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Two Abnormalities of Hexosaminidase A in Clinically Normal Individuals

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SUMMARY

Two abnormalities of beta-hexosaminidase A (HEX A) activity are described. One, found in two unrelated Jewish children, was characterized by the complete absence of HEX A activity in serum, but low levels of activity in leukocytes and fibroblasts using artificial substrate. The other, found in a non-Jewish man, was characterized by uniformly low levels of HEX A activity in leukocytes, fibroblasts, and serum against artificial substrate. In all cases, the pH optimum of HEX A was normal, there was no increased lability at 37°C, and no inhibitor was detected to account for the deficiency of activity. Cultured fibroblasts of these individuals were capable of synthesizing and processing alpha- and beta-subunits of HEX A and capable of cleaving G_{M2} ganglioside. The patients, ranging in age from 6 to 30 years, are clinically normal. They are probably genetic compounds carrying the classical Tay-Sachs gene and a differently mutated allele that imparts the anomalous phenotypic features observed.

INTRODUCTION

Expression of beta-hexosaminidase A (HEX A) activity requires three separate gene products: an alpha-subunit, a beta-subunit, and an activator protein, all

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encoded by genes located on different chromosomes. Various forms of Tay-Sachs disease have been described that can be traced to mutations in one or another of these three loci [1–3]. The net effect is usually a deficiency of HEX A activity (sometimes a concomitant HEX B deficiency) permitting a pathological accumulation of G_{M2} ganglioside in neurons. The extent of the deficiency apparently determines the rate of ganglioside accumulation and, hence, the time of onset and the clinical severity of the disease [4]. The most severe is the classical infantile form characterized by the complete absence of HEX A activity resulting from one of several different mutations that render the alpha-chain either absent, insoluble, labile, or of increased size [5]. Less severe forms have been described that are characterized by the presence of residual, but insufficient, levels of HEX A activity. These milder variants present as juvenile, chronic, or adult forms of the disease [1–3]. The defect in some of these patients appears to be the result of faulty association of alpha- and beta-subunits [6].

Not all hexosaminidase mutations result in disease, however. A few cases have been described of healthy individuals with an apparent HEX A deficiency [3, 7, 8]. We describe here three other individuals in this category, two of whom were unrelated Ashkenazi Jewish children, phenotypically characterized by low activity against 4-methylumbelliferyl-*N*-acetyl-beta-D-glucosaminide in leukocytes and fibroblasts and by the absence of such activity in serum and plasma. Since our first report of this finding [9], two other cases have been described that appear to have a similar defect [10]. The other variant reported here, occurring in a 30-year-old non-Jewish male, showed a low level of HEX A activity in leukocytes and fibroblasts as well as serum. HEX B and I appeared normal in all three individuals.

MATERIALS AND METHODS

Distribution and Quantitation of Beta-Hexosaminidases in Tissues

Beta-hexosaminidase isozymes in serum, leukocytes, and fibroblasts were separated by electrophoresis and DEAE cellulose chromatography. Electrophoretic methods were performed as described [11]. Details of DEAE chromatography are given in the legend of figure 1. Enzyme activity was measured using 4-methylumbelliferyl-*N*-acetyl-beta-D-glucosaminide.

Fibroblasts for this portion of the study were obtained from skin biopsies and cultured in MEM alpha medium containing Garamycin and 15% fetal calf serum. Harvested cells were washed and suspended in 0.9% NaCl, and enzyme was extracted by freezing and thawing five times. The suspension was centrifuged at 1,000 *g* for 10 min, and the supernatant fluid was used without further treatment. Leukocyte pellets were prepared by the method of Kaback et al. [12] and sonicated to prepare extracts. The sonicate was used directly without centrifugation.

Cleavage of Natural Substrate

The ability of cultured skin fibroblasts to cleave G_{M2} ganglioside in situ was studied according to the method of Raghavan et al. [13]. Confluent cells in 60-mm Petri dishes were maintained in culture for 10 days with sphingosine-labeled ^{3}H-G_{M2} ganglioside (20 μM; 40,000 cpm/nmol) in MEM containing 10% fetal calf serum and 2 mM glutamine. Triplicate dishes were examined for each patient. Lipids were then extracted from

harvested cells with chloroform:methanol (2:1). Undegraded radioactive G_{M2} ganglioside was partitioned into an aqueous upper phase by the addition of water. The labeled metabolites of G_{M2} were retained in the organic lower phase. The absolute amounts of the endogenous monosialogangliosides G_{M3} and G_{M2} in the cultured cells were quantitated by HPLC as described by Raghavan et al. [13].

Synthesis and Processing of HEX A Subunits

Fibroblasts were analyzed according to the method of Hasilik and Neufeld [14] for their ability to synthesize and process the alpha- and beta-subunits of HEX A. Three batches of fibroblasts were maintained for 3 hrs in medium containing [^{3}H]leucine (pulse), after which one was harvested and the others incubated in the presence of unlabeled leucine for an additional 5 or 20 hrs (chase). Total beta-hexosaminidase was precipitated with antiserum raised against HEX A. The immunoprecipitate was analyzed by SDS polyacrylamide gel electrophoresis, and the radioactive alpha- and beta-chains were visualized by fluorography.

RESULTS

Patient 1 was an Ashkenazi Jewish male who underwent prenatal diagnosis because both parents had been classified through screening as heterozygotes for the Tay-Sachs gene. The results of the prenatal diagnosis showed that HEX A comprised only about 3.5% of the total hexosaminidase activity in cell-free amniotic fluid and 25% in cells cultured from the amniotic fluid as determined by quantitative polyacrylamide gel electrophoresis (PAGE). The 3.5% HEX A activity in amniotic fluid was unusually low, but the activity in cultured cells was in the range expected for an unaffected but heterozygous fetus, and the pregnancy was allowed to go to term. However, cord blood at birth showed no HEX A activity in serum and was indistinguishable in all tests from serum of Tay-Sachs patients, suggesting that the pregnancy had been misdiagnosed. Distressed by this, the parents permitted no further tests, and it was not until the child was 4 months old that they agreed to another blood test, which again showed no HEX A activity in serum but, surprisingly, 19% HEX A in leukocytes. A clinical examination at the same time showed normal development and no cherry-red spot. His development continued to progress normally, and at age 2, his serum, plasma, leukocytes, and cultured fibroblasts were examined by DEAE cellulose chromatography, starch gel and Cellogel electrophoresis, and PAGE. Serum and plasma were still totally deficient in HEX A, but leukocytes and cultured fibroblasts showed the presence of HEX A, albeit at levels lower than usually found in heterozygotes (table 1). Up to the present (age 6), his development has been normal in all respects.

Patient 2 was an Ashkenazi Jewish female, whose parents also presented as Tay-Sachs heterozygotes and who was also diagnosed in utero. In contrast to patient 1, HEX A levels in both amniotic fluid and cultured amniotic fluid cells were very low: approximately 2% of the total in fluid and 8% in cells as determined by quantitative PAGE and starch gel electrophoresis, suggesting a possible juvenile variant of the disease (case 2 [11]). The parents elected to continue the pregnancy on the strength of the knowledge that the fetus did not appear to have classical Tay-Sachs disease, and at birth, cord blood showed no HEX A activity in serum but low levels in leukocytes. At age 5, HEX A activity was

TABLE 1

APPROXIMATE QUANTITATION OF HEX A, B, AND I IN PLASMA, LEUKOCYTES, AND FIBROBLASTS OF THE THREE PATIENTS USING DEAE CHROMATOGRAPHY AND PAGE

PATIENT	TISSUE	DEAE			PAGE	
		%A	%B	%I	%A	%B and I*
1 (at age 2)	Plasma	0	41	59	0	100
	Leukocytes				28	72
	Fibroblasts				13	87
2 (at age 5)	Plasma	0	28	72	0	100
	Leukocytes				24	76
	Fibroblasts				22	78
3 (at age 30)	Plasma	13	67	20	9	91
	Leukocytes				14	86
	Fibroblasts				8	92
Normal (no. = 4)	Plasma	66	17	17	60	40
Tay-Sachs (no. = 3)	Plasma	0	38	62	0	100

* HEX B and I are grouped together because this system does not separate them well enough to quantitate them accurately. In leukocytes and fibroblasts, HEX I is only a minor component.

still totally absent from serum but present in leukocytes and cultured fibroblasts at about the same level as patient 1 (table 1). Up to the present (age 8), her development has been normal in all respects.

Patient 3 was a 30-year-old, healthy, non-Jewish male. The serum HEX A deficiency was discovered through routine Tay-Sachs carrier screening. His leukocyte and fibroblast HEX A levels were also unusually low, but unlike the two children, he had detectable HEX A in plasma (table 1).

Figure 1 shows the hexosaminidase isozymes found in plasma from the three patients as determined by DEAE cellulose chromatography. Patients 1 and 2 had an isozyme pattern indistinguishable from that of Tay-Sachs patients, not only in the complete absence of HEX A, but also in the relatively high proportion of HEX I. Patient 3, at first thought to be phenotypically the same as the two children, differed by having measurable HEX A activity in serum (about 13% of the total). Also, the distribution of HEX I and B (low I, high B) was almost opposite that found in the children.

To rule out the possibility of a serum inhibitor, serum from each of the patients was incubated with normal serum, and total hexosaminidase was measured. The results were additive, showing that no inhibitor was present.

Because of the absence of any clinical symptoms, HEX A from these patients was tested for the ability to cleave ganglioside in vivo. Cultured fibroblasts were first analyzed for the presence of accumulated G_{M2} ganglioside. Cells of the patients contained essentially no stored G_{M2} ganglioside. In contrast, five Tay-Sachs cell lines averaged 2.01 nmol G_{M2} accumulated per mg protein, compared with an average of 0.14 nmol per mg protein in controls (table 2).

In a second study, fibroblasts from the three patients were incubated in the presence of sphingosine-labeled G_{M2} ganglioside according to the method of

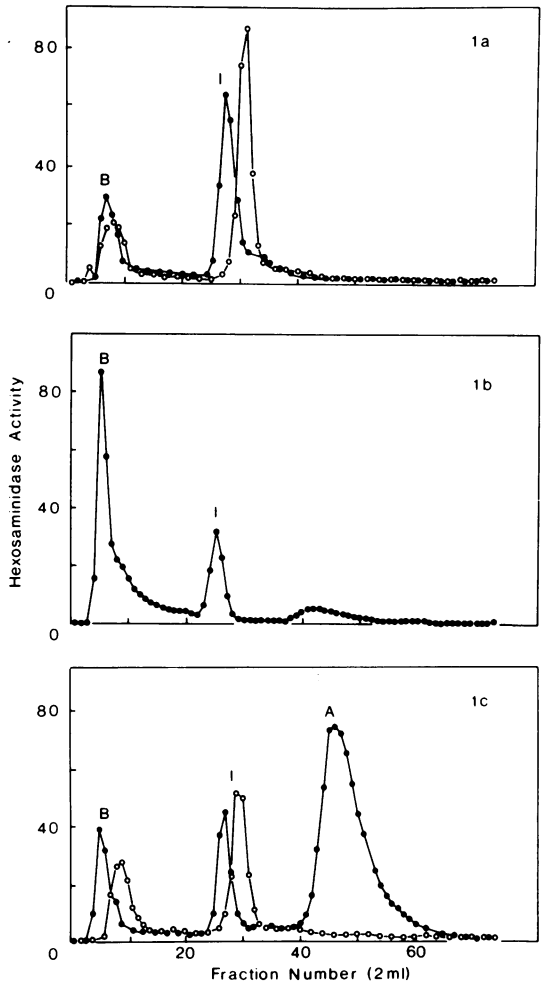

FIG. 1.—DEAE chromatography of plasma from a normal control, a Tay-Sachs patient, and from the three variants, showing the hexosaminidase isozyme patterns. *1a*, Patients 1 (●) and 2 (○); *1b*, patient 3; *1c*, classical Tay-Sachs patient (○) and a normal control (●). Plasma was dialyzed overnight against 0.01 sodium phosphate buffer, pH 6, centrifuged, and applied to a 21 × 100-mm column of Whatman DE-22 equilibrated with the same buffer. HEX B was eluted with 30 ml of the same buffer. HEX I and A were eluted with 100 ml of a 0 to 0.2 M NaCl gradient. Fractions were assayed in the same manner as PAGE slices [11]. Hexosaminidase activity is expressed in fluorometric units.

Raghavan et al. [13]. Fibroblasts from all three patients were able to cleave this exogenously supplied G_{M2} ganglioside. An average of 40% of the G_{M2} taken up was cleaved by the patient's cells compared to an average of only 10% cleaved by the five Tay-Sachs cell lines. Normal controls metabolized about 56% of the G_{M2} entering the cells (table 2).

To see if the anomalous isozyme pattern observed in these variants was in some way related to an inability to synthesize or process the subunits properly,

TABLE 2

G_{M2} GANGLIOSIDE METABOLISM

	ACCUMULATION OF ENDOGENOUS GANGLIOSIDE (nmols/mg PROTEIN)		DEGRADATION OF EXOGENOUS G_{M2} GANGLIOSIDE		
			RADIOACTIVITY (cpm/mg PROTEIN × 10^{-3})		Unhydrolyzed G_{M2} (% total radioactivity in upper phase)
	G_{M3}	G_{M2}	Upper phase	Lower phase	
Patient 1.....	0.56	0	81.5	58.5	58
Patient 2.....	...	...	176.9	133.2	57
Patient 3.....	1.19	0.08	181.0	100.0	64
Controls (4).....	1.05 ± 0.34	0.14 ± 0.08	127.1 ± 39.1	154.4 ± 31.9	44.2 ± 5.2
Tay-Sachs (5)	1.34 ± 0.44	2.01 ± 1.42	246.8 ± 70.5	25.7 ± 6.0	90.4 ± 1.5

fibroblasts from each patient were analyzed for this property by the method of Hasilik and Neufeld [14]. The cells from all three patients were shown to have the ability to synthesize both alpha- and beta-subunit precursors, to process them to the mature form, and to secrete unprocessed precursor into the medium (fig. 2). However, there was clearly a quantitative difference between the patients and the normal control. In normal cells, the 67-K (alpha) and 63-K and 61-K (beta) precursors appeared to be synthesized in approximately equal amounts and secreted into the medium in equal amounts, while cells from all three patients showed a marked reduction in the amount of alpha-precursor both intracellularly and extracellularly, with a corresponding reduction in the amount of mature alpha-subunit. Fibroblast extracts of Ashkenazi Tay-Sachs patients are known to exhibit no trace of cross-reacting material corresponding to either the alpha-chain precursor or the mature alpha-chain [14].

To determine the pH optimum, HEX A and B from fibroblast extracts were separated from one another by DEAE cellulose chromatography and assayed in citrate-phosphate buffer over a pH range of 2.4 to 6. Both HEX A and B from all patients had a pH optimum of 4.4, which is in agreement with that reported for the normal enzyme.

To determine if the absence of HEX A from serum was due to increased lability, HEX A activity at 37°C was measured in the following manner. Identically prepared tubes containing 50 μl of a HEX A solution, isolated from fibroblast extracts of patients 1 and 3 by DEAE chromatography, were incubated at 37°C. At 15-min intervals over a 2-hr period, duplicate tubes were removed and stored on ice. After 2 hrs, all tubes were assayed. HEX A from both patients and from a normal control showed no decrease in activity over the 2-hr incubation period.

DISCUSSION

Patients 1 and 2 share the same nonuniform distribution of HEX A, that is, HEX A was present in tissues but absent from serum. The intracellular HEX A levels were lower than normal but apparently sufficient to have prevented any detectable buildup of G_{M2} ganglioside and to have permitted normal develop-

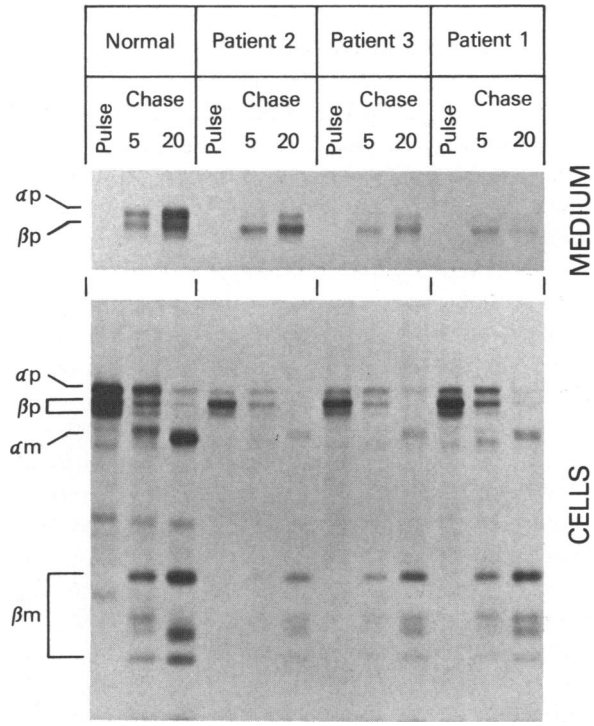

FIG. 2.—Autoradiogram showing synthesis and maturation of hexosaminidase subunits. *In the lower panel*, the "pulse" lane shows the incorporation of radioactive label into intracellular 67-K alpha (αp)- and 63-K and 61-K beta (βp)-chain precursors during a 3-hr exposure to [^{3}H]leucine. The presence of some faster migrating faint bands in this lane indicates that some processing to the mature forms has begun but is not complete for 20 hrs (see αm, βm). Secreted precursor chains appear in the medium during the chase (*upper panel*). The reduced amount of alpha-chain presursor in the patients' cells is evident in the "pulse" lane of each, in the precursor chains excreted into the medium, and in the reduced but finite amount of mature alpha-form, in the 20-hr chase.

ment to date. This decreased enzyme level as measured with artificial substrate was consistent with decreased G_{M2} degradation and decreased production of the alpha-subunit and is consistent with the presumption of heterozygosity for the classical Tay-Sachs gene [but it may also reflect subnormal enzyme production by the newly described allele(s)]. However, reduced enzyme production alone cannot readily explain the absence of HEX A in the children's serum, for the amount of HEX A measurable in serum is usually roughly equivalent to that found intracellularly. For example, the low cellular enzyme level of patient 3 (lower even than that of the children) was expressed in serum.

The presence of mature alpha-chain in an amount approximately equal to that of its precursor suggests that the problem is not faulty association of alpha- and beta-chains. Association of alpha- and beta-precursor chains appears necessary for transport of the alpha-chain to lysosomes, where proteolytic processing takes place [15].

Possible explanations for the absence of HEX A activity from serum of these patients may be lack of secretion, increased lability, or accelerated clearance

from the circulating blood. Although secretion from fibroblasts appeared normal, the first possibility cannot be ruled out because the cellular origin of serum HEX A is not known. The possibility that the absence of HEX A activity in serum is a transient function of the children's age is lessened by the appearance, in a 43-year-old woman, of an identical enzyme pattern [10].

Patients 1 and 2 are probably compound heterozygotes for both the classical Tay-Sachs allele and another allele responsible for the nonuniform distribution of HEX A activity described. It has not been established whether the mutation is identical in both. Patients 1 and 2 phenotypically resemble three other unrelated healthy individuals reported previously [10, 16] who share this same nonuniform distribution of HEX A activity. One is a child of Ashkenazi Jewish descent, while the other two are non-Jewish adults (one of whom is the 43-year-old woman referred to above).

Another mutation that we have described here, found in a 30-year-old man (patient 3), was characterized by a more uniform, and fairly severe, deficiency of HEX A activity in serum, leukocytes, and fibroblasts as measured with artificial substrate (roughly one-sixth the amount of activity found in normal tissues). However, there was no significant intracellular accumulation of G_{M2} ganglioside and the fibroblasts were capable of metabolizing exogenous G_{M2} ganglioside at a rate greater than half that of normal cells.

His fibroblasts were capable of synthesizing and processing alpha- and beta-subunits, but the synthesis of the alpha-subunit was decreased as in patients 1 and 2. This, along with reduced enzyme activity against exogenous G_{M2} ganglioside, would again be consistent with the presumption of compound heterozygosity for both the classical Tay-Sachs allele and this newly described allele.

Several similar healthy adults have been described who are presumed to be compound heterozygotes for the Tay-Sachs gene and a second mutation that diminishes or abolishes activity against the artificial but not the natural substrate [8, 17, 18]. A more meaningful comparison of such variants will be possible only when the molecular defects in either the protein or DNA can be detected and cataloged. Patient 3 clearly differs from patients with the adult form of Tay-Sachs disease, for while those patients have similarly low HEX A activity against artificial substrate, unlike patient 3 they accumulate G_{M2} ganglioside and are almost totally lacking the ability to cleave exogenously supplied G_{M2} ganglioside [3].

Despite the absence of clinical symptoms, the prognosis for these and other variants with decreased HEX A activity must still be guarded, for they may be functioning at a marginally balanced level of HEX A activity that may be tipped by other physiological or environmental factors. This possibility has been raised by the recent demonstration of clinical heterogeneity in sibs who presumably have the same HEX A defect. One sib has the clinical manifestations of chronic Tay-Sachs disease caused by failure of the alpha- and beta-subunits to associate. The other appears to have the same biochemical abnormality but is asymptomatic, suggesting that the difference in their clinical state must be caused by other factors that determine the adequacy of the residual HEX A activity [6].

Because heterozygotes for variant alleles are generally indistinguishable from alleles of phenotypically classical Tay-Sachs disease, they complicate screening for the detection of carriers. Greenberg and Kaback [19] estimated that one in 40 Jewish individuals diagnosed as carriers of the classical Tay-Sachs allele are actually carriers of a variant HEX A deficiency allele. Therefore, because the clinical prognosis of these variants cannot be predicted accurately, and their detection is difficult, they offer particular problems in prenatal diagnosis.

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AAAS REQUESTS SUGGESTIONS. The National Science Foundation's Division of International Programs has requested the American Association for the Advancement of Science and its Consortium of Affiliates for International Programs (CAIP) to assist in the development of a list of potential topics for consideration as new global initiatives of the Foundation. As a result of a meeting of a steering group, which included Consortium representatives, a solicitation of potential topics is being made to a wide variety of groups within the mainstream scientific and engineering community. Topics should meet three criteria: (1) be an important global problem; (2) have high potential scientific, technological, or intellectual content; and (3) involve the broadest possible range of disciplines. The steering group seeks suggestions of potential global initiatives from both individual scientists and engineers and from appropriate groups. Suggestions should include a title, a short description, and the potential of the program to meet the established criteria and should be not more than one page in length. Recommendations should be sent by April 1, 1986, to: Ms. Sandra M. Burns, Office of International Science, American Association for the Advancement of Science, 1333 H Street, N.W., Washington, DC 20005.