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Juvenile G_{M2} Gangliosidosis (A^MB Variant): Inability to Activate Hexosaminidase A by Activator Protein

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SUMMARY

Two siblings from a consanguineous Puerto Rican marriage were found to have a juvenile-onset type of lipidosis first noted at age 2½ by expressing difficulties with motor function and developmental delay. They continued to deteriorate, showing muscle atrophy, spasticity, and loss of speech, and death occurred at ages 7 and 8. Examination of the brains from these patients revealed that the concentration of G_{M2} ganglioside was about 56% of the total gangliosides. Hexosaminidase and percent hexosaminidase A (HEX A) and other lysosomal enzymes were normal in cultured skin fibroblasts, liver, and brain. The concentration of the activator protein required for the enzymatic hydrolysis of G_{M2} ganglioside was in high normal levels in the brain of the patient available. However, the HEX A from the patient's brain and liver as well as from skin fibroblast lysates could not be activated to hydrolyze G_{M2} ganglioside by the activator protein from a control or himself. The HEX A from a control could be activated by the activator protein from controls or this patient. These patients appear to have a defect in HEX A, which does not affect its heat stability, electrophoretic migration, and activity toward fluorogenic substrates, but may affect the binding of the activator protein required for G_{M2} ganglioside hydrolysis. We propose to call these patients the A^MB variant of G_{M2} gangliosidosis to denote the mutation in HEX A but with normal levels of HEX A and B with synthetic substrates. This is to distinguish these patients from those missing the activator protein and normal HEX A and B levels.

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INTRODUCTION

G_{M2} gangliosidosis is the name given to a group of genetic diseases in which G_{M2} ganglioside is stored, primarily in nervous tissue. These patients present in a variety of clinical forms ranging from the severely affected infant to the adult patient with mild spinocerebellar degeneration and/or psychosis [1, 2]. The mutations resulting in storage of G_{M2} ganglioside can be in the α chain of hexosaminidase resulting in a defect in HEX A [3], in the β chain resulting in a defect in HEX A and B [4], or in the activator protein required for the action of HEX A on G_{M2} ganglioside [5, 6]. In addition, Li et al. [7] described studies in a patient whose HEX A from brain could not be activated by activator protein isolated from the patient or a control. Typical Tay-Sachs disease (B variant) and Sandhoff disease (0 variant) patients can be diagnosed using the fluorogenic substrate to demonstrate a deficiency of HEX A activity in all tissues and fluids in the former and an almost total deficiency of hexosaminidase activity in the latter. Juvenile patients can be demonstrated to have a partial deficiency of HEX A activity. Patients with the so-called AB variant have normal HEX A and B in all tissues, and diagnosis requires the demonstration of increased G_{M2} ganglioside in brain samples, a decrease in activator protein concentration in tissues, an activation of the patient's HEX A by activator protein from control tissues, or a decreased turnover of radiolabeled G_{M2} ganglioside added in the medium to cultured skin fibroblasts.

Here we describe the clinical findings in two Puerto Rican siblings with a juvenile form of G_{M2} gangliosidosis. Brain samples obtained at autopsy from the children showed excess G_{M2} ganglioside. HEX A and B were normal in all tissues tested with fluorogenic substrate, and the G_{M2} activator protein concentration was normal or elevated in the liver and brain from the one sibling available. However, HEX A isolated from the patient's brain and liver and total hexosaminidase from fibroblast extracts were unable to be activated by the G_{M2} activator protein. These children probably have a mutation in the activator protein binding site on HEX A or in the catalytic site required for the hydrolysis of G_{M2} ganglioside.

FAMILY HISTORY

This Puerto Rican family originally consisted of five children born to first cousins once removed. The first pregnancy resulted in the birth of female twins who expired at age one month from gastroenteritis. Patient 1 (BD 4-22-72) had normal birth and development until age 2½, when the mother noted difficulty with walking and loss of her reflex grasp. At age 4, she was evaluated in Puerto Rico for her inability to walk or speak. At that time, she was only tube fed. Her liver was palpable 2 cm below the rib margin, but the spleen was not felt. A report of "negative urine values for arylsulfatase A" resulted in a diagnosis of metachromatic leukodystrophy (MLD). When the child was evaluated at age 7, she was thin and partially cachectic, and she did not walk or speak but did respond to the parents' efforts by signs of recognition. Her eyes moved and reacted normally except for some medial nystagmus. The fundi showed only some deposition of brown material under the maculi. The child was unresponsive neurologically except for very deep stimuli. There was diffuse atrophy of all muscle groups with spasticity. EEG showed almost continuous generalized epileptiform activity with anterior prominence and a moderately diffuse disturbance of cortical activity. Routine laboratory tests and a screen of lysosomal enzymes including arylsulfatase A were normal. She died at age 8 from progressive neurological degeneration complicated by a respiratory infection.

Patient 2 (BD 6-18-74) had an early clinical course identical with his older sister. At age 5, he was thin and walked poorly with an ataxic gait. He could communicate with his parents but did not speak to the examiner. The physical examination was within normal limits except for diminished neurological response throughout, moderate muscle atrophy, and moderate spasticity. The fundi showed a similar brownish discoloration to that of the sister. He had no seizure activity and a normal EEG. Study of his urine in Puerto Rico resulted in the diagnosis of MLD. A screen of lysosomal enzymes in leukocytes (in the U.S.) was normal. Increased problems with swallowing and feeding as well as with aspiration and respiratory infections were found. This patient died at age 7 from a respiratory infection.

Patient 3 (BD 8-20-77) had normal growth and development until her latest examination in July 1982. Her fundus was interpreted as showing a brownish discoloration similar to that of her siblings. Examination of her urine in Puerto Rico also resulted in the diagnosis of MLD. A screen of lysosomal enzymes in her leukocytes (in the U.S.) was found to be normal.

MATERIALS AND METHODS

Substrates

The 4-methylumbelliferyl (4MU) substrates for measuring β-galactosidase, α-L-fucosidase, α-mannosidase, β-glucuronidase, β-*N*-acetylglucosaminidase, and β-*N*-acetylgalactosaminidase were purchased from Research Products (Mt. Prospect, Ill.). 4MU-α-*N*-acetylneuraminic acid was synthesized according to the method of Warner and O'Brien [8]. Nitrocatechol sulfate for measuring arylsulfatase A was obtained from Sigma (St. Louis, Mo.). [^{3}H]galactose-labeled galactosylceramide was prepared in this laboratory by the method of Radin et al. [9]. [^{14}C]stearic acid-labeled sulfatide was synthesized in this laboratory according to the method of Dubois et al. [10]. G_{M2} ganglioside was isolated from the brain of a child who died with Tay-Sachs disease, and it was subsequently radiolabeled in the terminal *N*-acetylgalactosaminyl residue by oxidation with galactose oxidase and reduction with sodium [^{3}H]borohydride as described by Suzuki and Suzuki [11]. The final specific radioactivity was 1,200 cpm/nmol.

Assays with Synthetic Substrates and Natural Substrates Using Detergents

Beta-galactosidase, α-L-fucosidase, α-mannosidase, β-glucuronidase, and β-*N*-acetylglucosaminidase were assayed using the 4MU substrates as reported [12]. Arylsulfatase A and galactocerebrosidase activities were measured using nitrocatechol sulfate and [^{3}H]galactosylceramide, respectively [12]. Sialidase activity in cultured skin fibroblasts was assayed with the 4MU derivative as described by Warner and O'Brien [8]. Sulfatide sulfatase metabolism was determined in vitro and after uptake into cultured skin fibroblasts using the methods reported from this laboratory [13]. The ability of enzyme sources to hydrolyze G_{M2} ganglioside was determined using 20 nmol [^{3}H]G_{M2}, 200 μg sodium taurodeoxycholate (Calbiochem, La Jolla, Calif.), sodium acetate buffer (pH 4.6, 0.01 M final concentration), and either 100 μg fibroblast culture homogenate supernatant or 0.1 U of HEX A or B from liver and brain in a total incubation volume of 0.2 ml. In the assays with partially purified HEX A and B, 100 μg of human serum albumin (Sigma) were added to the assays. After incubation for 16 hrs (fibroblast supernatant) or 3 hrs (partially purified HEX A and B), the free [^{3}H]*N*-acetylgalactosamine was determined as follows. To the chilled mixture were added 0.8 ml of *N*-acetylgalactosamine (1 mM), 250 mg wet wt. AG50W-X8 resin (100–200 mesh, hydrogen form from BioRad, Richmond, Calif.), and 150 mg dry wt. AG3X4A resin (100–200 mesh, hydroxyl form, BioRad). After thorough mixing, the mixture was allowed to stand for 5 min followed by centrifugation at 800 *g* for 10 min. A 200-μl aliquot of the supernatant was placed in vials and counted in 10 ml Ready-Solv HP (Beckman, Fullerton, Calif.). After incubation with fibroblast extracts, the reactions were processed using DEAE-cellulose minicolumns as described by O'Brien et al. [14]. The enzyme activities obtained were almost identical with these two methods. Protein concentrations were determined according to the method of Lowry et al. [15] using bovine serum albumin as the standard.

Assays with G_{M2} Ganglioside Using Activator Protein

Into each test tube, 20 nmol of [^{3}H]G_{M2} were added followed by evaporation with nitrogen. The ganglioside was dissolved in 0.1 ml acetate buffer (pH 4.6, 0.01 M final concentration), and 100 μg of protein from the fibroblast extract were added along with either 50 μg partially purified activator protein from brain of a control or the patient, or 3.3 μg of more purified activator from control human brain. When HEX A and B were used separately as the enzyme sources, each tube contained 0.1 U of hexosaminidase activity. The [^{3}H]*N*-acetylgalactosamine released by the reaction was determined after incubation at 37°C for 3 hrs or 16 hrs using the methods described above for the detergent-stimulated reaction.

Enzyme Sources

Fibroblast cultures were started from forearm skin biopsy, and they were grown in Eagle's minimal essential medium supplemented with 10% fetal calf serum, glutamine (2mM), nonessential amino acids, Penstrep (100 μg/ml streptomycin and 100 U/ml penicillin). The fibroblast culture from case 1 described by Goldman et al. [16] was generously supplied by Dr. K. Suzuki. The confluent cells were harvested with trypsin and washed with phosphate buffered saline. On the day of assay, the cells were homogenized in distilled water using Duall homogenizers (Kontes, Vineland, N.J.). For most assays, the total homogenate was used, but for assay of G_{M2} ganglioside hydrolysis, the pellet of cells was frozen and thawed once, homogenized as above, and centrifuged at 11,000 *g* for 15 min, and only the supernatant was used.

HEX A and B were isolated from 250 g of control human liver and from 30 g of liver from patient 2. The thawed liver was homogenized in 5 vol of 0.01 M sodium phosphate buffer (pH 7.0) using a Waring blender. After centrifuging the homogenate at 10,000 *g* for 1 hr, the supernatant was brought to 30% saturation with ammonium sulfate and allowed to stand overnight at 4°C. The solution was centrifuged to remove the precipitate, and the supernatant was brought to 70% saturation with solid ammonium sulfate. After standing overnight at 4°C, the precipitate was collected by centrifugation and suspended in a small volume of sodium phosphate buffer (0.05 M, pH 7.0). After dialysis against the same buffer, the small amount of sediment was removed by centrifugation and the supernatant applied to a Sephadex G-200 column. The column fractions were monitored for hexosaminidase activity, and the active tubes were pooled and applied to a concanavalin A (Con A) Sepharose column. A small molecular weight fraction from the Sephadex G-200 column was saved for purification of activator protein. The hexosaminidase activity was eluted from the Con A column with 0.75 M α-methylmannoside. The active fractions were pooled, dialyzed against sodium phosphate buffer (0.05 M, pH 7.0), and applied to a DEAE-Sephadex A-50 column (Pharmacia, Uppsala, Sweden). HEX B was eluted unbound, and HEX A was eluted with sodium citrate buffer (0.05 M, pH 6.0, containing 0.05 M sodium chloride). The pooled fractions of HEX A and B were concentrated on a PM-10 membrane (Amicon, Lexington, Mass.) and dialyzed against sodium acetate buffer (0.01 M, pH 4.6). The control liver had a final specific activity of 4.9 U/mg protein for HEX A and 2.9 U/mg protein for HEX B. A unit of hexosaminidase activity is defined as μmol 4MU-β-NAcglc hydrolyzed per min at 37°C. HEX A was 66% of the total hexosaminidase activity. The liver from patient 2 had a final specific activity of 1.02 U/mg protein for HEX A and 0.85 U/mg protein for HEX B. HEX A was 55% of the total hexosaminidase activity.

Initially, HEX A and B were isolated from 10-g brain samples from a control and patient 2 using the procedure of Li et al. [7]. HEX A and B were further purified on Con A columns and separated by DEAE-Sephadex A-50 column chromatography. More HEX A and B plus activator proteins were purified from 75-g samples of brain using the method described for liver except that the initial homogenate in phosphate buffer was lyophilized and washed with cold acetone before rehomogenization in the starting buffer. The control brain had final specific activities of 1.8 U/mg protein and 1.5 U/mg protein for HEX A

and B, respectively. HEX A was 48% of the total hexosaminidase activity. Patient 2 had final specific activities of 1.5 U/mg protein and 0.89 U/mg protein for HEX A and B, respectively. HEX A was 54% of the total hexosaminidase activity.

Purification of Activator Proteins

The activator protein for G_{M2} ganglioside hydrolysis was partially purified from liver and brain from a control and from brain from patient 2. The fractions from the Sephadex G-200 columns, described above, which contained the activator protein, were applied to a DEAE-Sephadex A-50 column in sodium phosphate buffer (0.05 M, pH 7.0). The unbound fraction that contained the activator protein was pooled and applied to a column of octyl-Sepharose (Pharmacia). After washing with a large volume of distilled water, the activator was eluted with a 2% solution of octyl-glucoside (Calbiochem). The active fractions were pooled and dialyzed against sodium acetate buffer (0.01 M, pH 4.6). The small amount of precipitate was removed by centrifugation, and the supernatant fraction was used as the source of activator. The activator proteins from control liver and brain had specific activities of 424 activator U/mg protein and 980 activator U/mg protein, respectively. The activator protein from the brain of patient 2 had a specific activity of 5,559 activator U/mg protein. An activator unit is defined as the amount of activator that stimulates the hydrolysis of 1 nmol of G_{M2} ganglioside per hr/U of HEX A. The activator protein is stable at 4°C in the acetate buffer.

Polyacrylamide Gel Electrophoresis of Brain and Liver Extracts

Frozen samples of brain from a control, a child who died with typical Tay-Sachs disease, and patient 2 were thawed and homogenized in phosphate buffer (0.01 M, pH 6.0) using 1.7 ml buffer per g wet wt. tissue. Samples of liver from a child who died with Tay-Sachs disease and from patient 2 were treated similarly to brain. The homogenate was frozen and thawed twice and centrifuged at 25,000 *g* for 20 min. The supernatant was removed and recentrifuged at the same speed for 10 min. This supernatant fluid was used for polyacrylamide gel electrophoresis as described by Grebner and Jackson [17]. Slices were assayed for hexosaminidase activity using 3 mM 4MU-β-NAcglc as reported [17].

Electron Microscopy of Brain

One-cubic-mm pieces of cerebral cortex from patient 2 were fixed in 2% phosphate buffered glutaraldehyde and post-fixed in 1% osmium tetroxide. The tissues were dehydrated, embedded in Spurr resin, and sliced on a Sorvall MT-2 ultramicrotome. Sections were stained with uranyl acetate and lead acetate and examined with a Zeiss 9S-2 electron microscope.

Ganglioside Extraction

Previously frozen samples of total brain were homogenized in 10 vol of chloroform-methanol (2-1, by volume) and filtered through glass wool. After the addition of one-fifth vol of distilled water, the mixture was vortexed and centrifuged at 2,000 *g* for 10 min. The upper phase was removed, and an aliquot was used for thin-layer chromatography in chloroform-methanol-2.5 N ammonia (60-35-8, by volume). Gangliosides were visualized with resorcinol spray after heating 20 min at 110°C. Another aliquot was chromatograpned for estimating the amount of each ganglioside. After scraping the regions visualized with iodine vapor into test tubes, distilled water was added. After 10 min, resorcinol reagent was added according to the method of Svennerholm [18]. The percentage distribution of each ganglioside was based on the number of sialic acid residues per compound.

RESULTS

Initially, a small piece of frozen brain was received from patient 1 who died at age 8. Enzyme studies of the patient's urine by a laboratory in Puerto Rico gave the diagnosis of MLD because of "negative values for arylsulfatase A." The brain was extracted in this laboratory for enzyme studies and lipid analysis. Enzyme studies revealed normal levels for β-galactosidase, arylsulfatase A, sphingomyelinase, and hexosaminidase (and % HEX A). However, there was a great increase in the level of G_{M2} ganglioside approaching 56% of the total gangliosides (fig. 1). Other lipids appeared to be in the normal range. Fibroblast cultures were received from the two younger children who were also diagnosed as having MLD. A large number of lysosomal enzymes were studied, including hexosaminidase (and % HEX A), arylsulfatase A, and sulfatide sulfatase, and normal activities were measured (table 1). In addition, the metabolism of [^{14}C]stearic acid-labeled sulfatide was measured after the uptake from the medium as described by this laboratory [13]. There was completely normal metabolism of sulfatide, with only 10% remaining unhydrolyzed by day 4. This indicated that the children did not have a defect in sulfatide metabolism but perhaps a defect in the catabolism of G_{M2} ganglioside, as indicated by the G_{M2} storage in brain.

When patient 2 died at age 7, brain, spleen, and liver were obtained at autopsy and frozen. A sample of brain was examined by electron microscopy, and membranous cytoplasmic bodies were found that suggest a ganglioside storage disease (fig. 2). Extraction of the brain for gangliosides again demonstrated the increase

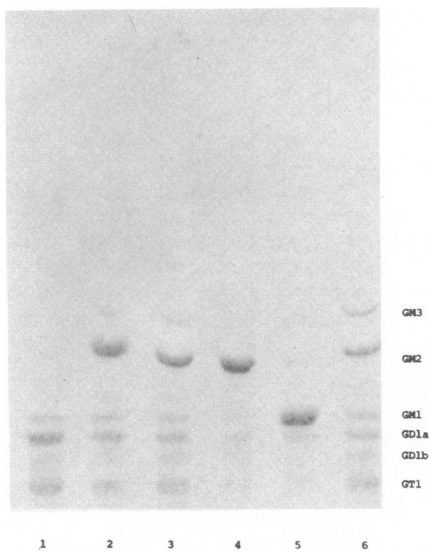

FIG. 1.—Pattern of gangliosides extracted from brain samples from a control and from patients. Gangliosides were extracted and chromatographed as described in MATERIALS AND METHODS. *Lane 1*—from a 10-year-old control; *lane 2*—from patient 1; *lane 3*—from patient 2; *lane 4*—from a child who died with Tay-Sachs disease; *lane 5*—from a child who died with G_{M1} gangliosidosis, type 1; *lane 6*—from a child who died with Neimann-Pick disease, type A.

TABLE 1

LYSOSOMAL ENZYME ACTIVITIES

Enzyme source Enzyme activity	Patient 2	Patient 3	Controls (mean ± SD)
Fibroblast homogenate:			
β-Galactosidase*	390	616	396 ± 91.2
α-L-Fucosidase*	47.3	48.0	47.9 ± 33.0
α-Mannosidase*	56.2	173	83.0 ± 29.1
β-Glucuronidase*	169	259	94.4 ± 41.8
Sialidase*	42.9	95.7	94.3 ± 40.2
Galactocerebrosidase†	2.18	2.30	4.48 ± 1.64
Arylsulfatase A‡	393	623	407 ± 175
Sulfatide sulfatase§	52.0	66.3	71.3 ± 28.6
β-*N*-acetylglucosaminidase*	2,672	4,335	2,885 ± 1,086
% HEX A	63.0	65.0	68.0
Liver supernatant fraction:			
β-Galactosidase*	17.2	NA‖	417 ± 231
β-*N*-acetylglucoaminidase*	854	NA	2,483 ± 563
% HEX A	59.5	· · ·	70.7
Brain supernatant fraction:			
β-Galactosidase*	105	NA	84.9 ± 35.0
β-*N*-acetylglucosaminidase*	1,257	NA	1,276 ± 839
% HEX A	67.8	· · ·	78.3

NOTE: Lysosomal enzyme activities are measured as nmol hydrolyzed/mg protein/hr.
* Measured with fluorogenic substrates.
† Measured with [^{3}H]galactosylceramide.
‡ Measured with nitrocatechol sulfate.
§ Measured with [^{14}C]sulfatide.
‖ NA = not available.

in G_{M2} ganglioside (fig. 1). G_{M2} ganglioside was 56% of the total gangliosides as compared to 5.7% in brain from a 10-year-old control, 86% in the brain from a patient with typical Tay-Sachs disease, and 38% in the brain from a child with Niemann-Pick disease, type A. The lipid content of liver and spleen was found to be near normal with no obvious storage of globoside or G_{A2} (asialo G_{M2} ganglioside).

The liver and brain were extracted with distilled water and assayed for β-galactosidase and β-*N*-acetylglucosaminidase activities (table 1). Both organs had normal hexosaminidase (and % HEX A), and the brain had normal β-galactosidase activity, agreeing with the findings in brain from patient 1. The liver extracts (total homogenate and supernatant) from patient 2 were found to be deficient in acid β-galactosidase activity. The deficiency was not as low as we find in liver from cases of G_{M1} gangliosidosis but was lower than we have found in livers from patients with Hurler syndrome, Hunter syndrome, and Sanfilippo syndrome (our unpublished data, 1980).

Since the brain was demonstrated to contain excess G_{M2} ganglioside and normal hexosaminidase isoenzymes, portions of liver and brain were extracted for isolation of hexosaminidase and for activator protein. The patient was found to have a normal total hexosaminidase and % HEX A and B in liver and brain when measured with 4MU-β-*N*-acetylgalactosaminide and 4MU-β-*N*-acetylglucosaminide. Polyacrylamide gel electrophoresis of brain revealed a pattern similar to control as compared with a sample of brain from a child with Tay-Sachs disease that had

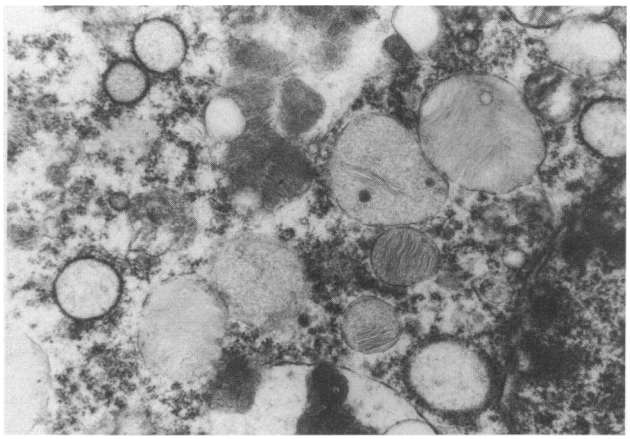

FIG. 2.—Electron microscopy of cerebral cortex from patient 2 showing the membranous cytoplasmic bodies (magnification × 15,400).

a clear deficiency of HEX A activity (fig. 3*A*). In liver, there was also a normal pattern of hexosaminidase activity as compared with Tay-Sachs disease (fig. 3*B*).

The HEX A and B fractions from liver samples from patient 2 and a control were then tested for their ability to hydrolyze G_{M2} ganglioside using sodium taurodeoxycholate (200 μg per assay) (fig. 4). There was a dramatic effect of adding human serum albumin to the assay when purified HEX A was used as the enzyme source. The activity of HEX A, but not HEX B, from control liver was stimulated more than fivefold by the addition of 100 μg of human serum albumin. The HEX A activity from patient 2 was stimulated less than twofold by the addition of the human serum albumin. When human serum albumin is not added to the assays with partially purified HEX A and B, there is almost identical specific activities toward G_{M2} ganglioside by both isozymes from the control and the patient.

HEX A and B also were partially purified from brain samples from the patient and a control. Figure 5 shows a summary of the results from liver and brain HEX A and HEX B using sodium taurodeoxycholate and activator proteins purified from brain samples from the patient and the control. The ability of HEX A from control liver to be activated by sodium taurodeoxycholate (in the presence of 100 μg of human serum albumin) is compared with the low activity found for the HEX A from the patient and HEX B from the control and the patient. A similar result is found for HEX A and B from brain, although the patient's value is about 30% of control HEX A. When sodium taurodeoxycholate is replaced by activator proteins purified from the brains of the patient and the control, the severe deficiency in the ability of HEX A from the patient to be activated is observed. HEX B is not activated at all by activator protein, and the data are not included in figure 5. The activator protein from the patient's brain is more active than the same concentration of activator protein (50 μg/0.2 ml assay) from the control brain in stimulating the HEX A from the control liver and brain. This figure clearly shows the defect in the ability of the HEX A from the patient to be activated by activator

protein and the ability of the activator protein from the patient to activate the HEX A from the control liver and brain.

In an attempt to develop a method for diagnosing such patients in cultured skin fibroblasts, activator protein purified from samples of brain from control and patient were incubated with G_{M2} ganglioside and fibroblast lysates. The cultured skin fibroblasts from controls could hydrolyze an average of 1.35 nmol of G_{M2} ganglioside per 16 hrs/mg fibroblast supernatant protein when activator from control brain was utilized. Cells from patient 2, case 1 of Goldman et al. [16], and a case of typical Tay-Sachs disease could only metabolize 0.06, 0.19, and

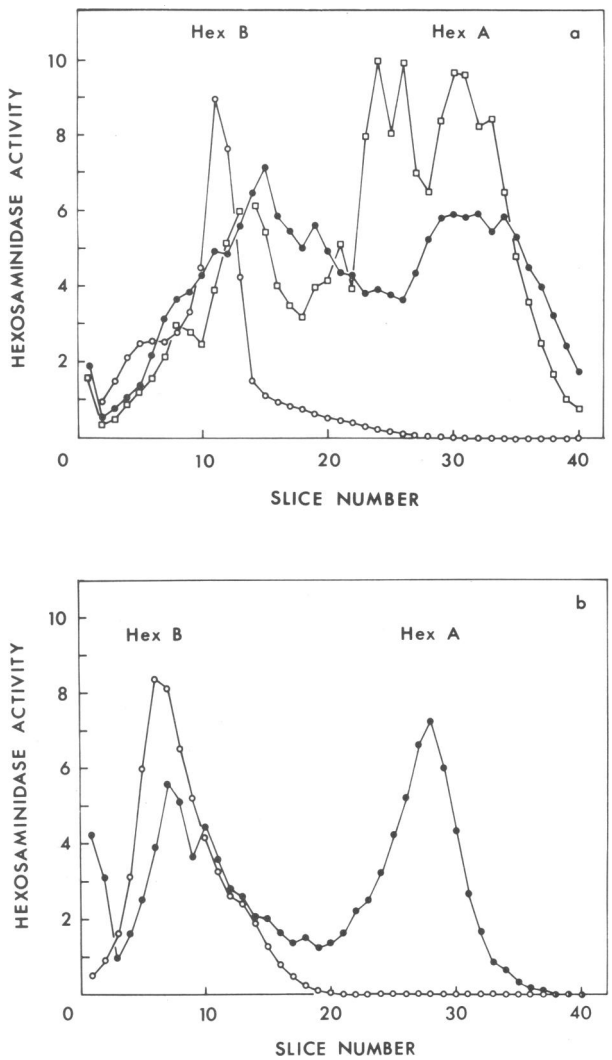

FIG. 3.—Polyacrylamide gel electrophoresis of brain (*a*) and liver (*b*) from patient 2 (●—●), a child who died with typical Tay-Sachs disease (○—○), and a control (□—□). Hexosaminidase activity is expressed in arbitrary units.

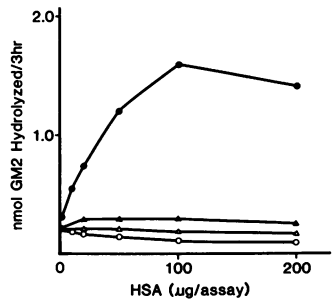

FIG. 4.—Effect of human serum albumin (HSA) on the hydrolysis of G_{M2} ganglioside by liver HEX A and B from a control and patient 2. All samples contain 20 nmol [^{3}H]G_{M2}, 200 µg sodium taurodeoxycholate, acetate buffer (0.01 M, pH 4.6), and 0.1 U of HEX A or B. Control HEX A (●), control HEX B (○), patient 2 HEX A (▲), patient 2 HEX B (△).

0.13 nmol of G_{M2} ganglioside per 16 hrs/mg fibroblast supernatant protein, respectively. Patient 3, who is clinically normal at this time, has a value of 1.00, which is in the low normal range. At this time, we have not studied the cultured skin fibroblasts from the parents or other obligate heterozygotes to determine if carrier testing is possible. However, it is now possible to positively diagnose these types of patients with an in vitro test in cultured skin fibroblasts.

DISCUSSION

Two patients were suspected of having some type of G_{M2} gangliosidosis because of the finding of increased G_{M2} ganglioside in brain (fig. 1) and pathological findings consistent with a ganglioside storage disease (fig. 2). They, along with their clinically normal sister, were previously diagnosed as having MLD by a test for urinary arylsulfatase A by another laboratory. Their clinical pictures, neuropathology, and G_{M2} ganglioside storage levels (56% of the total gangliosides) are typical of other patients with juvenile G_{M2} gangliosidosis with a partial deficiency of HEX A [19–23]. In addition, other patients with a chronic form of G_{M2} gangliosidosis with a low total hexosaminidase or low percent HEX A have been described [24–29]. However, the patients described here have normal total hexosaminidase and percent HEX A in cultured skin fibroblasts, liver, and brain (table 1).

Extraction of the activator protein required for the enzymatic catabolism of G_{M2} ganglioside from brain of patient 2 revealed elevated levels when tested with HEX A isolated from control sources. This appeared to rule out a diagnosis of AB variant of G_{M2} gangliosidosis as described by Conzelmann and Sandhoff [5] and Hechtman et al. [6]. However, when these activator proteins, purified from liver and brain of controls and brain from patient 2, were tested with HEX A from the liver and brain of the patient, near zero activity was measured. A similar finding was made by Li et al. [7] using only brain samples from case 1 described in a paper by Goldman et al. [16].

Further studies were done to determine if this type of patient could be diagnosed in cultured skin fibroblasts. Cultured cells from our patient 2, case 1 described

by Goldman et al. [16], an Ashkenazi Jewish case of Tay-Sachs disease, and nine controls were grown using our usual conditions and treated as described in MATERIALS AND METHODS. Using the G_{M2} ganglioside activator protein purified from brain from either a control or patient 2, significant activity for G_{M2} ganglioside hydrolysis could be measured in fibroblast lysates of controls. Cells from patient 2, case 1 described by Goldman et al. [16], and a case of Tay-Sachs disease had very low activity toward G_{M2} ganglioside when activator protein was used in place of sodium taurodeoxycholate. Using bile salts, it was difficult to differentiate these patients from controls because of the stimulation of HEX B to hydrolyze G_{M2} ganglioside (our unpublished data, 1982). HEX B cannot hydrolyze the ganglioside in an activator protein-mediated reaction. Previously, Erzberger et al. [30] demonstrated the use of G_{M2} activator protein for assays of G_{M2}-*N*-acetylgalactosaminidase activity in fibroblast lysates. Our methods differ in the source of activator protein (kidney vs. brain), concentrations of G_{M2} ganglioside, cell protein, activator protein, and buffer (100 mM citrate, pH 4.2, vs. 10 mM acetate, pH 4.6), and incubation time. However, either method could be used to diagnose those patients with a defect in HEX A, whether it be in the catalytic site or the activator binding site.

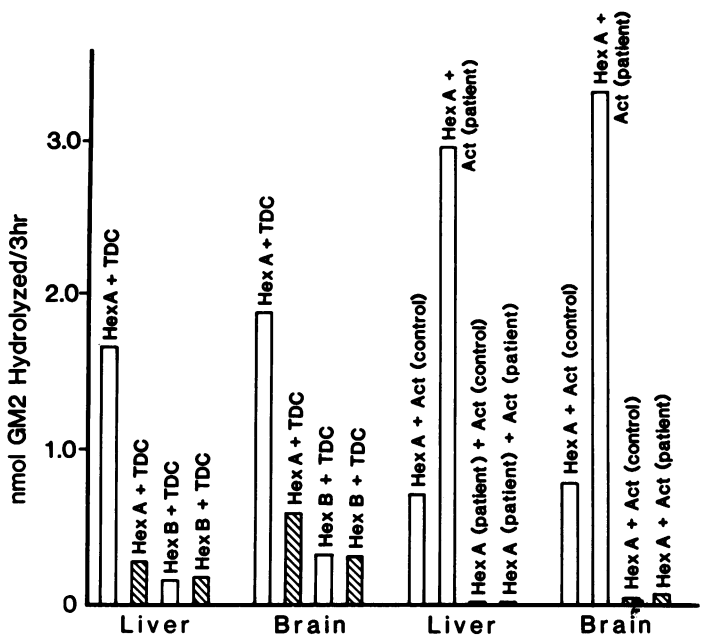

FIG. 5.—Effects of sodium taurodeoxycholate (TDC) and activator proteins from human brain on HEX A and B from patient 2 and controls. All assays were performed as described in MATERIALS AND METHODS using 0.1 U of hexosaminidase activity. Assays with TDC were carried out with 100 μg human serum albumin, and those with partially purified activator proteins from 10 g brain samples were carried out using 50 μg protein per assay. The source of the activator protein is described *on top of each bar* and the source of HEX A and B is given *on the abscissa*. *Open bars* are from control tissues and *crosshatched bars* are from patient 2 tissues. The results using HEX B and activator protein are not presented because HEX B is not activated by this activator protein.

The two patients described in this manuscript have some similarities to the patient described by Goldman et al. [16]. The unsteady gait noted at ages 2–2½, loss of speech, loss of muscle mass, and spasticity later in the disease process, and absence of motor seizures with significant EEG changes are found in all three patients. The fact that both families are from Puerto Rico, although not known to be related, is interesting. There are some differences, including the presence of bilateral macular cherry-red spots only in the patient of Goldman and deposition of some brown material under the maculi in these two patients. Patient 1 also had a slightly enlarged liver. Case 1 of Goldman et al. [16] died at age 4 years, 8 months, while the two patients described here died at ages 7 and 8. However, it is safe to assume from the report of Li et al. [7] and our research here that these patients have a similar mutation resulting in the storage of G_{M2} ganglioside.

The precise nature of the mutation in this type of G_{M2} gangliosidosis is not known; however, we might suspect that a mutation in HEX A results in a failure to bind activator protein and thereby prevents interaction with the natural substrate. Alternatively, a mutation in the binding site for G_{M2} ganglioside may be demonstrable only by using an activator protein-stimulated reaction. The ability of the "mutant" HEX A to catalyze the hydrolysis of 4MU-β-*N*-acetylglucosaminide and 4MU-β-*N*-acetylgalactosaminide is not affected in these patients. In addition, the heat stability and electrophoretic properties are comparable to normal. The so-called AB variant of G_{M2} gangliosidosis delineated by Conzelmann and Sandhoff [5] appears to have a defective activator protein and normal HEX A [31] and HEX B. Our patients and case 1 of Goldman et al. [16] appear to have a mutation in HEX A demonstrable only by using an activator protein-stimulated reaction. Perhaps they should be called G_{M2} gangliosidosis, $A^{M}B$ variant, to indicate a mutation in HEX A. The designation AB variant should be reserved for those patients having a defect in the G_{M2} activator protein [5]. The identification of these types of patients will require additional careful observation by the clinician and new tests, such as those described, by the laboratories doing diagnostic testing.

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