6	20.4	55.0
25 Days						
Control	7.8	40.9	48.4	10.7	24.5	49.0
Control	8.7	42.7	46.0	11.3	21.7	56.6
Twitcher	5.1	46.5	42.4	11.0	22.3	52.6
Twitcher	8.8	43.9	45.1	11.0	25.0	49.6
42 Days						
Control	14.8	34.5	44.6	20.9	20.1	40.6
Control	15.2	34.9	49.3	15.8	18.5	41.8
Twitcher	5.3	39.6	35.8	24.5	20.3	42.6
Twitcher	8.5	44.7	35.3	20.0	17.5	40.8

* Per animal, irrespective of pooling for analysis.

TABLE 2
Major Constituents of Sciatic Nerve

Age and animal	Dry weight* (mg)	Protein* (mg)	"Total lipid" (% of dry weight)	Cholesterol (free) (% of "total lipid")	Phospholipid (total) (% of "total lipid")
16 Days					
Control	1.5	0.16	34.0	14.1	26.2
Control	0.8	0.19	60.0	13.5	28.0
Twitcher	1.8	0.46	34.6	14.6	33.6
Twitcher	1.4	0.47	44.9	14.2	32.2
25 Days					
Control	0.9	0.26	66.1	18.2	38.0
Control	1.6	0.45	47.9	19.2	44.8
Twitcher	1.6	0.66	38.8	15.1	39.2
Twitcher	2.5	1.03	36.3	16.8	39.2
42 Days					
Control	3.7	—	51.4	17.9	27.3
Control	3.6	—	50.0	15.9	31.3
Twitcher	5.0	—	34.0	18.2	32.3
Twitcher	4.0	—	30.0	20.1	33.0

* Bilateral nerves.

TABLE 3
Major Constituents of Kidney

Age and sex	Water content (%)	C-M insoluble residue (% of dry weight)	"Total lipid" (% of dry weight)	Upper phase solid (% of dry weight)	Cholesterol (free) (% of "total lipid")	Phospholipid (total) (% of "total lipid")
16 Days						
Control, male	76.3	68.2	16.6	15.3	12.8	64.0
Control, male	79.8	73.2	16.0	10.7	12.8	65.0
Twitcher, male	76.8	67.7	17.1	15.3	10.3	58.6
Twitcher, male	78.8	69.1	16.3	14.7	12.9	64.6
25 Days						
Control, male	71.5	70.5	19.5	9.9	10.0	51.6
Control, ?	72.3	71.0	17.4	11.6	11.4	60.6
Twitcher, male	71.1	70.1	19.1	10.8	9.5	46.0
Twitcher, male	69.2	73.1	16.4	10.4	11.1	57.0
42 Days						
Control, female	67.9	71.0	17.6	11.4	10.5	56.6
Control, male	69.4	71.3	19.0	9.7	11.1	50.0
Twitcher, female	63.6	74.7	16.8	8.5	12.0	57.6
Twitcher, male	67.3	73.0	15.8	11.2	11.4	61.6

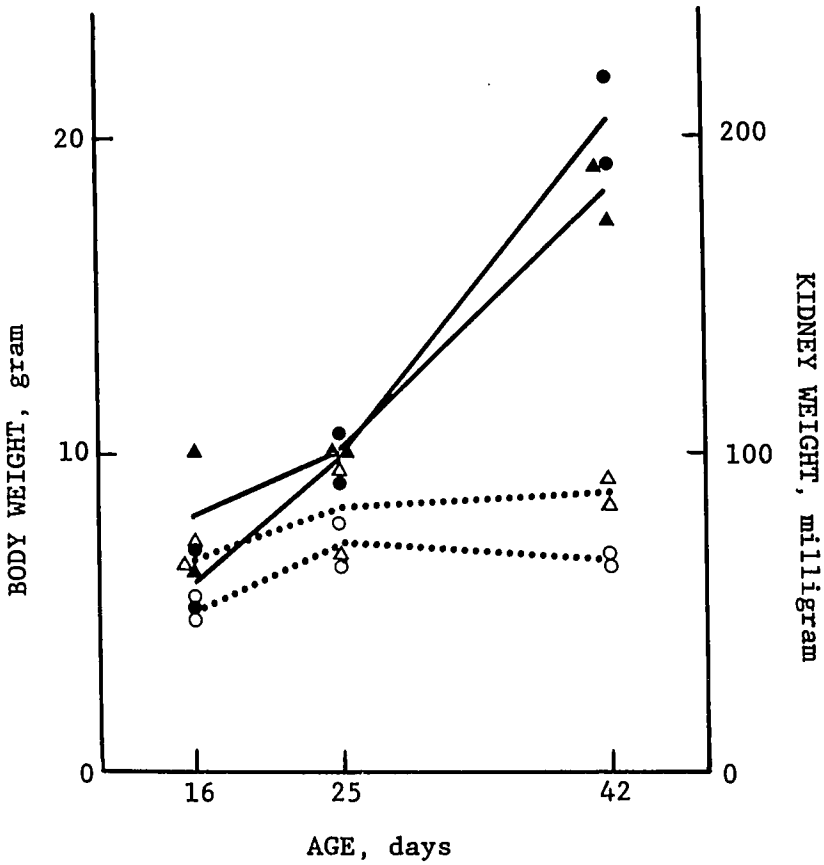

Fig. 1. Body weight and the weight of the kidney in the twitcher mouse. The organ weight is for the bilateral kidneys. Closed circles are body weight of normal mice and open circles are body weight of affected mice. Closed triangles are the weight of control kidneys and the open triangles are those of twitcher kidneys.

days. In contrast, some abnormalities were apparent in the sciatic nerve at all of the three time points. Although dry weight was slightly increased only at 42 days, the protein content of twitcher nerves appeared consistently greater throughout. On the basis of protein, the total lipid in twitcher nerves was generally less than normal. As in the spinal cord, the proportion of free cholesterol and total phospholipids in the total lipid fraction was not abnormal.

Kidney (Table 3, Fig. 1): The size of the kidney of affected mice was approximately 50% of the controls at 42 days. However, the size of their kidney was proportionately greater than normal relative to the body weight which was reduced to one-third of the normal size at this age (Fig. 1). Otherwise, the water content and the major constituents of twitcher kidney were essentially normal at all of the three ages examined (Table 3).

Liver and Lung (Table 4): The size of the liver of affected mice was not different from normal at 25 days, both in the absolute weight and relative to the body weight. At 42 days, however, it was only 20–25% of the normal size, smaller than the control

TABLE 4
Major Constituents of Liver and Lung

Animal and organ	Organ weight (mg)	Organ/ body (%)	Water content (%)	C-M insoluble residue	"Total lipid"	Upper phase solid	Cholesterol (free)	Phospholipid (total)
				(% of dry weight)			(% of "total lipid")	
Liver, 25 days								
Control	282	3.7	70.5	74.4	14.4	11.3	5.1	62.0
Control	481	5.7	72.0	79.4	11.0	9.6	5.9	70.0
Twitcher	227	4.0	70.8	72.6	15.8	11.5	5.9	54.5
Twitcher	287	4.7	71.0	78.1	13.4	8.6	6.1	61.5
Liver, 42 days								
Control	1,005	5.8	67.0	70.8	17.0	12.3	3.8	47.0
Control	984	5.5	70.4	68.6	15.5	15.9	4.9	60.0
Twitcher	205	3.8	68.6	75.2	12.5	12.3	8.0	56.5
Twitcher	203	4.2	68.1	74.3	11.8	13.9	6.5	62.0
Twitcher	279	4.4	70.1	72.5	13.8	13.7	5.6	58.0
Lung, 42 days								
Control	141*	0.8	74.9	74.3	17.0	8.7	11.2	54.0
Control	141*	0.8	75.4	73.9	17.8	8.3	10.2	55.5
Twitcher	62.3*	1.2	72.3	76.8	14.3	8.9	15.3	54.0
Twitcher	49.5*	1.0	72.6	71.1	16.6	12.3	12.9	50.5

* Bilateral lungs.

size even relative to the body weight. At 42 days the twitcher lungs weighed approximately 40% of the controls. Similar to the kidney and in contrast to the liver, the lungs were slightly larger than controls relative to the body weight. Except for the abnormal size of the organs, the water content and other major constituents of the liver and lungs were essentially normal even at the terminal stage.

Glycolipid Composition

Spinal Cord: Both galactosylceramide and sulfatide in the spinal cord of affected mice were normal at 16 and 25 days (Fig. 2). At 42 days, galactosylceramide was slightly lower than the controls on the basis of total lipid. Since the total lipid in the twitcher spinal cord was one-third of the normal level at this age (see above), there were substantial decreases of both galactosylceramide and sulfatide to 25–30% of the controls in the whole tissue. This finding was similar to that for the brain (9) except for the quantitatively greater degree of the decrease of these galactolipids in the spinal cord. On the other hand, lactosylceramide in the control spinal cord was below the level of reliable determination but it was always present in clearly increased amounts in the twitcher spinal cord. There was no significant abnormality in glucosylceramide.

Sciatic Nerve: On the basis of total lipid, there were small increases in galactosylceramide and sulfatide in the twitcher sciatic nerve at 42 days (Fig. 3). These increases were only relative because the total lipid was decreased by 30–40%. In the whole sciatic nerve, therefore, the galactolipids were not increased. Similar to the

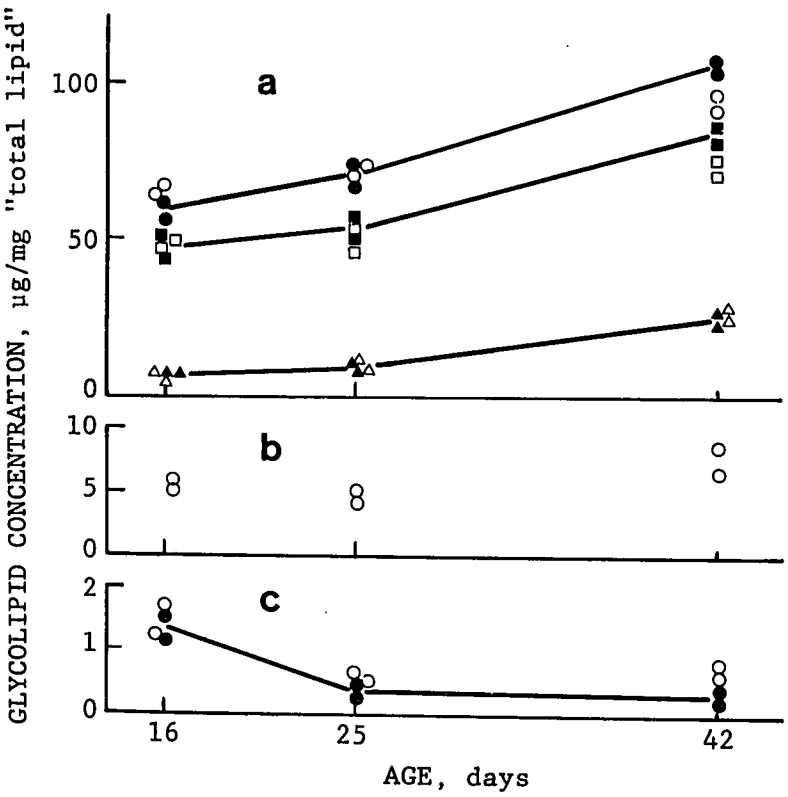

Fig. 2. Glycolipids in the spinal cord. a: galactosylceramide and sulfatide. Circles are for HFA-galactosylceramide; squares are for NFA-galactosylceramide; triangles, sulfatide. Closed symbols are controls and open symbols are twitchers. b: lactosylceramide. Lactosylceramide in control spinal cords was too low for reliable determination and only the data for affected mice are given. c: glucosylceramide. Closed circles, control; open circles, twitcher.

spinal cord, lactosylceramide was present in clearly increased amounts in the twitcher nerve at the three time-points. On the other hand, glucosylceramide was abnormally high in the twitcher nerve, particularly in later stages, contrary to the findings in the spinal cord.

Kidney: Striking analytical abnormalities were observed in the glycolipid composition of the twitcher kidneys (Fig. 4). Both HFA- and NFA-galactosylceramides were definitely increased even at 16 days, but both of them increased rapidly as the disease progressed. At 42 days, HFA-galactosylceramide was increased 50-fold, and the NFA-galactosylceramide 5 to 7 fold. It should be noted that the ratio of the HFA- and NFA-compounds in the normal kidney is approximately 1:2, while HFA-galactosylceramide is present in great excess over the NFA-compound in the twitcher kidney. There were moderate increases of sulfatide and lactosylceramide in the twitcher kidney only at 42 days.

The glycolipid composition of the kidney is known to be complex. It should be cautioned that identification of the renal glycolipids is tentative, based on the four thin-layer chromatographic systems and that rigorous chemical identification is yet to be done. This caution is particularly pertinent to lactosylceramide. Normal kidney contains a substantial amount of digalactosylceramide which is difficult to separate

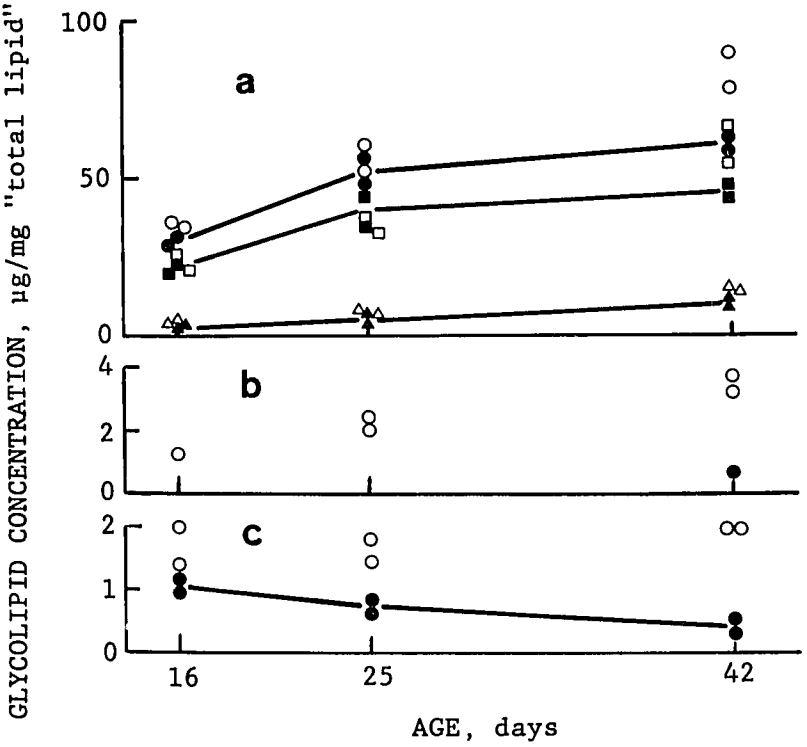

Fig. 3. Glycolipids in the sciatic nerve. The symbols are the same as for Figure 2.

by thin-layer chromatography. The values given as lactosylceramide, particularly those for the normal control kidneys, may well include a significant porportion of digalactosylceramide. Similarly, normal kidney contains small amounts of monohexosylceramides and sulfatides with phytosphingosine as the long-chain base. NFA-galactosylceramide and NFA-sulfatide that contain phytosphingosine may have thin-layer chromatographic mobility similar to those of HFA-galactosylceramide and HFA-sulfatide, respectively, in all solvent systems utilized.

Another potentially complicating factor is the known sex differences in the renal glycolipids in the mouse. However, the phenomenon is strain-dependent. We earlier observed essentially no difference either in the size of the kidney or in the capacity to synthesize galactosylceramide between males and females in this particular strain of C57BL/6J (23). We further took the precaution of analyzing only male kidneys at 16 and 25 days, and male and female kidneys separately at 42 days. Differences between the sexes at 42 days were very small, relative to the large differences in galactosylceramide, lactosylceramide and sulfatide between the control and the affected mice.

Liver and Lung: Galactosylceramide was present in normal mouse liver and lung often at concentrations barely detectable but not sufficient for reliable quantitation. However, it was present in the twitcher organs at readily quantifiable amounts (Table 5). As in the kidney, HFA-galactosylceramide was 2–3 times higher than the NFA-compound in the twitcher liver and lung. Lactosylceramide was also moderately increased but glucosylceramide in the twitcher liver and lung was normal.

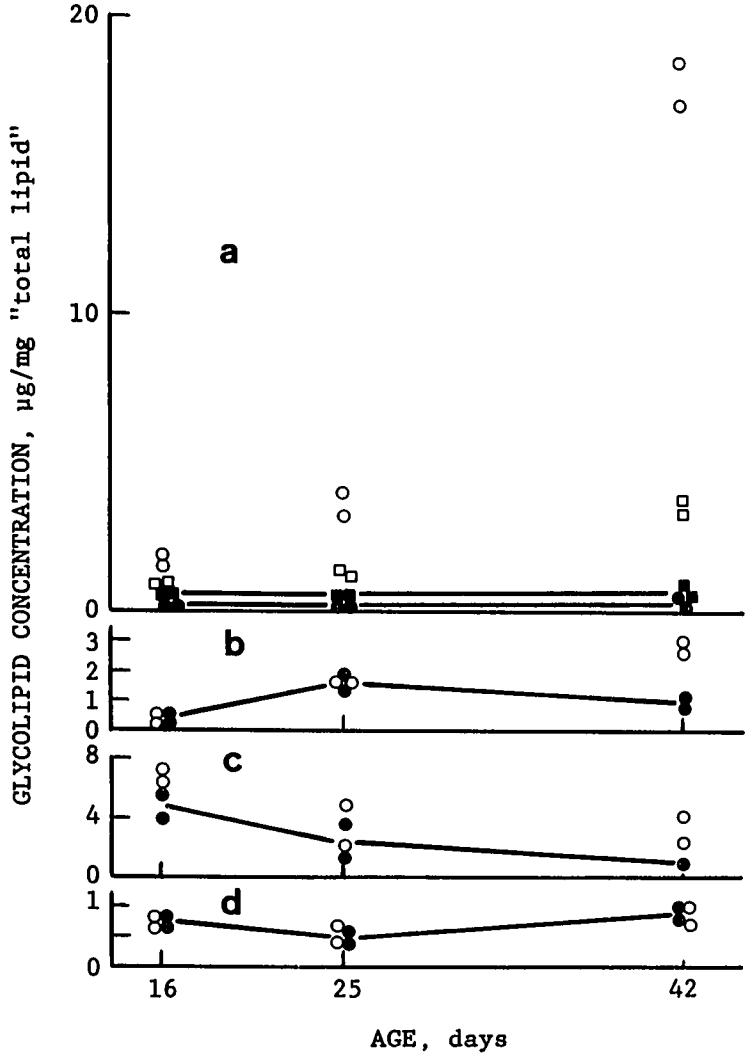

Fig. 4. Glycolipids in the kidney. Closed symbols are controls and the open symbols are twitchers. a: galactosylceramide. Circles, HFA-galactosylceramide; squares, NFA-galactosylceramide. b: sulfatide. c: lactosylceramide. d: glucosylceramide. For 16- and 25-day-old specimens only males were used. The two specimens each for the control and affected mice at 42 days are male and female. Even at 42 days, differences between males and females are very small.

Thin-layer chromatograms of the organ glycolipids at 42 days are shown in Figures 5 and 6. The nearly normal galactolipid composition in the twitcher nervous system, the increase of glucosylceramide in the twitcher sciatic nerve, and the large increases in galactosylceramide and lactosylceramide in the systemic organs of twitcher mice are demonstrated in these chromatograms. The composition of non-saponifiable lipids in the kidney is much more complex than that of the nervous system. Many bands on the chromatogram remain unidentified.

TABLE 5
Glycolipids of Liver and Lung

Animal and organ	NFA-gal-cer	HFA-gal-cer	Lact-cer	Glc-cer
	(mg/g "total lipid")			
Liver, 25 days				
Control	0.083	0.147	1.21	1.17
Control	0.039	0.064	0.97	1.12
Twitcher	0.116	0.189	4.28	1.06
Twitcher	0.293	0.401	—	1.00
Liver, 42 days				
Control	n.d.*	n.d.	1.60	0.688
Control	trace	trace	1.33	0.655
Twitcher	0.122	0.385	3.64	0.674
Twitcher	0.036	0.113	2.51	0.724
Twitcher	0.256	0.698	2.36	0.761
Lung, 42 days				
Control	0.137	0.144	2.79	1.61
Control	0.134	0.131	1.97	1.42
Twitcher	0.421	0.800	4.41	1.29
Twitcher	0.382	0.671	2.92	1.25

* n.d.: not detectable.

DISCUSSION

The glycolipid composition of the spinal cord was similar to that of the brain (9). Reflecting the normal myelination in early stages of development, galactosylceramide was normal at 16 and 25 days but was decreased at 42 days. Decrease in total lipid was observed already at 25 days in the twitcher sciatic nerve. This is consistent with the early and severe involvement of the PNS in the murine model. The lack of abnormal accumulation of galactosylceramide in the nervous system can be explained, as in the human disease, on the basis of the devastating pathological lesions. The relative preservation of galactosylceramide in the sciatic nerve probably reflects the numerous infiltrating macrophages–globoid cells which contain characteristic inclusions (6). The increase of glucosylceramide in the sciatic nerve is also compatible with the pathological changes. On the other hand, lactosylceramide was increased more prominently in the spinal cord. This cannot be explained simply by the infiltration of the mesodermal cells. Since in the normal CNS white matter, lactosylceramidase I, which is deficient in the twitcher, is relatively more active than lactosylceramidase II, the genetic enzymatic defect may be a factor for the lactosylceramide accumulation in the spinal cord.

The findings in the twitcher kidney were unexpected. In human globoid cell leukodystrophy, despite the normal histological features and function, no specific accumulation of galactosylceramide occurs in the kidney (13). Svennerholm and Vanier also analyzed kidney specimens from 12 human patients and observed no significant increase in galactosylceramide (personal communication). Dawson also found no increase in one case (24). In sharp contrast, galactosylceramide was already abnormally high in the 16-day-old twitcher kidney and increased enormously as the disease progressed. At the terminal stage, there was concomitant increase in sulfatide. Ida

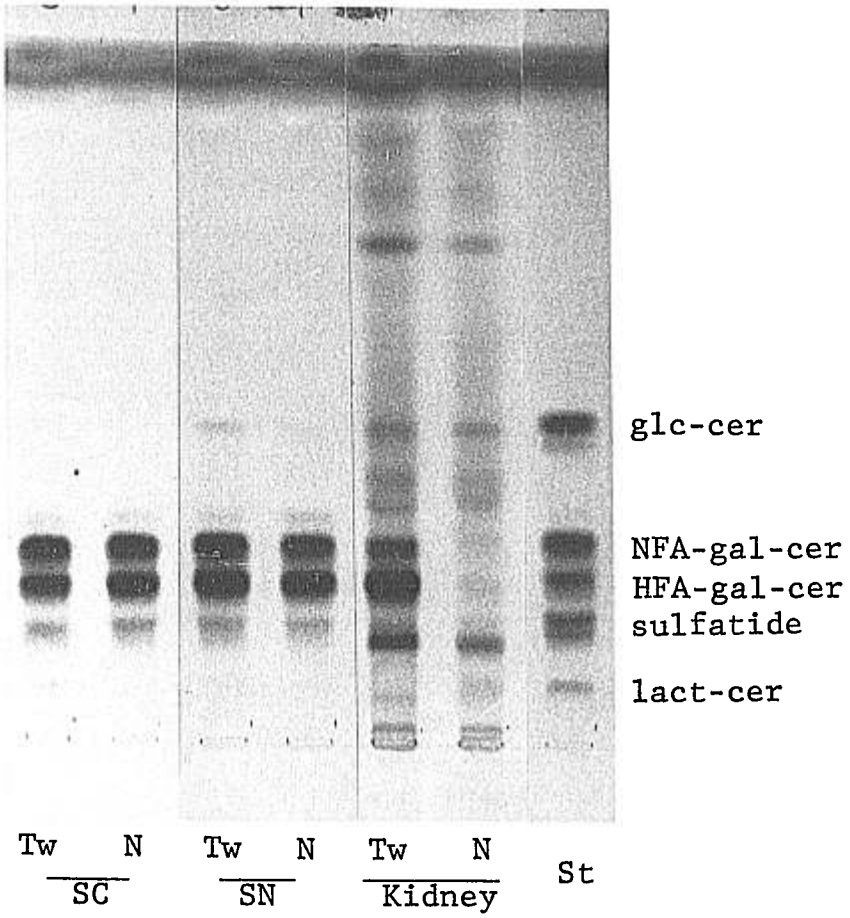

Fig. 5. Thin-layer chromatogram of mercuric chloride–base-treated lipid fraction from the 42-day-old mouse spinal cord, sciatic nerve and the kidney. The chromatographic system IV as described in the method section was used. SC: spinal cord, SN: sciatic nerve, St: standard glycolipids, Tw: twitcher, N: normal. The amounts of applied lipids were equivalent to 0.1 mg (spinal cord and sciatic nerve) and 0.5 mg (kidney) of untreated total lipid, respectively.

et al (11) recently reached the same conclusion with respect to the abnormal accumulation of galactosylceramide in the twitcher kidney. Although the absolute amounts are miniscule, galactosylceramide was also clearly increased in the liver and lung of affected mice. In the liver of human patients, Dawson (24) reported 5–15-fold increase of galactosylceramide in three of the four cases, although Svennerholm et al (25) did not find such an accumulation. Thus, at least for the systemic organs, the twitcher mutant is truer to the concept of a lysosomal storage disease.

While the degrees of galactosylceramide accumulation in the twitcher systemic organs are enormous, it should be cautioned that the absolute amounts are exceedingly small, particularly in the lung and liver. However, the amount in the kidney appears to be sufficiently high to be observed as the characteristic abnormal inclusions in the renal tubular epithelial cells (10). Such pathological alterations have not been found in the kidney of human patients.

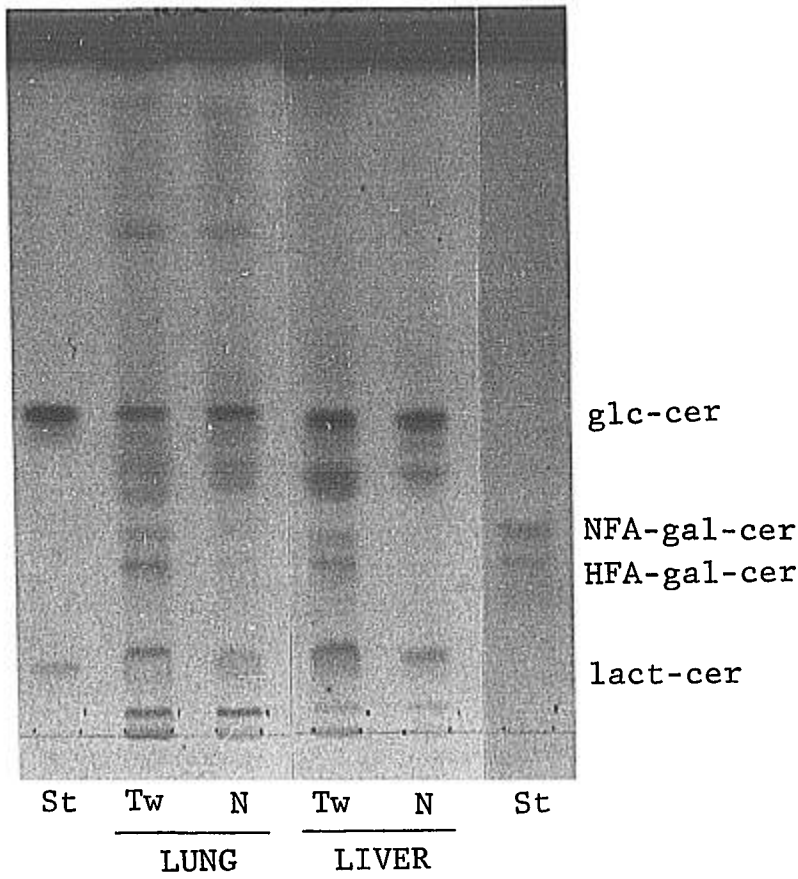

Fig. 6. Thin-layer chromatogram of mercuric chloride-base-treated lipid fraction from the 42-day-old mouse liver and lung. Symbols are the same as for Figure 5. The chromatographic system IV, as described in the method section, was used. The amounts of the applied lipids were equivalent to 1 mg (liver) and 0.5 mg (lung) of untreated total lipid, respectively.

It is noteworthy that HFA-galactosylceramide was always predominant over the NFA-galactosylceramide in all systemic organs of affected mice. HFA-galactosylceramide is characteristically the compound in the nervous system. This raises a question regarding the source of the accumulating galactosylceramide in these organs. It can be endogenous in the respective organs. We know that, at least in the twitcher kidney, the synthetic enzyme for galactosylceramide, UDP-galactose : ceramide galactosyltransferase, remains normal throughout (23). The predominant accumulation of HFA-galactosylceramide may be due to different turnover rate or differential substrate specificity of the mutant galactosylceramidase (26). On the other hand, we cannot exclude the possibility that it originates in the nervous system. In an extremely unusual phenotype of the human disease, the patients may have typical onset of the disease but then their deterioration slows down. At the time of death, which can occur in ten years or even later, the white matter can be almost completely devoid of globoid cells (1). The presence of globoid cells in early stages can be inferred from one instance where identical twins were affected. One patient died early and showed typical pathological features with globoid cells. The other survived for more than

ten years and showed no globoid cells in the white matter at the time of death. Therefore, some mechanism seems to exist that allows galactosylceramide, either by itself or contained within the globoid cells, to be slowly eliminated from the nervous system. With this possibility in mind, we examined galactosylceramide levels in whole blood. They were below the reliable detection limit in both normal and affected mice (Igisu and Suzuki, unpublished data).

The observations reported here are a warning against tacit assumptions that, when the underlying genetic defect is the same, its consequences should be the same. Clearly there are morphological and biochemical species differences which must be taken into consideration when results on "authentic" animal models are to be interpreted. We interpreted the normal galactosylceramide-synthesizing activity in the twitcher kidney on the assumption that galactosylceramide accumulation did not occur in the mouse model analogous to the human disease (23). We cautioned then that analytical data did not exist as to whether or not galactosylceramide accumulated in the twitcher kidney. The results of that series of experiments must now be interpreted in view of the enormous accumulation of galactosylceramide in the twitcher kidney.

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