Am J Hum Genet 35:900–913, 1983

Ganglioside G_{M2} *N*-Acetyl-β-D-Galactosaminidase Activity in Cultured Fibroblasts of Late-Infantile and Adult G_{M2} Gangliosidosis Patients and of Healthy Probands with Low Hexosaminidase Level

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SUMMARY

A sensitive assay was developed to assess the ability of extracts from cultured fibroblasts to catabolize ganglioside G_{M2}, in the presence of the natural activator protein but without detergents. This method, which permitted the reliable determination of residual activities as low as 0.1% of normal controls, was then used to measure ganglioside G_{M2} hydrolase activities in fibroblasts from several hexosaminidase variants. The residual activities thus determined correlated well with the clinical status of the respective proband: infantile Tay-Sachs (0.1% of normal controls), late-infantile (0.5%), and adult G_{M2} gangliosidoses (2%–4%) and healthy probands with "low hexosaminidase" (11% and 20%). In contrast, β-hexosaminidase A levels as measured with the synthetic substrate 4-MU-GlcNAc could not be relied on for diagnostic purposes (the late-infantile patient studied retained 80% of the activity of controls).

INTRODUCTION

G_{M2} gangliosidosis is an inherited disorder of lysosomal glycolipid catabolism, characterized by the massive accumulation of ganglioside G_{M2} and glycolipid G_{A2} in neuronal tissue [1, 2]. Biochemically, three enzymic variants can be distinguished: patients of variant B lack β-hexosaminidase A [3, 4], the enzyme responsible for the degradation of the stored glycolipids [5], whereas in patients with variant 0, both major lysosomal hexosaminidase isoenzymes, A and B, are

Received September 7, 1982; revised December 13, 1982.

This study was supported by grants Sa 257/7 and Sa 257/10 from the Deutsche Forschungsgemeinschaft and by the fund for basic research, administrated by the Israel Academy of Science and Humanities.

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missing [6, 7], presumably due to the defect of their common subunit [8, 9]. The third variant, AB, is not caused by the deficiency of an enzyme but by the absence of an activator protein required for the interaction of the water-soluble glycosidases with their lipid substrates [10].

Besides the severe infantile forms with complete absence of the respective isoenzyme activities, juvenile and adult forms of variants B and 0 are known with variable residual activities of hexosaminidase isoenzymes (for a review, see [2]). On the other hand, a number of apparently healthy individuals possessing only very low hexosaminidase levels have been found [11–17]. Some of the cases reported in the literature as apparently healthy adults have, later on, developed progressive neurological symptoms [18], whereas others still seem to be unaffected.

Most of the studies on the residual levels of hexosaminidase activities have employed only artificial water-soluble substrates, such as 4-methylumbelliferyl glycosides, but have not investigated the capability of these enzymes to degrade the stored glycolipids. In the few studies where the ganglioside G_{M2} hexosaminidase was assayed, it was found that this activity did not parallel the residual activity toward water-soluble substrates [19, 20]. However, even these measurements do not allow the reliable estimation of the respective tissues' ganglioside degrading capacity, since they had to use detergents to enable the interaction between the enzymes and the lipid substrates. In the meantime, it has become clear that, in vivo, this interaction is accomplished by nonenzymic protein cofactors, termed "activator proteins" [5, 10, 21–23], and that the interaction of these activators with the glycolipid hydrolases is highly specific and determines the enzymes' substrate specificity to a great extent [5, 24]. Therefore, an assay system was developed to assess the ganglioside G_{M2}-cleaving capability of cultured fibroblasts, employing the natural activator protein instead of detergents. This system could be shown to be greatly superior to detergent-containing assays in differentiating between G_{M2} gangliosidosis patients, carriers, and healthy controls [24].

We describe here the application of an improved version of the activator-based ganglioside G_{M2} hydrolase test to the determination of the residual ganglioside G_{M2}-cleaving capabilities of fibroblasts from late-infantile and adult G_{M2} gangliosidosis patients and from healthy individuals with low levels of hexosaminidase activity.

THE PATIENTS

The patients include: (1) three siblings from an Ashkenazi Jewish family with the adult form of G_{M2} gangliosidosis. These patients had initially been diagnosed as apparently healthy individuals with low hexosaminidase (HEX) A [11, 14] but had later developed speech and gait difficulties [18]; (2) two apparently healthy probands, a father and his daughter, with low β-HEX level described by Dreyfus et al., called the "Paris variant" [13, 16]; (3) a late-infantile patient with G_{M2} gangliosidosis, possessing a nearly normal level of HEX A, as determined with the fluorogenic substrate; (4) and an infantile Tay-Sachs patient.

MATERIALS AND METHODS

Materials

The fluorogenic substrates 4-MU-GlcNAc and 4-MU-GalNAc as well as *N*-acetyl-D-galactosamine were from Koch-Light, Colnbrook, England. Bovine serum albumin (more

than 99% pure) was purchased from Serva, Heidelberg, West Germany. DEAE-cellulose (DE 52) from Whatman, Maidstone, England; scintillation fluid (Aqualuma) from Baker, Deventer, The Netherlands, and carrier ampholytes for isoelectric focusing (IEF) from LKB, Bromma, Sweden.

Ganglioside G_{M2} was isolated from Tay-Sachs brain by the method of Svennerholm [25] and tritiated in the *N*-acetylgalactosamine moiety by the galactose oxidase-NaB $^3H_4$ method according to Suzuki and Suzuki [26]. Its specific radioactivity was 13 Ci/mol. After isolation from the reaction mixture, the labeled ganglioside was repurified by preparative thin-layer chromatography on silica gel 60 plates (Merck, Darmstadt, West Germany) with the solvent system chloroform/methanol/15 mM aqueous $CaCl_2$ (60/40/9, v/v/v). The band containing the ganglioside was scraped off and eluted with 10 ml of chloroform/methanol/water (60/40/9, v/v/v). This ganglioside solution (containing some 2 μmol ganglioside) was then loaded onto a 10-ml column of DEAE-cellulose in methanol. The column was washed with 2 vol of methanol and then eluted with 4 × 1 vol of 0.1 mol/l ammonium acetate in methanol. Radioactive fractions were combined, the solvent was evaporated under a stream of N_2, and the residue dissolved in 2 ml of water and dialyzed against several changes of distilled water for 72 hrs. After determination of sialic-acid content by the modification of Miettinen and Takki-Luukkainen [27] of the method of Svennerholm [28], the solution was adjusted with concentrated citrate buffer to give a solution of 1 mmol/l of ganglioside G_{M2} in 80 mM citrate buffer, pH 4.0. In the standard assay (see below) without enzyme, this preparation gave a background of 0.15% of the radioactivity employed.

Activator Protein

The activator protein for the degradation of ganglioside G_{M2} by HEX A was purified from postmortal human kidney as described [5]. One activator unit (U) was defined as the amount of activator protein that stimulates ganglioside G_{M2} degradation by HEX A under the conditions used to assay the amount of activator [5] (100 mM citrate buffer, pH 4.2, 37°C) by 1 nmol/hr per enzyme U. One mg of purified activator corresponds to approximately 4,000 activator U.

Cell Culture

Skin fibroblast cultures were provided by Dr. Galjaard, Rotterdam (healthy proband, daughter), Drs. Poenaru and Boué, Paris (healthy proband, father), and Dr. Maire, Lyon (late-infantile patient). Skin biopsies of the other probands were taken by us. The cells were cultured with Eagle's modified medium (Gibco, Grand Island, N.Y., cat. no. H 16) containing 10%–20% fetal calf serum and maintained in a 5% CO_2 atmosphere. After 6–8 weeks (four subcultures), confluent cells were harvested with trypsin and frozen at −20°C until used. Of each cell line, at least two different samples were analyzed.

Preparation of Cell Extracts

Fibroblasts were homogenized in water (10% v/v) in an ice bath by sonication (Branson sonifier B-12, Branson, Danbury, Conn.) equipped with a microtip, at 40 W (2 × 10 seconds). The cell suspensions were homogenized by sonication rather than by repeated freezing and thawing to avoid the risk of destroying the (possibly more freeze-labile) mutated enzyme. Aliquots of the homogenates were assayed for total β-HEX activity with the fluorogenic substrate 4-MU-GlcNAc as described below and for protein content (see below). The homogenates were then centrifuged at 100,000 *g* for 30 min, and the supernatant was again assayed for total β-HEX activity with the fluorogenic substrate to determine the percentage of β-HEX extracted.

Determination of Enzyme Activity

With fluorogenic substrates. Appropriately diluted samples of the enzymes were added to incubation mixtures containing 0.2 μmol of either 4-MU-GlcNAc or 4-MU-GalNAc

and 10 μmol of citrate buffer to give a total volume of 0.2 ml. After 30 min of incubation at 37°C, the reaction was terminated by the addition of 1 ml 0.2 M glycine/0.2 M Na_2CO_3 solution. Fluorescence of the liberated 4-methylumbelliferone was read in a filter fluorimeter (Locarte, London, England) and compared with that of a standard solution of known concentration. One enzyme U is defined as the amount of enzyme that splits 1 μmol of 4-MU-GlcNAc per min under these conditions.

With ganglioside G_{M2}. The assay mixture contained 20 μl of cell extract (100,000 g supernatant; approximately 20 μg protein), 5 μl of substrate solution (corresponding to 5 nmol of ganglioside G_{M2} and 0.4 μmol of citrate buffer, pH 4.0), and 15 μl of the activator protein solution (7.5 activator U and 1.5 μg of bovine serum albumin). After incubation for 13 hrs at 37°C, the reaction was stopped by addition of 0.5 ml of an ice-cold solution of 1 mM *N*-acetylgalactosamine in water. To determine the amount of [^{3}H]GalNAc liberated, the assay mixture was passed over a 1-ml column of DEAE-cellulose in water. The column was eluted with 2 ml of 1 mM aqueous *N*-acetylgalactosamine, the combined effluents mixed with 10 ml of scintillation liquid, and their radioactivity quantified by scintillation counting. All assays were run in duplicates, with excellent agreement.

Isolation of HEX A by Ion-exchange Chromatography

Fibroblast extracts were loaded onto 0.5-ml columns of DEAE-cellulose that had been equilibrated with 10 mM phosphate buffer, pH 6.0, with 50 mM NaCl. Nonadsorbed material, including HEX B, was eluted with 3 column vol of the same buffer. HEX A was eluted with 3 vol of 500 mM NaCl in buffer. Enzyme-containing fractions were exhaustively dialyzed against distilled water.

IEF

IEF was performed in a 110-ml column (LKB, Bromma, Sweden) using carrier ampholytes, pH 3.5–pH 10, in a linear sucrose gradient from 35% to 0% (w/v). Cathode solution was 1.5% (w/v) of ethylene diamine in a 50% (w/v) aqueous sucrose solution; the anode solution was 0.1% (w/v) aqueous H_2SO_4. After 72 hrs at 500 V, focusing was terminated and fractions of 1.5 ml were collected. The pH value of each fraction was determined in 65-μl aliquots using a microelectrode (Radiometer, Copenhagen, Denmark), and hexosaminidase activity was measured with 4-MU-GlcNAc as described above.

Heat Inactivation

HEX A, isolated by IEF from normal and from mutant fibroblasts and dialyzed against distilled water, was incubated in 40 mM citrate buffer, pH 4.5, containing 1 mg bovine serum albumin/ml. After various times up to 60 min, samples were withdrawn and analyzed for their β-HEX content with the fluorogenic substrate 4-MU-GlcNAc as described above.

Protein Determination

Protein content was measured by the method of Lowry et al. [29], using crystalline bovine serum albumin as standard.

RESULTS

The original assay system for the determination of the ganglioside G_{M2}-cleaving capability of cultured skin fibroblasts, employing the natural activator protein, as proposed by Erzberger et al. [24], proved to be too insensitive for the reliable detection of the very low residual activities of adult G_{M2} gangliosidosis variants. Therefore, the following modifications were introduced: (1) repurification of the [^{3}H]ganglioside G_{M2} substrate as detailed in MATERIALS AND METHODS to reduce unspecific background to less than 0.15% of the radioactivity employed; (2) reduction of buffer concentration from 100 mM to 10 mM increased degradation

rates more than eightfold [30]; (3) reduction of ganglioside G_{M2} concentration from 0.25 mM to 0.125 mM reduced the background proportionally, but had little effect on the degradation rates observed (cf., [5]); (4) the amount of activator employed was increased to 7.5 activator U/assay, approaching saturation of the enzyme; and (5) cell homogenates were centrifuged at 100,000 *g* to remove practically all particulate material, since it had been found that homogenates gave much less reproducible data, especially in the case of infantile Tay-Sachs fibroblasts. Altogether, these modifications improved the sensitivity of the assay system some 20-fold, permitting the reliable determination of residual activities as low as 0.1% of the control values.

The improved assay was employed to measure the ganglioside G_{M2} β-D-galactosaminidase activity of cultured fibroblasts from normal controls, from a Tay-Sachs patient, and from the patients with various levels of HEX A as determined with the artificial fluorogenic 4-MU substrates.

Supernatants obtained from fibroblasts of the different probands as well as of healthy controls were analyzed for their capability to degrade the ganglioside G_{M2} in the presence of the natural activator protein. In all cases examined, the reaction proceeded linearly for up to 14 hrs (fig. 1). Therefore, the incubations were run overnight (13 hrs) in order to determine the small residual activities as accurately as possible.

The degradation rates measured were divided by the extraction factors determined with the fluorogenic substrate as described in MATERIALS AND METHODS to give the values that would have been measured if all β-HEX had been extracted from the cells. These corrected degradation rates were then related to the protein content of the respective homogenates. Of each cell line, at least two different samples were analyzed; the results were always in good agreement. The data of a typical series are shown in table 1, together with the β-HEX activities determined with the artificial substrate.

It is evident that in some cases the residual HEX A activity toward the fluorogenic substrate roughly parallels the ganglioside G_{M2} hydrolase capacity measured with the activator protein, whereas in others there is some discrepancy. The most conspicuous case is that of the late-infantile patient who has a nearly normal amount of 4-MU-GlcNAc-β-hexosaminidase A activity (fig. 2*c*) with normal heat stability (fig. 3), but whose HEX A is obviously not able to degrade the ganglioside G_{M2} in the assay described here (table 1).

The residual activities of ganglioside G_{M2} *N*-acetyl-β-D-galactosaminidase in cells from the adult G_{M2} gangliosidosis patients described by Navon et al. [18] ranged between 2% and 3.6% of the control values. No correlation could be established between residual activity in these cases and severity of the disease or age of onset. Fluctuations seemed, rather, due to variations in cell culture conditions, confluency, and other such parameters. In contrast, cells derived from infantile Tay-Sachs patients had residual activities of some 0.1% of the control values.

Since these studies had been performed on cell pellets that had been kept frozen for some time, the possibility of inactivation of the mutated enzymes by freezing or storage had to be ruled out. To this end, some of the experiments were repeated

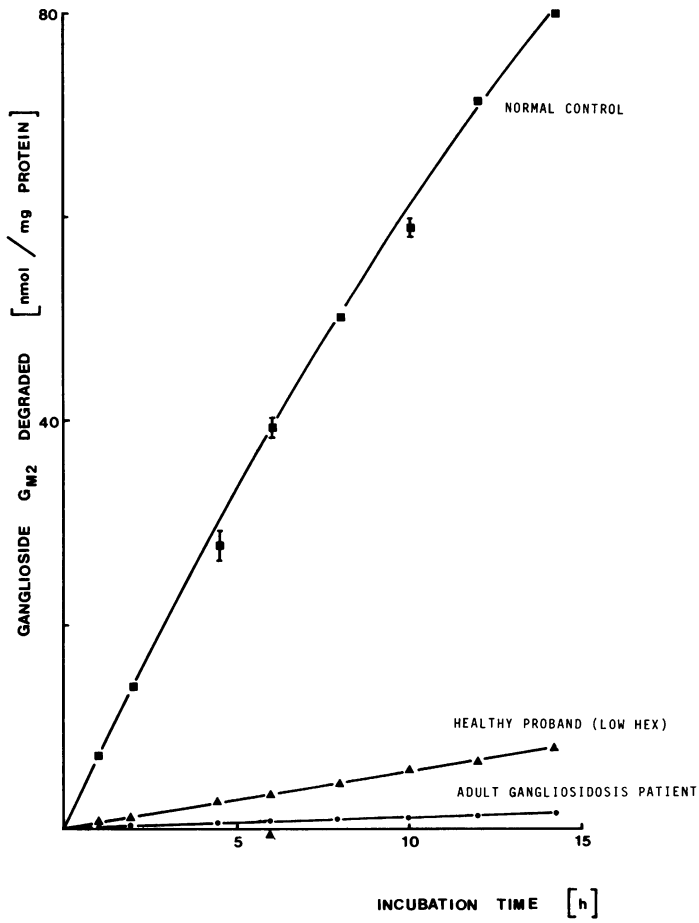


FIG. 1.—Degradation of ganglioside G_{M2} by extracts of various fibroblasts. Fibroblast extracts were prepared and incubated with ganglioside G_{M2} and activator protein as described under MATERIALS AND METHODS. Incubation was terminated after the *times indicated* and liberated GalNAc was determined. ■—■, normal control; ▲—▲, apparently healthy proband (father); ●—●, adult G_{M2} gangliosidosis.

with freshly harvested cells. The values thus obtained were essentially the same as with frozen cell pellets (data not shown).

The apparently healthy probands with very low β-HEX activity, a girl born in 1973 and her father, were reported to have the same genotype, both possessing residual β-HEX activities of 10% of the control values as estimated with the fluorogenic substrate 4-MU-GlcNAc [16]. In the present study, we found essentially the same level of total β-HEX activity, but IEF revealed that practically all residual β-HEX corresponded to HEX A (fig. 2*c*). In extracts of the father's cells, a substantial amount of HEX S could also be detected (table 1).

The residual ganglioside G_{M2}-cleaving activity of cells from these probands, as determined in the assay reported here, was severalfold above that of the adult G_{M2} gangliosidosis patients, reaching some 20% of the normal values (table 1).

TABLE 1

DEGRADATION OF GANGLIOSIDE G_{M2} BY FIBROBLAST HOMOGENATES IN THE PRESENCE OF THE NATURAL ACTIVATOR PROTEIN

PROBAND*	AGE (YRS)	4-MU-GLCNAC-β-HEXOSAMINIDASE		GANGLIOSIDE G_{M2} DEGRADATION	
		Total activity nmol/min × mg	% HEX A†	pmol h × mg × activator U	% of control
Adult patient 1 (f)	44	38.2	7.2[a,b]	19.2	2.87
Adult patient 2 (m)	38	33.7	6.8[b]	24.1	3.60
Adult patient 3 (f)	35	45.2	8.3[b]	13.1	1.96
Healthy probands with low hexosaminidase:					
Father (m)	35	4.6	67[a]‡	74.9	11.2
Daughter (f)	7	5.4	98[a,b]	134.4	20.1
Late-infantile patient (m)	5	55.0	51.3[a]	3.4	0.51
Infantile Tay-Sachs (m)	...	74.4	0[b]	0.78	0.12
Three controls (m + f)	...	44.6 (33.2–57.2)	76.6[a,b] (70.3–84.1)	670 (540–760)	100

NOTE: Fibroblast supernatants (100,000 g) were used in the assays, and the values measured were corrected with the extraction factors determined as described in the text.

*m: male; f: female.

†a: determined by IEF; b: determined by ion-exchange chromatography.

‡The remaining activity focused at the IEP of HEX S, pH 4.7; HEX B was not detectable.

These data suggested that the residual HEX A of the healthy probands' cells is decreased to some 10%–20% of normal, but has still the same ratio of ganglioside G_{M2} degradation vs. cleavage of the artificial substrate as the normal enzyme. To investigate this point further, the HEX A of the fibroblasts from one of the probands (daughter) was isolated by IEF and was compared with normal HEX A, which had been isolated the same way. For both preparations, the ratio of ganglioside G_{M2} cleavage (in the presence of the activator protein) vs. degradation of 4-MU-GlcNAc was practically identical (15.4 nmol ganglioside G_{M2} cleaved/[hr × enzyme U × activator U]).

A slight difference in thermal stability could be demonstrated by heat inactivation at 50° C, pH 4.5: at this temperature, the normal enzyme had a half-life of some 15 min, whereas for the mutant enzyme, a half-life of approximately 10 min was found (fig. 3). This is in contrast to the results of Dreyfus et al. who had found that under the same conditions normal as well as the proband's HEX A were completely inactivated within 5 min at 50°C, whereas at 42°C, the mutant enzyme was considerably less stable than the normal one [16].

The K_M values of both enzyme preparations for the synthetic water-soluble substrates were very similar, with values of 0.50 mM (normal) and 0.71 mM (proband) for 4-MU-GlcNAc and 0.090 mM (normal) and 0.084 mM (proband) for 4-MU-GalNAc.

DISCUSSION

The inherited deficiency of HEX A causes accumulation of ganglioside G_{M2} in the lysosomes, mainly in those of neuronal cells. The severity of the resulting neurological impairments depends probably on the amount of storage material deposited in the neurons, and it is generally assumed that the rate at which the ganglioside G_{M2} accumulates is related to the residual activity of HEX A in the patients' tissues: complete absence of HEX A activity leads to the severe infantile forms of G_{M2} gangliosidosis with onset of the symptoms before 1 year of age and death usually occurring by 2–4 years of age. On the other hand, late-infantile, juvenile, and adult forms of variants 0 and B of G_{M2} gangliosidosis have been described with later onset and slower progression of the disease (for a review, see [2]). Some of the adult patients are only moderately handicapped at the age of 40 [18]. The residual HEX A activities in such cases have been measured with artificial water-soluble substrates or with ganglioside G_{M2} in the presence of detergents. Both methods have, however, serious disadvantages: a mutation may alter an enzyme's substrate specificity in such a way that it still cleaves water-soluble substrates but is inactive toward glycolipids. On the other hand, if ganglioside G_{M2} is used as substrate in a detergent-containing system, this detergent may alter the isoenzymes' substrate specificities [24, 31] and/or may denature the enzyme [24]. The latter effect may be more severe for a more labile mutated enzyme than for a normal one, rendering all values thus determined incomparable.

Therefore, it has hitherto been impossible to correlate the residual HEX A activity measured in vitro with the severity of the disease; for example, residual activities of juvenile patients ranged from 5% up to 50% of normal controls (for a review, see [2]). On the other hand, healthy adult probands have been found

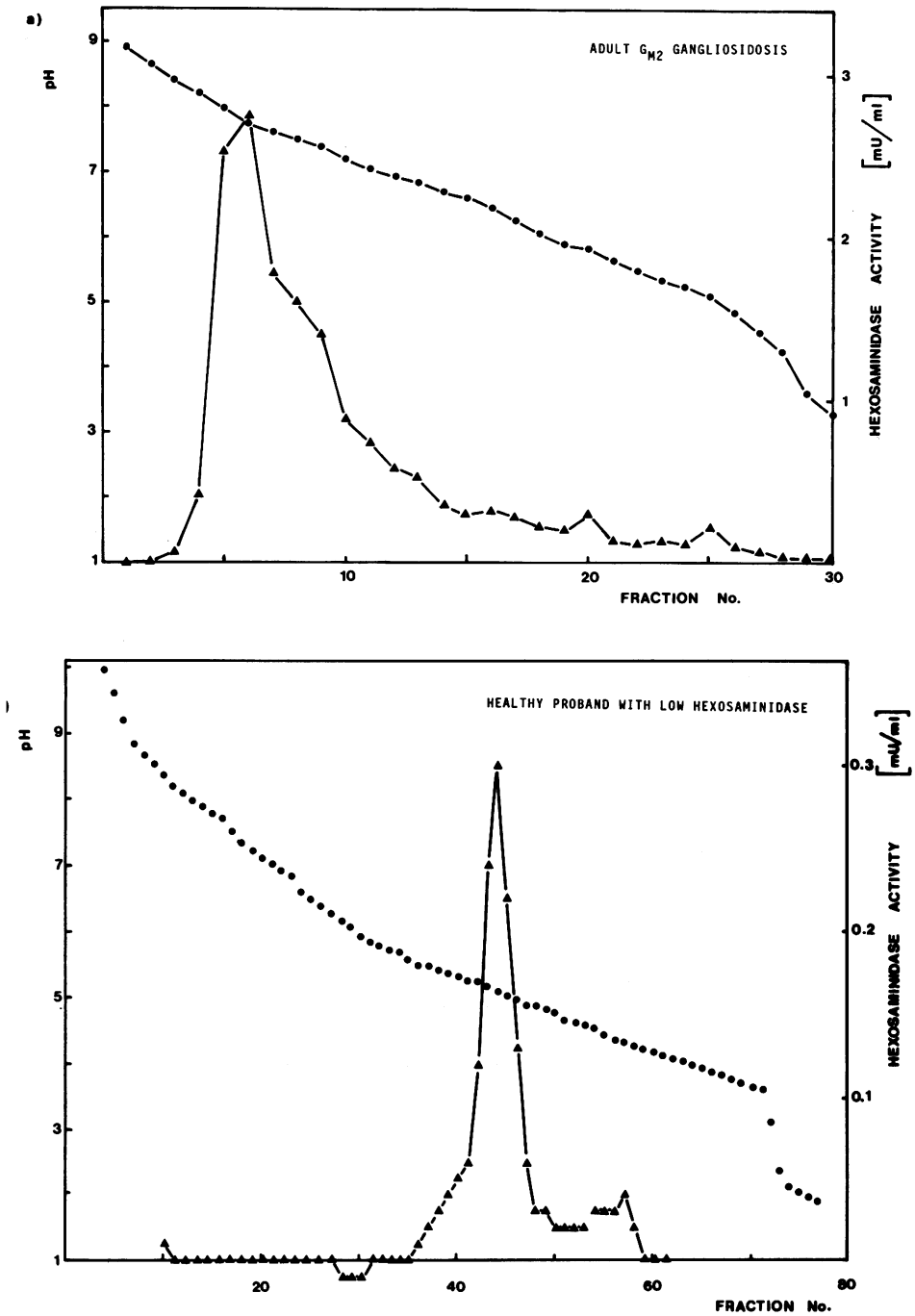
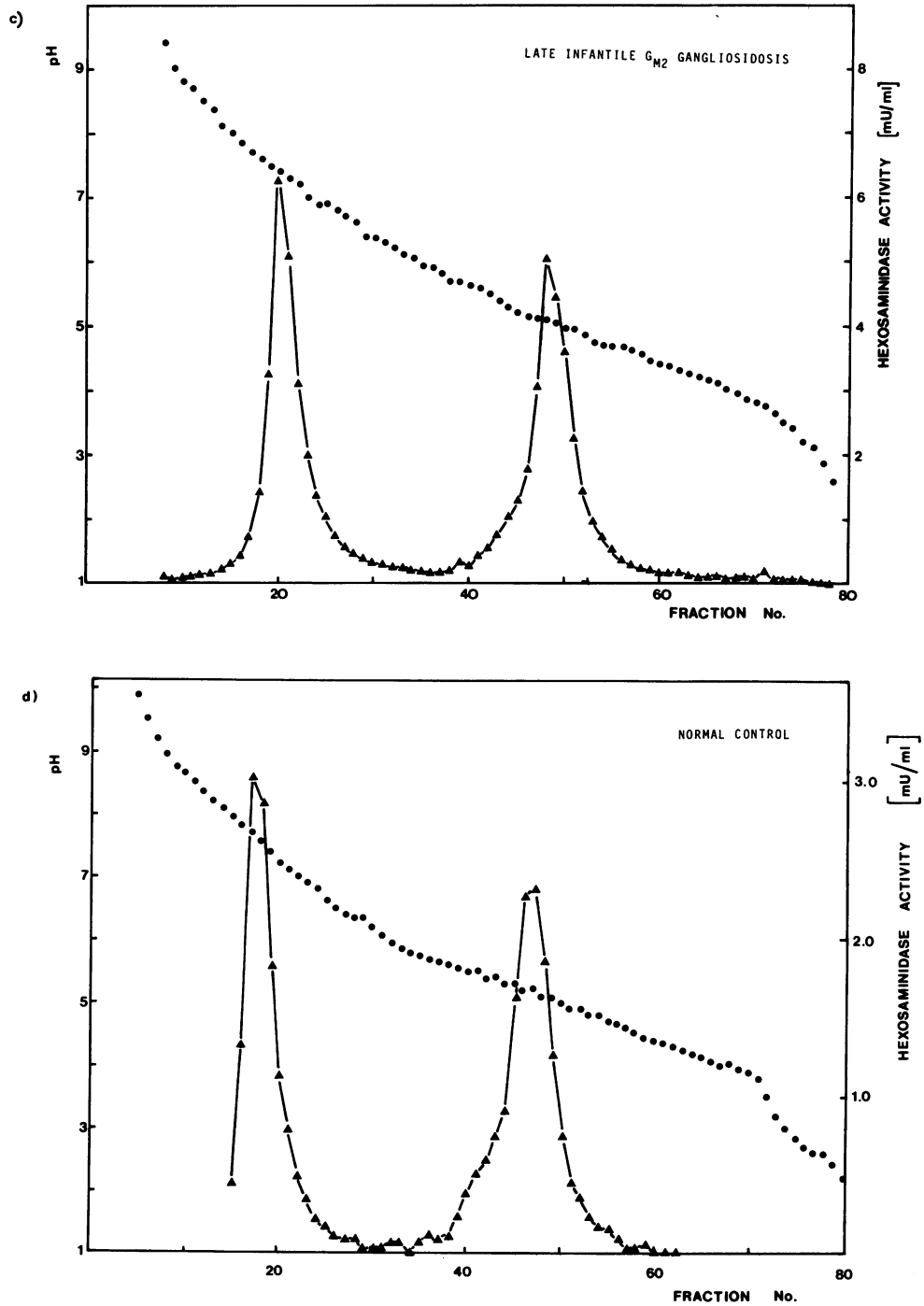


FIG. 2.—Hexosaminidase patterns after IEF of fibroblast extracts from various probands. Extracts of fibroblasts from the probands listed below were prepared and subjected to IEF as described under MATERIALS AND METHODS. Hexosaminidase activity in the fractions was monitored with 4-MU-GlcNAc



as substrate. *a*, adult G_{M2} gangliosidosis patient; *b*, apparently healthy proband (daughter); *c*, late-infantile G_{M2} gangliosidosis patient; *d*, normal control.

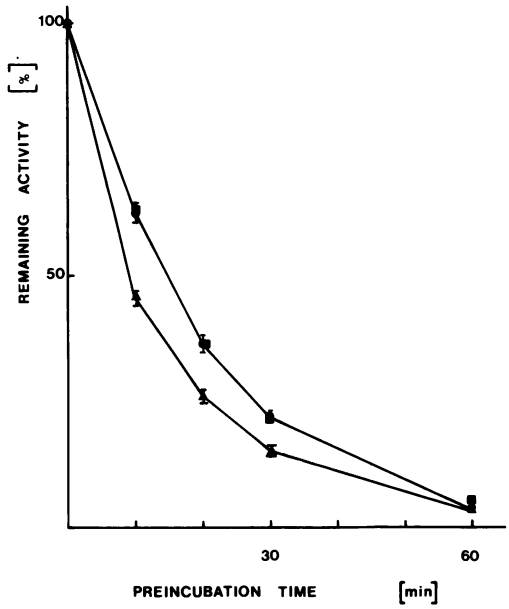


FIG. 3.—Heat inactivation of the HEX A of various probands. HEX A was isolated from fibroblast extracts by IEF as described under MATERIALS AND METHODS. The enzymes were dialyzed against distilled water and incubated at 50°C in 40 mM citrate buffer, pH 4.5, containing 1 mg of bovine serum albumin/ml. After *the times indicated*, samples were withdrawn and analyzed for their hexosaminidase activity with 4-MU-GlcNAc as substrate. ●—●, normal control; ▲—▲, apparently healthy proband with low hexosaminidase (daughter); ■—■, late-infantile patient with G_{M2} gangliosidosis.

with hexosaminidase levels of less than 10% of normal [13, 16]. It has recently been demonstrated that the activity of water-soluble lysosomal glycosidases on their membrane-bound glycolipid substrates depends on the presence of nonenzymic protein cofactors, which are called activators (for a review, see [23]) and which serve to solubilize the glycolipids to facilitate the attack by the enzyme, and that the interaction between these activator proteins and the respective enzyme is highly specific [5]. To assess the true capability of a patient's HEX A to degrade the ganglioside G_{M2}, this specificity must be taken into consideration. Therefore, such assays should use the ganglioside G_{M2} as a substrate, in the presence of the specific activator protein but without detergents. An assay system based on these principles has previously been shown to allow a clear-cut differentiation between G_{M2} gangliosidosis patients and normal controls [24].

In our present study, a substantially improved version of this test is demonstrated to enable the estimation of the residual ganglioside G_{M2} β-D-hexosaminidase activities of fibroblasts from G_{M2} gangliosidosis patients of widely differing ages and from apparently healthy probands with low β-HEX levels.

The HEX A levels determined with artificial water-soluble substrate after separation of the isoenzymes may be extremely misleading, as evidenced by the late-infantile case in this study (fig. 2*b* and table 1). A similar case has been reported by Li et al. [32]. Obviously, the HEX A of these patients is mutated in

such a way that it is still nearly normally active on the water-soluble 4-MU glycosides while having lost its ability to interact with the ganglioside G_{M2}/activator complex (which seems to be the true substrate of this enzyme [30]). In such cases, only an assay based on the principle outlined above, with ganglioside G_{M2} as substrate in the presence of the activator, will yield diagnostically meaningful results.

In the other cases studied here, there seems to be at least some gross correlation between HEX A activity toward synthetic substrates and ganglioside G_{M2} hydrolase activity (table 1).

The fibroblasts from adult G_{M2} gangliosidosis patients contain some 10% of the normal amount of 4-MU-GlcNAc HEX A but exhibit only 2%–4% of the normal ganglioside G_{M2} galactosaminidase activity. These findings are in contrast to those of Tallman et al., who, using ganglioside G_{M2} in the presence of detergents, found heterozygote values in the leukocytes of the same patients [19]. Since the test system described here meets the specificity requirements outlined above, the values of table 1 may be presumed to correlate with the true capabilities of these cells to degrade ganglioside G_{M2}. It seems that a residual ganglioside G_{M2} hexosaminidase activity between 2% and 4% of the normal controls degrades most but not all of the ganglioside G_{M2} delivered to the lysosomes so that the ganglioside accumulates at a very slow rate, leading to neurological symptoms only in adulthood. An even higher residual activity (e.g., more than 10% of normal) may therefore be compatible with normal life. From these considerations, it seems likely that the "apparently healthy" probands whose fibroblasts were found to possess ganglioside G_{M2} hydrolyzing activities of 11% and 20% of normal, respectively (table 1), will remain unaffected for all their lives. The father is already 35 years old but did not yet exhibit any sign of G_{M2} gangliosidosis [16].

The adult patients as well as the healthy probands described in this study were initially discovered when their respective families were screened for β-HEX levels because some cases of infantile G_{M2} gangliosidosis had occurred in the families. Evaluation of the pedigrees thus obtained revealed that all of the probands described here must be compound heterozygotes, that is, carrying the Tay-Sachs or Sandhoff gene, respectively, on one chromosome and a less severe mutation in the corresponding gene locus on the other chromosome [11, 14, 16].

In the adult patients described here, only HEX A is deficient whereas the B isoenzyme is present in normal or elevated amounts [11], indicating that the mutations reside in the subunit which is unique to HEX A (variant B of G_{M2} gangliosidosis).

In the cells of the healthy probands, on the other hand, the total β-HEX activity as measured with synthetic fluorogenic substrates is strongly depressed [16], indicating a mutation of the β subunit that is common to both HEX A and B. Preliminary immunochemical experiments demonstrated the presence of some material (approximately 40% of normal) cross-reacting with an anti-HEX B antiserum and of a higher level (60% of normal) of material cross-reactive with an antiserum specific for the α subunit of HEX A [33]. The results of the IEF experiment demonstrating the complete absence of HEX B activity (fig. 2*b*) may be explained in a number of ways; for example, by the following model: The

mutation of the β subunits only partially affects the activity of the enzymes formed but renders the β subunits unable to aggregate to the homopolymeric B enzyme. Interaction with the α subunits to form the heteropolymeric HEX A is less impaired, so that some HEX A is formed, but is less stable. This would also explain the difference in heat stability between normal and mutant HEX A (fig. 3).

In the case of the late-infantile patient with nearly normal HEX A level, it cannot be stated with certainty which polypeptide chain, α or β, is affected, since the roles of both in ganglioside degradation by the intact HEX A are not yet clearly understood. From the specific activities of the purified HEX A and B toward synthetic water-soluble substrates [31, 34] and from the observation that this activity is deficient in variant 0 but normal in variant B of G_{M2} gangliosidosis it may be speculated that the activity toward water-soluble substrates is mainly contributed by the β subunit. On the other hand, only the heteropolymeric HEX A interacts with the activator/glycolipid complex [5].

Further studies of these variants may be expected to provide more insight into the respective roles of hexosaminidase subunits and their interaction with the activator protein, in normal condition as well as in disease.

ACKNOWLEDGMENTS

We are greatly indebted to Dr. Galjaard, Rotterdam, Drs. Poenaru and Boué, Paris, and Dr. Maire, Lyon, for providing the fibroblast cultures of the various probands. We thank Mrs. Ruth Gil, Miss Gabriele Weiß, and Mr. Otto Küpper for their skilled technical assistance, and Mrs. Waltraud Rau and Miss Karin Lempke for the preparation of the manuscript.

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