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ABNORMAL ACCUMULATION OF GALACTOSYLCERAMIDE IN THE KIDNEY OF TWITCHER MOUSE

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The kidney tissue of the twitcher mice, a neurological mutant caused by a genetic deficiency of galactosylceramidase, contains enormously increased amounts, up to 50 times normal, of galactosylceramide. The finding is in sharp contrast with those in the enzymatically equivalent human disease, globoid cell leukodystrophy (Krabbe disease), in which no specific abnormal accumulation of galactosylceramide occurs despite the same genetic block in the catabolic pathway. This indicates that the same genetic defect can result in entirely different consequences in different species. Caution must be exercised even when "authentic animal models" are utilized for studies of human diseases.

Globoid cell leukodystrophy (Krabbe disease) in humans is caused by a genetic deficiency of galactosylceramidase activity (EC 3.2.1.46)(1). One of the unusual features of the human disease is the lack of abnormal accumulation of galactosylceramide despite the genetic block in its degradative pathway (2). While the lack of accumulation in the central nervous system can be explained on the basis of the devastating pathology, particularly the loss of myelin and the oligodendroglia, the lack of specific accumulation in the histologically and functionally normal kidney is difficult to explain (3). An earlier report on galactosylceramide accumulation in the liver of human patients (4) has been contradicted by a more recent report (5).

The twitcher is a genetic murine leukodystrophy with clinical and pathological features similar to human globoid cell leukodystrophy (6). The underlying genetic cause has been identified as a deficiency of galactosylceramidase activity (7). Therefore, the twitcher mutant is a genetically, clinically, pathologically and enzymatically authentic model of the human disease. An

Abbreviations used: HFA-, hydroxy-fatty acid-containing; NFA-, non-hydroxy-fatty acid-containing.

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analytical study of the twitcher brain during development showed, similar to the human disease, no abnormal accumulation of galactosylceramide (8). The capacity for galactosylceramide synthesis as determined by the activity of UDP-galactose:ceramide galactosyltransferase, was severely reduced in the central nervous system after 25 days, consistent with the severe pathology, but it remained normal in the kidney throughout the life-span of affected mice (9). We have therefore examined the galactosylceramide content of twitcher kidney and have unexpectedly found an enormous accumulation, in sharp contrast to the human disease.

Materials and Methods

The colony of the twitcher mutant is maintained in our institution. The genetic status of each mouse was determined by galactosylceramidase assays on clipped tails (10). The diagnosis of homozygous affected mice could be readily confirmed clinically after 25 days. Two each of affected and normal mice were killed at 42 days by decapitation and both kidneys were removed. The weighed kidneys were extracted with 19 vol of a mixture of chloroform and methanol (2:1, v/v) in a motor-driven all-glass Potter-Elvehjem homogenizer (11). The tissue was fractionated into the chloroform-methanol-soluble and insoluble fractions (12). The chloroform-methanol soluble fraction was partitioned into the water-soluble upper phase fraction and the chloroform-soluble lower phase fraction, which was dried, weighed, and was used for analysis of glycolipids without elimination of the very small amount of chloroform-methanol soluble proteins ("total lipid fraction").

Identification of glycosphingolipids was by co-migration with authentic standards in three different systems of thin-layer chromatography on the high-performance silica gel 60 plates (E. Merck, purchased from American Scientific Products, Rochester, NY): I, total untreated lipid in chloroform-methanol-water (65:25:4, by volume); II, mercuric chloride-alkaline-treated lipids in chloroform-methanol-conc ammonia (65:25:5, by volume); and III, mercuric chloride-alkaline-treated lipid on borate-impregnated plates in chloroform-methanol-conc ammonia (65:25:5, by volume). Spots were visualized by the cupric acetate spray and heating (13). The mercuric chloride-alkaline treatment was according to Abramson et al. (14). After the treatment, which eliminated all glycerophospholipids, the remaining lipid was subjected to silicic acid column chromatography according to Norton and Autilio (15). 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 bulk of cholesterol in the first fraction, left sphingomyelin on the column, and improved the chromatographic resolution of the eluted glycolipids. The borate-impregnated plates were prepared by suspending the plate in a chromatographic chamber for 20 hrs over a mixture of methanol and 4% aqueous sodium tetraborate (3:1, v/v). They were activated at 120^{o} for 60 min prior to use. This impregnation by the vapor phase was far superior to that by the liquid phase in yielding uniform impregnation and better resolution of glycolipids.

Quantitation was done by densitometry at 350 nm with a Shimadzu CS-910 scanner and a CR-1B data processor. Series of known amounts of standards were always included in each plate to correct for plate-to-plate variations. The chromatographic system II was used for quantitation of sulfatide and lactosylceramide, and the system III for others because of the optimal separation of the respective compounds.

Results

The enormous accumulation of galactosylceramide, both hydroxy-fatty acid-containing (HFA-) and non-hydroxy-fatty acid-containing (NFA-), was readily evident on the thin-layer chromatography (Fig. 1). Quantitative determination (Table 1) indicated that, at 42 days, the accumulation was proportionately much greater for the HFA-galactosylceramide (X50 normal) than the NFA-galactosylceramide (X5 normal). Sulfatide, which is degraded through galactosylceramide, was also moderately increased, and so was lactosylceramide. On the other hand, glucosylceramide, a compound analogous to galactosylceramide but unrelated to the genetic defect, was not abnormal in the kidney of affected mice.

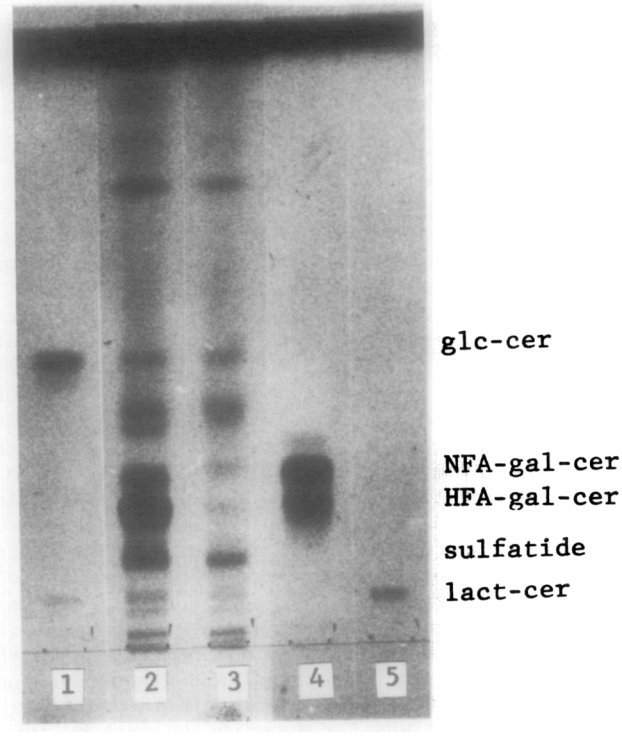

Fig. 1. Thin-layer chromatograph of murine renal glycolipids. The "total lipids" were subjected to the mercuric chloride-saponification procedure and to the silicic acid column chromatography as described in the text. Samples equivalent to 0.5 mg of the starting "total lipid" were applied to the borate-impregnated plate, developed in chloroform-methanol-conc ammonia (65:25:5, by vol) and visualized by the cupric acetate spray. 1. standard glucosylceramide; 2. twitcher kidney; 3. normal kidney; 4. standard galactosylceramide; 5. standard lactosylceramide.

Table 1. Glycolipids in the Kidney of Twitcher Mouse

constituents	control		twitcher	
	female	male	female	male
	(% of wet weight)			
water	67.9	69.4	63.6	67.3
	(% of dry weight)			
"total lipid"	17.6	19.0	16.8	15.8
	(μg/mg "total lipid")			
NFA-gal-cer	0.4	1.0	3.8	3.4
HFA-gal-cer	0.2	0.4	18.6	17.1
sulfatide	1.1	1.0	2.8	3.0
lact-cer	0.8	---*	2.4	4.0
glc-cer	1.0	0.8	1.0	0.8

* Could not be determined reliably.

Discussion

Studies of human globoid cell leukodystrophy are constrained by the rarity of patients and ethical considerations. The twitcher mutant therefore provides an invaluable experimental tool, together with the canine form of globoid cell leukodystrophy (2), both of which are caused by the genetic defect in galactosylceramidase activity, as in the human disease. Despite the same fundamental genetic defect, some significant species differences have been noted in the clinical and pathological findings between the human and the murine diseases (16). Such species differences must always be taken into consideration when experimental results obtained with an animal model are extrapolated to the human disease.

The kidney tissues of affected mice show characteristic abnormal inclusion bodies, most notably in the epithelial cells of the loop of Henle, while such abnormality could not be found in the kidney of a human patient (Takahashi et al., unpublished). The analytical results reported here are consistent with the difference in the morphological findings and point to another important difference in the genetic galactosylceramidase deficiency in the two species.

The abnormal accumulation of galactósylceramide in the twitcher kidney came as a surprise to us. We had anticipated to find no accumulation in analogy to the human disease. Many genetically and enzymatically "authentic animal models" of human diseases are being utilized extensively for studies of human

genetic disorders. They are useful in overcoming the limited availability, ethical considerations, and other constraints in studying human patients. Tacit assumptions are often made that the results obtained in one species can be readily extrapolated to another species. Our findings indicated clearly that the assumption of the same genetic defect producing the same consequences cannot be made. Despite the inherent advantages, caution must be exercised when experimental results obtained with animal models, no matter how "authentic", are interpreted in relation to human diseases.

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