

## Apparent Deficiency of Hexosaminidase A in Healthy Members of a Family with Tay-Sachs Disease

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### INTRODUCTION

Tay-Sachs disease (TSD) is a lethal autosomal recessive inborn error of lipid metabolism. It is characterized by accumulation of the ganglioside G<sub>M2</sub> in the brain, leading invariably to death during early childhood. Okada and O'Brien [1] have shown that component A of N-acetyl- $\beta$ -D-hexosaminidase is absent in all examined tissues of the affected homozygous infants, whereas in normal people both components A and B are present [2]. Among obligate heterozygous parents of TSD infants, a significant decrease in the activity of component A has been demonstrated in serum [3, 4], leukocytes [4-6], cultured skin fibroblasts [7], amniotic fluid cells [8-10], and urine [11]. In rare cases overlapping was noted between enzymic activities of heterozygotes and those of normal controls, but no case has been reported of an overlapping between heterozygotes and affected homozygotes. These observations have led to studies that confirmed the reliability of prenatal diagnosis of TSD, when performed on cultured amniotic cells [9, 10].

Recently we have encountered an apparently complete deficiency of hexosaminidase A in a healthy father of a deceased TSD infant. This exceptional finding prompted an examination of all available members of this family. The preliminary results of these examinations are reported and discussed in this paper.

### THE FAMILY

The pedigree, incorporating some of the laboratory findings, is shown in figure 1. The proband (II-3) is the son of unrelated Ashkenazi Jewish parents, whose families originated in different regions of Poland. His wife, as well as his sisters' husbands, are of unrelated Jewish families. All these individuals are in good health. The deceased TSD infants, who died between the ages of 1½ and 2½ years, had not been studied biochemically, but they presented the typical symptoms and course of the disease. The living sons of the proband (III-9 and III-10, born

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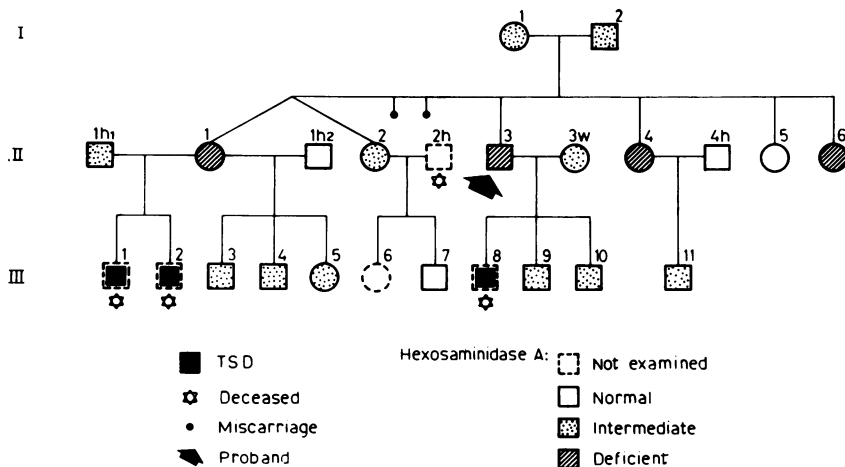


FIG. 1.—Family pedigree, including levels of hexosaminidase A in peripheral leukocytes

1970 and 1972, respectively) were diagnosed prenatally as heterozygous for TSD and are developing normally.

#### MATERIALS AND METHODS

Starch gel electrophoresis of homogenated cultured skin fibroblasts was run for 10 hr at 4° C and 5 v/cm in 0.04 M citrate-phosphate buffer, pH 6. The components of hexosaminidase are located through fluorescence in ultraviolet light after incubation of the gel at 37° C for 1 hr with the substrate 4-methyl-umbelliferyl-N-acetylglucosaminide, and subsequent spraying with sodium hydroxide-glycine buffer, pH 10.6.

The activity of hexosaminidase A was estimated by the heat-inactivation method in peripheral leukocytes, serum, cultured skin fibroblasts, urine, and cultured amniotic fluid cells. The method is based on the observation [2] that upon incubation at 50° C, pH 5, the activity of component A of the enzyme begins to decline immediately. After incubation for 3 hr, component A disappears whereas component B remains virtually unchanged [5]. The total amounts of hexosaminidase (A + B) expressed in terms of nanomoles substrate cleaved per milligram protein (or number of cells or milliliters of serum) vary widely among individuals, with overlapping of all genotypes [3]. The proportion of isozyme A is expressed, therefore, as percentage activity at zero time ( $A + B = 100\%$ ) minus the activity at the end of a 3-hr incubation period.

Full details of the materials and procedures for the spectrofluorimetric estimation of the released 4-methyl-umbelliferone are given elsewhere [5, 10, 11]. Each enzymic determination was performed in triplicate, and the results, which were usually very close, were averaged.

All electrophoretic and enzymic assays of members of the family were performed concurrently with samples from normal controls, from known heterozygotes, and from affected TSD infants.

#### RESULTS

Upon electrophoresis, the extracts from skin fibroblasts of four healthy siblings of generation II had only one fluorescing band at the cathode end of the plate, corresponding to component B of hexosaminidase (fig. 2). This pattern is indis-

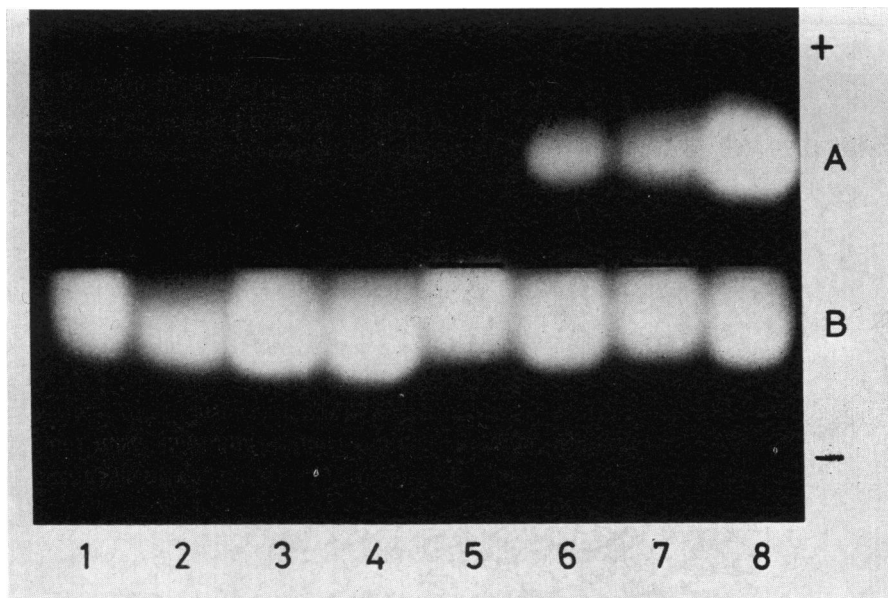


FIG. 2.—Starch gel electrophoresis of cultured skin fibroblast extracts. (1) TSD patient; (2-6) family members II-1, II-6, II-3, II-4, II-2; (7) unrelated obligate heterozygote; (8) normal control.

tinguishable from that seen in TSD patients, and is sharply contrasted with the finding of a strongly fluorescing "A" band at the anode end in normal controls, and a band of intermediate intensity in obligate heterozygotes, as well as in the sister (II-2).

The proportions of hexosaminidase A found in various tissues of all members of the family are given in table 1, along with levels recorded in various control groups. The results of the leukocyte tests are also depicted in figure 3. It will be noted that the same four siblings (II-1, 3, 4, 6) have very low levels of hexosaminidase A, which are again indistinguishable from those found in TSD patients.

#### DISCUSSION

It has been generally assumed that hexosaminidase A is essential for the degradation of the  $G_{M2}$  ganglioside [12]. If this is indeed the case, then the absence of the enzyme in the four healthy adults in this family must be apparent rather than real. It may be argued that whereas TSD infants do not synthesize isozyme A at all, these people do have minute quantities that are not detectable by our methods. This seems unlikely, however, since it has been shown that juvenile  $G_{M2}$  gangliosidosis develops in patients in whom the activity of hexosaminidase A has reached 12% of the total [13]. Alternatively, the four sibs in this family may have a sufficient amount of a variant of isozyme A, which acts normally *in vivo* but is unable to cleave the synthetic substrate used by us *in vitro*. It is also conceivable that in these individuals the apparent absence of isozyme A is due to

TABLE 1

LEVELS OF HEXOSAMINIDASE A IN FAMILY MEMBERS AND CONTROLS

Individual	Sex	Age	Leukocytes	Serum	Skin Fibroblasts	Urine	Amniotic Cells
I-1	F	56 years	49	40	...	42	...
I-2	M	63 years	48	36	...	32	...
II-1	F	35 years	10	0	9	10	...
II-1h <sub>1</sub>	M	42 years	46	26	...	40	...
II-1h <sub>2</sub>	M	42 years	61	48	...	42	...
II-2	F	35 years	37	36	...	44	...
II-3	M	31 years	0	12	2	10	...
II-3w	F	30 years	47	35	...	35	...
II-4	F	27 years	8	0	1	5	...
II-4h	M	32 years	55	55	...	67	...
II-5	F	26 years	60	57	45	54	...
II-6	F	22 years	5	8	8	3	...
III-3	M	10 years	46	...	...	29	...
III-4	M	4 years	38	...	...	28	...
III-5	F	3 years	50	28	...	31	...
III-6	F	14 years	...	...	...	53	...
III-7	M	12 years	64	47	...	50	...
III-9	M	Prenatal	...	...	...	...	28
III-10	M	1 year	40	32	48	...	38
III-11	M	Prenatal	...	...	...	...	...
Normal controls	M + F	1 day (cord)	43	22	...	49	...
		1 day (cord)	35	21	...	56	...
		Prenatal	...	...	...	...	51-72 (N = 8)
		1 day	48-59 (N = 4)	...	...	60-78 (N = 6)	...
		Adults	65 ± 5 (N = 97)	54 ± 5 (N = 24)	42-62 (N = 5)	58 ± 7 (N = 34)	...
Obligate heterozygotes	M + F	Adults	46 ± 6 (N = 52)	37 ± 6 (N = 21)	...	38 ± 6 (N = 35)	...
TSD	M + F	Prenatal	...	...	...	...	0-12 (N = 5)
		Infants	0-15 (N = 11)	0-10 (N = 4)	...	0-11 (N = 9)	...

NOTE.—All levels are expressed as percentage hexosaminidase A of total hexosaminidase (A + B). For controls, means ± SD are given; when number examined is small, the range of results is indicated.

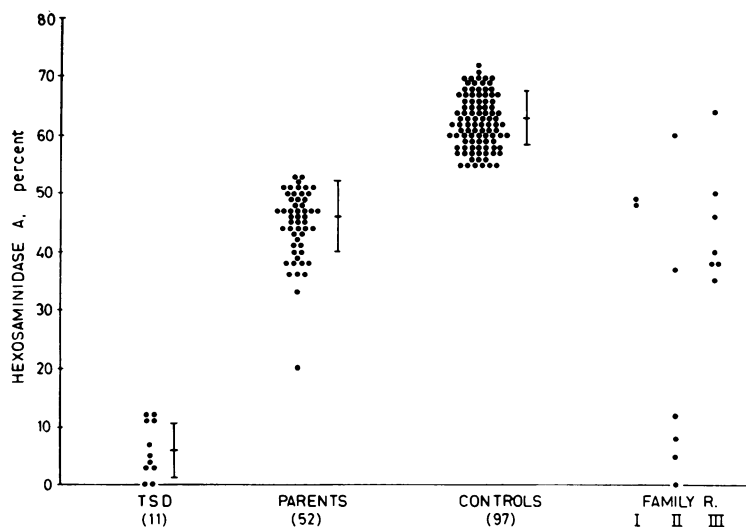


FIG. 3.—Levels of hexosaminidase A in leukocytes of TSD patients, obligate heterozygous parents, normal controls, and 15 members of the family.

an enzyme that affects the rate of its conversion to isozyme B, or the postulated conversion of B to A. All these possibilities are currently being explored.

Another possibility is that isozyme A is indeed deficient in the tissues examined so far, but that sufficient activity may be present in other tissues to prevent the accumulation of the  $G_{M2}$  ganglioside in the brain. An analogous situation exists with regard to other genetic diseases, for example, galactosemia [14]. A severe deficiency of galactose-1-phosphate uridyl transferase, which characterizes all tissues of the affected galactosemic babies, was found in the blood of healthy adults. These people, however, are able to metabolize galactose by the enzyme found in their livers. It has been postulated that this phenotype was due to a rare variant allele, and this may well be the case also in TSD.

At least two of the four exceptional sibs of generation II, those who have had TSD offspring (II-1, II-3), must be heterozygous for the common mutant gene responsible for TSD. It is suggested that their other allele at that locus is a rare variant. It is postulated, therefore, that there are three alleles at this locus:  $T$ , the normal (dominant) one;  $t_1$ , the common mutant TSD allele; and  $t_2$ , the hypothesized rare variant. The six possible genotypes resulting from this system would probably produce five phenotypes:  $TT$ , clinically and biochemically normal;  $Tt_1$  and  $Tt_2$ , indistinguishable from each other and clinically normal with reduced hexosaminidase A activity;  $t_1t_1$ , TSD with absence of hexosaminidase A;  $t_1t_2$ , the exceptional phenotype of the four sibs reported here; and  $t_2t_2$ , as yet unknown.

Under this hypothesis the genotypes in this family could be as shown in figure 4. The parental mating (generation I) is presumably between two types of heterozygotes: one with the usual mutant ( $Tt_1$ ) and one with the rare ( $Tt_2$ ). In generation II, three of the four possible genotypes occur: one  $TT$  person, one  $Tt_1$  or  $Tt_2$ , and

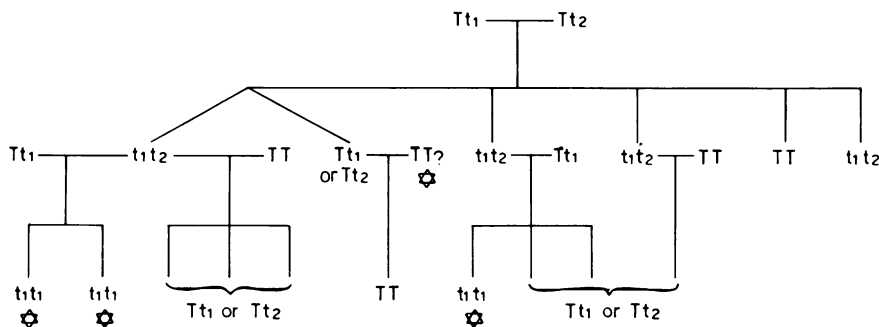


FIG. 4.—Proposed genetic interpretation of the findings

four  $t_1t_2$ . The fact that all six non-TSD offspring of the deficient ( $t_1t_2$ ) individuals have intermediate enzymic levels seems to support this interpretation; had these parents (II-1, II-3, II-4) had one normal  $T$  allele, some of their offspring could be homozygous normal ( $TT$ ). Under this assumption, the probability that all six offspring were heterozygous by chance alone is only about 3%.

As long as we are unable to distinguish between  $Tt_1$  and  $Tt_2$  individuals, the latter will be misclassified (for example, in population screening) as TSD heterozygotes and subject to whatever limitations this might impose. In marriages between them and genuine  $Tt_1$  heterozygotes (for example, the parental mating in our family),  $t_1t_2$  fetuses will be falsely diagnosed as having TSD and therefore subjected to unnecessary abortion. The same may be the case in marriages of  $t_1t_2 \times Tt_1$  individuals (such as two couples in generation II), half of whose hexosaminidase A-deficient offspring could be non-TSD ( $t_1t_2$ ). Fortunately this problem did not arise in the two amniocenteses performed thus far in the woman at risk.

#### SUMMARY

Deficiency of hexosaminidase A, indistinguishable from that found in patients with Tay-Sachs disease (TSD), is reported for the first time in healthy adults. Of four such siblings, two have had TSD offspring. The parents and all six living offspring of these individuals have intermediate enzymic levels. Possible explanations for the findings are discussed. It is suggested that the exceptional phenotype may be due to compound heterozygosity for the common mutant TSD gene and a rare allele which causes apparent enzymic deficiency but does not lead to TSD. It is pointed out that in such families there exists the possibility of a false-positive prenatal diagnosis of TSD.

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