Tay-Sachs Disease: Generalized Absence of a Beta-D-N-Acetylhexosaminidase Component

Abstract. *Two hexosaminidase components, separable by starch-gel electrophoresis and possessing both β-D-N-acetylglucosaminidase and β-D-N-acetylgalactosaminidase activity, are present in human tissues. One of these, hexosaminidase component A, is absent in brain, liver, kidney, skin, cultured skin fibroblasts, blood plasma, and leukocytes from nine patients with Tay-Sachs disease. Hexosaminidase assay may facilitate the early diagnosis of individuals homozygous for Tay-Sachs disease.*

Tay-Sachs disease is a hereditary disorder transmitted as an autosomal recessive trait involving the massive cerebral accumulation of a specific ganglioside, GM_2 (*1*). The disease is invariably fatal; progressive cerebral degeneration results in death by the second, third, or fourth year of life (*2*). The ganglioside has a carbohydrate and a fatty acid composition identical to that of the normal cerebral ganglioside GM_2 (*1, 3*), namely, *N*-acetylgalactosaminyl-(1 → 4)-[(2 → 3)-*N*-acetylneuraminyl]-galactosyl-(1 → 4)-glucosyl-(1 → 1)-[2-*N*-acyl]-sphingosine.

The enzyme defect in Tay-Sachs disease is unknown. A degradative enzymic failure is a likely possibility (*4*). In generalized gangliosidosis, another ganglioside storage disease in which ganglioside GM_1 accumulates (*5*), Okada and O'Brien (*6*) have demonstrated a profound deficiency of a β-galactosidase which cleaves the terminal galactose from the stored ganglioside. Absence of a lysosomally localized hexosaminidase which cleaves the terminal *N*-acetylgalactosamine from the oligosaccharide moiety of ganglioside GM_2 could account for the degree and the localization of ganglioside accumulation in Tay-Sachs disease on a similar basis. Despite the satisfying nature of this explanation, hexosaminidase activities in cerebral tissues of patients with Tay-Sachs disease are elevated rather than deficient (*7*, Table 1) when assays are performed with synthetic substrates.

It seemed worth while to explore the physical properties and the electrophoretic behavior of hexosaminidases in Tay-Sachs disease in order to determine whether abnormalities of this enzyme might be present. This report documents the absence of a β-D-*N*-acetylhexosaminidase component in frozen organs, in blood plasma, and in living cells from patients with Tay-Sachs disease.

Tissues were available from seven patients (2 to 6 years of age, four boys, three girls) who died from Tay-Sachs disease. All tissues were stored at −20°C prior to analysis; the activities of all enzymes studied here were not affected by prolonged (1 to 5 years) frozen storage of control tissues. Control tissues were obtained from 14 patients who died of disorders not involving the central nervous system, from two patients with late infantile amaurotic idiocy (Jansky-Bielschowsky type), from six patients with the Hurler's syndrome (mucopolysaccharidosis types 1 to 3), and from two patients with generalized gangliosidosis. Many of the controls were similar in age to the Tay-Sachs patients, and all tissues were stored for similar periods (1 to 5 years). Control patients with generalized gangliosidosis and late infantile amaurotic idiocy had taken anticonvulsants and antibiotics during the course of their illness, as had the Tay-Sachs patients. Venous blood was obtained from two living patients, a 14-month-old boy and a 4-year-old girl, with Tay-Sachs disease and from five healthy adult subjects; leukocytes were isolated from 5 ml of whole blood after the erythrocytes were precipitated with dextran (*8*).

β-D-*N*-Acetylglucosaminidase and β-D-*N*-acetylgalactosaminidase activities were assayed at *p*H 4.4 in citrate-phosphate buffer (0.1*M*) with both *p*-nitrophenyl and 4-methylumbelliferyl derivatives (Pierce Co.) of the corresponding β-D-*N*-acetylhexosaminides as substrates (*9*). β-Galactosidase and β-glucosidase activities were assayed at *p*H 5.0 in acetate buffer (0.1*M*) with both the *p*-nitrophenyl and the 4-methylumbelliferyl derivates of each β-D-hexopyranoside (Pierce Co.) as substrates (*6, 10*). Assays were performed on tissue homogenates in distilled water and on supernatant fractions obtained by high speed centrifugation (100,000*g* for 60 minutes) of homogenates. Vertical starch-gel electrophoresis was performed by the method of Smithies (*11*) with the use of commercially available starch and apparatus (Otto Hiller, Madison, Wisconsin). The electrode buffer was varied between *p*H 5.0 and 7.6 (citrate-phosphate buffer 0.04*M*). After development, the gels were incubated with 4-methylumbelliferyl-β-D- derivatives of each substrate, and fluorescent regions of enzyme activity were located by viewing the gels under ultraviolet light after they were sprayed with alkali (glycine-carbonate buffer 0.25*M*, *p*H 9.8) to enhance fluorescence. Violet areas of hexosaminidase activity were obtained by incubating the gels at 37°C with the naphthol AS-BI derivatives of β-D-*N*-acetylglucosaminide (in-

Table 1. Acid hydrolase activity in Tay-Sachs disease (TSD). Values in brain, liver, and kidney are expressed as nanomoles of substrate cleaved per milligram of wet tissue per hour. Values in skin are expressed as nanomoles of substrate cleaved per milligram of protein per hour. Enzyme values in parentheses are the average values for each group.

Subjects	No.	β-Glucosidase	β-Galactosidase	β-*N*-Acetyl-glucos-aminidase	β-*N*-Acetyl-galactos-aminidase
			Cerebral cortex		
Controls	9	0.42–1.32 (0.82)	1.87–2.56 (2.19)	30.7–78.6 (50.5)	7.4–18.4 (12.9)
TSD	4	0.71–1.66 (1.30)	4.6–8.3 (5.8)	126.5–246.9 (157.9)	86.0–118.2 (98.6)
			Liver		
Controls	17	1.3–13.7 (5.8)	16.3–46.0 (30.3)	121–563 (323)	19.9–54.1 (41.0)
TSD	3	7.8–14.1 (10.9)	41.1–47.6 (44.2)	77–157 (106)	10.4–19.7 (14.4)
			Kidney		
Controls	2	4.0–7.6 (5.8)	42.0–47.2 (44.6)	273–344 (309)	35.3–54.9 (45.1)
TSD	3	9.5–12.2 (11.0)	66.7–76.2 (71.6)	136–287 (190)	14.5–36.0 (23.4)
			Skin		
Controls	22			52.8–285.6 (159)	16.4*
TSD	3			63.6–148.6 (105.8)	9.2–15.4 (12.8)

* A single assay only.

cubation time, 1 hour) and β-D-*N*-acetylgalactosaminide (incubation time, 4 hours) at *p*H 4.4 in 0.1*M* citrate-phosphate buffer according to Hayashi (*12*); violet spots appeared when the released naphthol AS-BI was coupled with Fast Garnet GBC salt (*o*-aminoazotoluene, diazonium salt, Sigma Co.) (1 mg/ml) in the same buffer, but at *p*H 7.5, for 1 hour. Quantification of each colored spot was made by densitometric analysis of the gels, with commercially available equipment (Photovolt densitometer). We used the densitometric method to quantify the relative proportions of individual hexosaminidase components in tissues and body fluids.

The activities of β-D-*N*-acetylglucosaminidase, β-D-*N*-acetylgalactosaminidase, β-glucosidase, and β-galactosidase were elevated in the cerebral cortex of Tay-Sachs patients compared to controls (Table 1). The average activities of the β-D-*N*-acetylglucosaminidase and β-D-*N*-acetylgalactosaminidase in liver, kidney, and skin were lower in the Tay-Sachs patients compared to controls, but there was overlap between the two groups. The *p*H optimum of β-D-*N*-acetylglucosaminidase in liver tissue from controls and Tay-Sachs patients was identical (*p*H 4.4 in both groups).

The sedimentation properties of β-D-*N*-acetylglucosaminidase and β-D-*N*-acetylgalactosaminidase in Tay-Sachs tissues differed markedly from the controls. After high speed centrifugation (100,000*g* for 60 minutes) of brain homogenates, which had been frozen and thawed many times and diluted 1 to 20 with distilled water, a large proportion (85 percent) of the hexosaminidase activity from controls remained in the supernatant fraction. After the same treatment only 20 percent of the hexosaminidase activity in the brain homogenate from Tay-Sachs patients was present in the supernatant; 80 percent of the activity was in the sediment. Similar results were obtained with kidney tissue. The reason for this behavior became obvious after starch-gel electrophoresis of hexosaminidases.

Starch-gel electrophoresis studies demonstrated that two major hexosaminidase components were present in all organs from control subjects (Fig. 1); one hexosaminidase (component A) migrated rapidly toward the node, and the other hexosaminidase (component B) migrated slowly toward the cathode at *p*H 6.0. Incubation of supernatant fractions or tissue homogenates overnight in 2*M* urea or 1*M* sodium chloride at 4°C did not result in an alteration of the relative proportions of the two components. Both components possessed β-D-*N*-acetylglucosaminidase and β-D-*N*-acetylgalactosaminidase activity, as demonstrated by incubating starch gels with the 4-methylumbelliferyl, *p*-nitrophenyl, and naphthol AS-BI derivatives of each β-D-*N*-acetylhexosaminide. However, the activity of β-D-*N*-acetylgalactosaminidase was approximately one-eighth that of β-D-*N*-acetylglucosaminidase. After high speed centrifugation of brain or kidney homogenates from control subjects as described above, component B occurred predominately in the sediment while component A occurred predominantly in the supernatant solution. Robinson and Stirling (*13*) have reported similar results from a study of hexosaminidase in human spleen and have demonstrated that the two hexosaminidase components possess similar K_m (Michaelis constant) values, are readily separated by chromatography on diethylaminoethyl-cellulose, and are both present in the lysosomal fraction. They also found that specificity for orientation on the 4-carbon was not absolute; both components isolated from human spleen possessed β-D-*N*-acetylglucosaminidase and β-D-*N*-acetylgalactosaminidase activity. Our results with human liver, brain, skin, and kidney agree with theirs.

Starch-gel electrophoresis studies of the hexosaminidases in Tay-Sachs disease demonstrated that component A was absent in all frozen tissues available (brain, liver, kidney, and skin). The absence of component A in the Tay-Sachs patients' tissues was demonstrated with both the *p*-nitrophenyl and

Table 2. Activity of hexosaminidase components in Tay-Sachs disease. Values are expressed as nanomoles of substrate cleaved per milligram of wet tissue per hour. Values in parentheses are the percentages of the total hexosaminidase activity of each component.

Subjects	No.	β-*N*-Acetylglucosaminidase		β-*N*-Acetylgalactosaminidase	
		Component		Component	
		A	B	A	B
		Cerebral cortex			
Controls	9	38.9 (77)	11.6 (23)	9.9	3.0
Tay-Sachs	4	0	157.9 (100)	0	98.6
		Liver			
Controls	17	129.2 (40)	193.8 (60)	16.4	24.6
Tay-Sachs	3	0	106 (100)	0	14.4
		Kidney			
Controls	2	123.6 (40)	185.4 (60)	18.0	27.1
Tay-Sachs	3	0	190 (100)	0	23.4

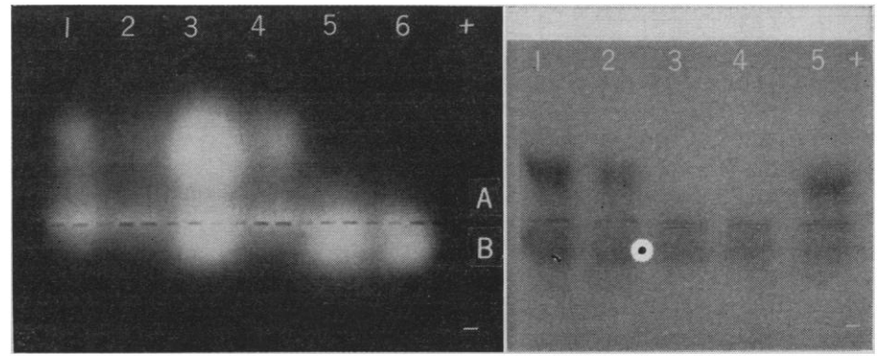

Fig. 1. Separation of β-D-*N*-acetylhexosaminidase components by starch-gel electrophoresis. The A components appear above the origin; the B components appear below the origin. (Left) Pattern in cerebral cortex (4-methylumbelliferyl-β-D-*N*-acetylglucosaminide as substrate) from patients with (1) late infantile amaurotic idiocy (aged 5); (2) renal disease (aged 6); (3) generalized gangliosidosis (aged 2); (4) carcinoma of lung (aged 59); (5) Tay-Sachs disease (aged 3); and (6) Tay-Sachs disease (aged 5). (Right) Pattern in leukocytes (naphthol-AS-BI-β-D-*N*-acetylglucosaminide as substrate) from (1) normal subject (aged 23); (2) normal subject (aged 22); (3) Tay-Sachs patient (aged 4); (4) Tay-Sachs patient (aged 14 months); and (5) normal subject (aged 23). Fifty microliters of a 1 to 20 homogenate was applied to each slot.

the naphthol AS-BI derivatives of both β-D-*N*-acetylglucosamine and β-D-*N*-acetylgalactosamine as substrates, as well as 4-methylumbelliferyl-β-D-*N*-acetylglucosaminide. The activity of hexosaminidase component B was markedly increased in cerebral cortex from the Tay-Sachs patients compared to controls (Table 2), explaining the high levels of hexosaminidase activity in this tissue (Table 1), but hexosaminidase A was not detected.

In three other neuronal lipid storage disorders, generalized gangliosidosis, Hurler's syndrome, and late infantile amaurotic idiocy, no deficiency of hexosaminidase component A was found (Fig. 1). These results indicated that ganglioside storage in itself, neuronal lipidosis, slowly progressive fatal cerebral degeneration, and prolonged storage of frozen tissues were not responsible for the deficiency of hexosaminidase component A in the brain of Tay-Sachs patients.

It seemed unlikely that storage of ganglioside GM_2 in itself accounted for the deficiency of hexosaminidase A, since the deficiency was the same in brain, where ganglioside storage is massive, and in liver, kidney, and skin, where ganglioside storage is minimal.

When brain or liver homogenates from controls and Tay-Sachs patients were mixed in equal proportions, the activity of hexosaminidase component A in the mixed sample was the average of the control and Tay-Sachs activities. This finding indicated that soluble endogenous inhibitors (including ganglioside GM_2 which was soluble in the buffers used) were not responsible for the complete inactivity of component A.

Starch-gel electrophoretic studies of other glycohydrolases from cerebral gray matter and liver tissues of the Tay-Sachs patients revealed no alteration of enzyme patterns. This was true of β-galactosidase, which can be separated into three or four components in gray matter and in liver (*14*), as well as β-glucuronidase and β-glucosidase, which can be separated into two or three components (*15*).

Studies on fresh venous blood demonstrated that component A was the major hexosaminidase in normal human plasma and only traces of component B were present. Component A was absent in fresh plasma obtained from two living patients with Tay-Sachs disease; all the hexosaminidase activity was due to component B.

Leukocytes from control subjects contained both hexosaminidase components in the ratio of 73 percent A to 27 percent B. Leukocytes obtained fresh and assayed immediately from both living patients with Tay-Sachs disease contained component B; component A was absent (Fig. 1). Hexosaminidase A was absent in cultured skin fibroblasts (greater than ten cellular generations) obtained from three patients with Tay-Sachs disease; skin fibroblasts cultured from control subjects contained high concentrations of hexosaminidase A. Tay-Sachs fibroblasts contained normal concentrations of hexosaminidase B.

The absence of a β-D-*N*-acetylhexosaminidase component which possesses both *N*-acetyl-β-D-glucosaminidase and *N*-acetyl-β-D-galactosaminidase activity could provide a satisfactory explanation for the ganglioside storage in Tay-Sachs disease. A block in the catabolism of ganglioside GM_2, which contains a terminal *N*-acetylgalactosamine residue, could result from the absence of such a hydrolytic lysosomally localized enzyme. Before acceptance of this explanation, it must be demonstrated that hexosaminidase component A participates in the catabolism of ganglioside GM_2. Nonetheless, the fact that the deficiency occurs in all Tay-Sachs tissues studied and that levels of hexosaminidase A in plasma and leukocytes in heterozygous carriers of the Tay-Sachs gene are intermediate between homozygous affected patients and controls (*15*) suggests that the enzyme deficiency is closely related to the genetic defect.

The immediate practical importance of our discovery is that hexosaminidase assay provides a means for the diagnosis of homozygotes. We have found (*15*) that both hexosaminidase components are present in normal fetal amniotic fluid cells obtained by amniocentesis early in pregnancy. If component A is absent in fetal amniotic cells derived from individuals homozygous for Tay-Sachs disease, as appears likely, the intrauterine diagnosis of this fatal human disease will be possible.

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Energized Configurations of Heart Mitochondria in situ

Abstract. *Changes in the configurational state of the cristal membranes of rat heart mitochondria within the living cell can be induced by imposing energizing conditions. An exact correlation has been established between the configurational states of the cristal membrane and the energy states of the mitochondrion. The configurational changes observed in mitochondria* in situ *are comparable to those established for beef heart mitochondria in vitro and are consistent with the postulate of the conformational basis of energy transductions in membrane systems. The formation of paracrystalline arrays is one of the noteworthy features of configurational changes of mitochondria* in situ.

In earlier communications (*1, 2*) a correlation has been established between the configurational state (*3*) of cristal membrane (as determined by electron microscopy or by light scattering) and the energy state (*4*) of the beef heart mitochondrion in vitro. The extensive configurational changes that have been induced by experimental means (*5*) in isolated mitochondria did