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Tay-Sachs Disease: Prenatal Diagnosis

Abstract. *Fifteen pregnant women with a 25 percent risk of delivering a child with Tay-Sachs disease were monitored by amniocentesis and hexosaminidase A assays of amniotic fluid, uncultured amniotic cells, and cultured amniotic cells. Tay-Sachs disease was diagnosed prenatally in six fetuses; the diagnosis was confirmed in one child after birth and in five fetuses after therapeutic abortion. Prenatal diagnosis indicated the absence of Tay-Sachs disease in nine other fetuses; this diagnosis was confirmed postnatally in six, three are still in utero.*

Tay-Sachs disease is a fatal cerebral degenerative disorder transmitted in an autosomal recessive manner involving the accumulation of a specific lipid, ganglioside GM_2. The primary enzymic defect appears to be the absence of the enzyme β-D-*N*-acetylhexosaminidase A (*1*) which apparently participates in cleaving the terminal *N*-acetylgalactosamine residue from the stored ganglioside (*2*). Its absence leads to massive cerebral ganglioside storage, profound mental and motor deterioration, and death by age 2 to 4 years.

Hexosaminidase A is absent in all tissues studied from patients with Tay-Sachs disease (*1*). A partial reduction of the enzyme occurs in blood serum of heterozygous carriers (*3*). The enzymic defect persists in cultured skin fibroblasts after many cellular generations (*1*, *4*). The enzyme is present in normal amniotic cells (*1*), an indication that the prenatal diagnosis of Tay-Sachs disease is possible by hexosaminidase assay. Indeed, Schneck *et al.* (*5*) diagnosed Tay-Sachs disease prenatally in one fetus at 20 weeks of pregnancy, and found deficient hexosaminidase A in amniotic fluid and uncultured amniotic cells; they terminated the pregnancy, and confirmed the diagnosis by analysis of fetal tissues.

In order to be useful, prenatal diagnosis must be free of error. We now present data on 15 women who had previously delivered one or more af-

Table 1. Clinical data in high-risk pregnancies.

Case	Location	Amniocentesis* (weeks)	Prenatal fetal diagnosis	Outcome of pregnancy
1	California	18	Not Tay-Sachs	Clinically normal girl at 12 months of life; intermediate reduction of serum hexosaminidase A at 9 months.
2	New York	18	Probably heterozygous	Normal boy at 7 months; intermediate reduction of serum hexosaminidase A at 7 weeks.
3	Israel	25	Normal homozygote	Normal girl at 9 months; good activity for hexosaminidase A in serum at birth.
4	Israel	28	Normal homozygote	Normal boy at 11 months; good activity for hexosaminidase A in leukocytes at 3 months, normal activity of hexosaminidase A in cultured skin fibroblasts.
5	South Africa	28	Tay-Sachs disease	Affected girl with signs and symptoms of Tay-Sachs disease, bilateral cherry red spots at 9 months, and nearly absent serum hexosaminidase A.
6	California	18	Normal homozygote	Normal girl at 9 months; good activity for hexosaminidase A in serum at birth.
7	California	17	Normal homozygote	Good activity of hexosaminidase A in serum at birth.
8	California	16, 17	Tay-Sachs disease	Fetus aborted by saline infusion at 18 weeks; absent hexosaminidase A in organs, serum, urine, and cultured skin fibroblasts; 49-fold increase in cerebral ganglioside GM_2, neuronal lipidosis, and cytoplasmic membranous bodies in neurons.
9	New York	16	Normal homozygote	Due March 1971.
10	Colorado	17 19	Tay-Sachs disease	Fetus aborted by saline infusion at 20 weeks; neuronal lipidosis; cytoplasmic membranous bodies in neurons, 35-fold increase in cerebral ganglioside GM_2, and absent hexosaminidase A in skin.
11	New York	17	Normal homozygote	Due March 1971.
12	New York	18	Tay-Sachs disease	Fetus aborted by saline infusion at 20 weeks; neuronal lipidosis; 15-fold increase in cerebral ganglioside GM_2, absent hexosaminidase A in visceral organs.
13	New York	18	Normal homozygote	Due April 1971.
14	Australia	17	Tay-Sachs disease	Fetus aborted by saline infusion at 20 weeks; neuronal lipidosis, 16-fold increase in cerebral ganglioside GM_2, absent hexosaminidase A in visceral organs.
15	Baltimore	17	Tay-Sachs disease	Fetus aborted by saline infusion at 21 weeks; neuronal lipidosis, 22-fold increase in cerebral ganglioside GM_2, absent hexosaminidase A in visceral organs.

* Dates given are weeks after first day of last menstrual period.

fected children with Tay-Sachs disease, and who had a 25 percent risk for bearing another affected child.

The diagnosis of Tay-Sachs disease in each kindred was suggested by clinical examination of all available affected children and confirmed by assay of serum hexosaminidase (*3*). In some families where probands were deceased, hexosaminidase assays of serum of parents or relatives established the diagnosis by demonstrating heterozygosity.

Amniocentesis was carried out by transabdominal needle aspiration of amniotic fluid between week 16 and week 28 after day 1 of the patient's last menstrual period (Table 1). The optimum time for this procedure is 17 weeks of pregnancy. Amniotic fluid was obtained from 45 control subjects who were between 15 and 20 weeks of pregnancy. All were undergoing therapeutic abortions, by saline infusion, for psychiatric reasons; no family history of genetic disease was elicited. All amniotic fluid samples obtained from the women at risk for Tay-Sachs disease were clear, straw-colored, and free from visible contamination with blood.

In four instances, amniocentesis for Tay-Sachs disease was performed in La Jolla, and amniotic fluid (8 to 22 ml) was placed in a sterile container and taken directly to our laboratory. In 11 instances, amniotic fluid was sent to us by airmail in tightly stoppered sterile containers from New York (five cases), Denver (one case), Israel (two cases), Baltimore (one case), Australia (one case), and South Africa (one case). Some overseas samples took as long as 6 days to arrive in La Jolla, but successful growth of amniotic cells was obtained in 10 of the 11 samples sent by mail. The samples were kept at the prevailing temperatures during shipment.

A 5-ml portion of amniotic fluid was centrifuged at 3000*g* for 10 minutes and the supernatant was carefully aspirated without disturbing the sedimented cells; sedimented cells were stored at −20°C prior to enzyme assay. The remaining amniotic fluid (3 to 10 ml) was centrifuged at 600*g* for 10 minutes, the supernatant was aspirated, and 8 ml of nutrient medium F-10 (Gibco) containing fetal calf serum (15 percent), penicillin (100 unit/ml), streptomycin (100 μg/ml), and fungizone (0.25 μg/ml) was added to the sediment. The sediment was suspended by agitation and 2-ml portions were placed in four plastic culture dishes [35 mm inside diameter (Falcon Plastic)]. The dishes were kept at 37°C in an atmosphere of 5 percent carbon dioxide and 95 percent air saturated with water vapor. After 1 week, one-half of the medium was replaced, and cultures were fed thereafter with fresh medium three times a week.

Amniotic cells were not cultured in two instances, because of contamination with microorganisms. One culture sent from Australia failed to grow. Successful cell growth was obtained in the remaining 12 cultures. Skin fibroblasts from five patients with Tay-Sachs disease, eight of their parents, and 20 control subjects were obtained by culturing skin biopsies (*4*). Enzyme assays were performed in the skin fibroblast cultures at 21 days after subculture.

Hexosaminidase A and B in cell-free amniotic fluid were estimated by the heat denaturation method (*3*). Cultured skin fibroblasts and cultured amniotic fibroblasts were harvested in isotonic saline by scraping; a volume of cells equal to one-half the surface area of a culture dish (approximately 500,000 cells) was sufficient for enzyme assay. Uncultured amniotic cells and cultured amniotic cells were homogenized in 30 to 70 μl of distilled water in a ground glass homogenizer. Portions (2 μl) of the homogenate were diluted with 18 μl of 0.04*M* citrate-phosphate buffer (*p*H 4.3) containing 0.15 percent human serum albumin (grade III, Sigma) (*4*); incubated for 1, 2, 3, and 4 hours at 50°C to selectively denature hexosaminidase A; and frozen after heating. Triplicate portions were taken to determine activity prior to heating. 4-Methylumbelliferyl-β-D-*N*-acetylglucosaminide (Pierce) dissolved in 20 μl of the buffer described (but without albumin) was added to each portion. After incubation at 37°C for 30 and 60 minutes, 2.5 ml of glycine-carbonate buffer (0.17*M*, *p*H 9.8) was added. Fluorescence was determined in a Tur-

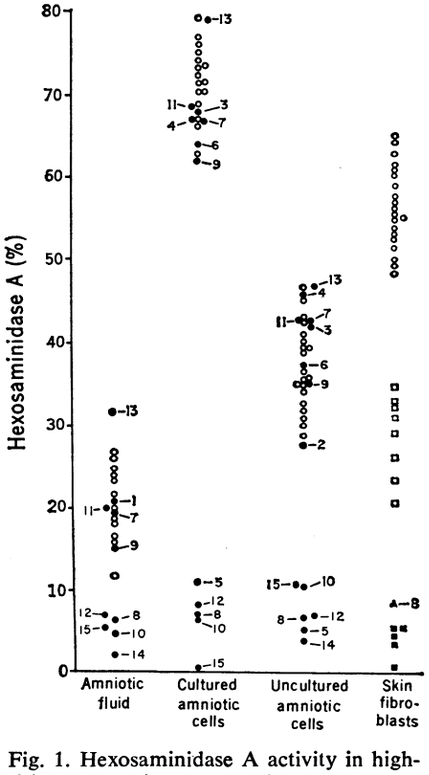

Fig. 1. Hexosaminidase A activity in high-risk pregnancies expressed as percentage of total hexosaminidase. Values from the 15 high-risk pregnancies (closed circles, same case numbers as Table 1) for amniotic fluid, uncultured amniotic cells, and cultured amniotic cells are compared to controls (open circles). Values from cultured-skin fibroblasts from patients with Tay-Sachs disease (closed squares), their parents (open squares), controls (open circles), and case 8 (closed diamond) are also shown.

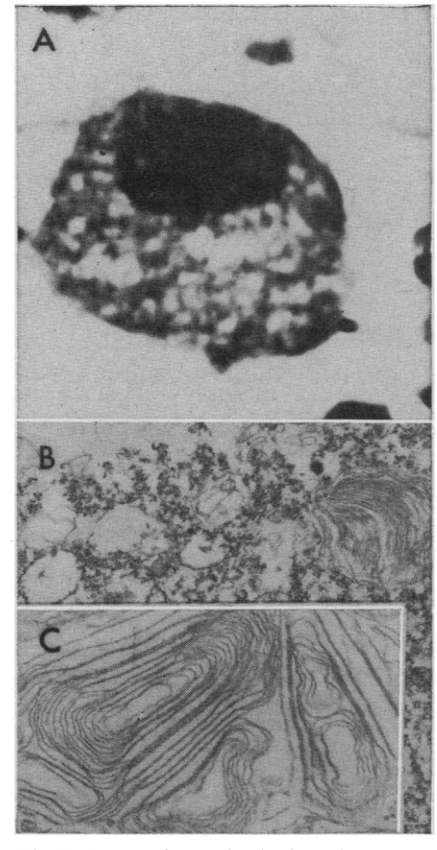

Fig. 2. Morphology of spinal cord neurons in Tay-Sachs fetus (case 8). Electron micrograph of spinal cord neuron at 18 weeks gestation demonstrates unstained cytoplasmic vacuolations (A) (× 1250), cytoplasmic lamellar inclusions (B) (× 12,000), and a single lamellar inclusion (C) (× 70,000). Nearly identical morphologic abnormalities were found in the other four cases.

ner fluorometer at an absorption wavelength of 365 nm, and an emission wavelength of 450 nm.

The activity of hexosaminidase A was calculated as that activity destroyed by heating for 3 and 4 hours (average). A separate portion from each cell-containing specimen was taken to determine protein concentration (*6*). Starch-gel electrophoresis, carried out as described (*1*), was also used to demonstrate both hexosaminidase components in tissue samples. Assay of β-galactosidase and β-glucosidase were carried out as described (*7*).

Hexosaminidase A and B were both present in amniotic fluid, uncultured amniotic cells, and cultured amniotic cells from control subjects (Fig. 1 and Table 2). In controls, 11 to 26 percent of the total hexosaminidase activity in amniotic fluid was due to hexosaminidase A, in uncultured cells 30 to 47 percent, in cultured amniotic cells 62 to 79 percent, and in cultured skin fibroblasts 50 to 65 percent.

In the high-risk pregnancies, a marked deficiency of hexosaminidase A was found in amniotic fluid, uncultured amniotic cells, and cultured amniotic cells in six cases 5, 8, 10, 12, 14, and 15 (Table 1). Amniocentesis was repeated in cases 8 and 10; hexosaminidase A was again markedly deficient in amniotic fluid and uncultured amniotic cells (Table 2). The degree of deficiency was similar to that found in cultured skin fibroblasts from infants with Tay-Sachs disease (Fig. 1). The prenatal diagnosis of Tay-Sachs disease in all six fetuses was made.

The diagnosis of Tay-Sachs disease was confirmed in each instance. In case 5, amniocentesis had been carried out at 27 weeks of pregnancy, too late for safe termination. A female infant, now 11 months old, has bilateral retinal cherry red spots and is developing the neurological signs of Tay-Sachs disease. In cases 8, 10, 12, 14, and 15, therapeutic abortions were carried out between 18 and 21 weeks of pregnancy; fetuses were delivered between 17 and 52 hours after intrauterine infusion of hypertonic saline. Portions of brain and spinal cord from each fetus were fixed for electron microscopy. Portions of brain and liver were frozen from each fetus. In some cases, portions of kidney, spleen, lung, heart, skin, placenta, serum aspirated from the heart, and urine aspirated from the bladder were obtained and frozen. Unfortunately, fetal organs from case 10 were placed in glutaraldehyde, so that enzyme assays were not possible. However, frozen skin was saved for enzyme assay. Portions of skin from case 8 were placed in tissue culture and fibroblast growth occurred; no fibroblast growth occurred in the other cases.

Table 2. Hexosaminidase activity in high-risk pregnancies. Total hexosaminidase in amniotic fluid is expressed as nanomoles of 4-methylumbelliferyl-β-D-*N*-acetylglucosamine cleaved per milliliter per hour and in cells, as nanomoles per milligram of protein per hour. Hexosaminidase A is expressed as a percentage of the total hexosaminidase.

Subject	Amniotic fluid		Uncultured amniotic cells		Cultured amniotic cells	
	Total	A (% of total)	Total	A (% of total)	Total	A (% of total)
1	269	20				
2				28		
3			269	42	1628	69
4			179	46	1853	68
5			111	5		12
6			279	38	2586	62
7	414	19	231	43	1688	67
8	506	6	383	8	1058	6
	508	4	221	10		
9	445	15	346	36		61
10	642	4	221	10	1268	6
	603	8	306	8		
11	427	18	184	43		70
12	383	8	283	7	3527	9
13	900	32		46	1563	79
14	305	3		5		
15	726	5		10	1286	1
Controls	392 ($n = 13$) (283–592)	21 (11–26)	348 ($n = 21$) (141–826)	38 (30–47)	2154 ($n = 16$) (1207–4916)	73 (62–79)

In the five aborted fetuses, striking cytoplasmic inclusions were evident on microscopic examination of nearly all spinal cord neurons (Fig. 2). By electron microscopy, cytoplasmic inclusion bodies were found in neurons. These were lamellar membranous bodies, often spirally wound but less compact than those found at later stages of Tay-Sachs disease (*8*). Evaluation of cortical neurons revealed morphologic abnormalities similar to those in spinal cord neurons. Preservation was very poor in all cases because of the prolonged interval between fetal death, delivery, and fixation.

Ganglioside analyses of the brain from all five aborted fetuses (*9*) revealed a striking increase in the concentration of cerebral ganglioside GM_2 compared to control fetuses aborted between 14 and 16 weeks gestation (Fig. 3). Ganglioside GM_2 comprised 23, 26, 11, 10, and 18 percent of the total brain ganglioside sialic acid in cases 8, 10, 12, 14, and 15, compared to 0.8 to 1.5 percent in the controls. Total gangliosides were increased to 1.3 to 2.1 times normal in the aborted fetuses. Elevations of cerebral ganglioside GM_2 were 49, 35, 15, 16, and 22 times normal in cases 8, 10, 12, 14, and 15, respectively.

Hexosaminidase A was nearly absent in all tissues examined from all five aborted fetuses including liver, brain, spleen, kidney, heart, and lung as well as serum and urine. Hexosaminidase A was nearly absent from cultured fetal skin fibroblasts (case 8) (Fig. 4). Hexosaminidase B and other lysosomal hydrolases, including β-galactosidase and β-glucosidase, were active in all tissues studied from each fetus, demonstrating the specificity of the deficiency for hexosaminidase A. The results of these morphological, chemical, and enzymic studies demonstrate that all five fetuses had Tay-Sachs disease.

In the remaining nine pregnancies, prenatal hexosaminidase assays indicated the absence of Tay-Sachs disease. Six of these infants have been born and range in age from 1 to 11 months. All are clinically normal. Hexosaminidase assays of serum or urine from each gave substantial activity for hexosaminidase A (Table 1), confirming the absence of Tay-Sachs disease. Three pregnancies have not yet come to term.

This study demonstrates that the prenatal diagnosis of Tay-Sachs disease is feasible and accurate. Cultured amniotic cells and uncultured amniotic cells appear to be the most reliable samples for assay, the greatest difference between controls and patients' values being found therein (Fig. 1). Amniotic fluid is less useful because of a smaller differential between the values from controls and affected fetuses (Fig. 1). Results from amniotic fluid and uncultured amniotic cells are obtained within 2 days after amniocentesis. Re-

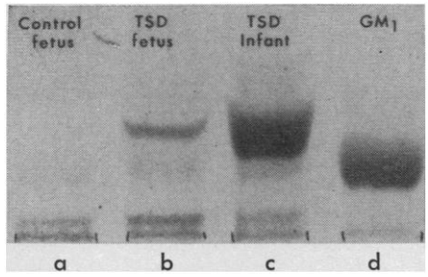

Fig. 3. Chromatography of fetal brain gangliosides. Thin-layer chromatography of cerebral gangliosides, stained with orcinol; (a) control fetus of 16 weeks gestation; (b) Tay-Sachs fetus (case 8) (TSD fetus); (c) child who died with Tay-Sachs disease at 3 years of age (TSD infant), and (d) purified ganglioside GM_1. Note the storage of ganglioside GM_2 in the Tay-Sachs fetus compared with the control fetus, but the much greater storage in the infant with Tay-Sachs disease. Similar elevations of ganglioside GM_2 were found in the other four cases.

sults from cultured amniotic cells in this study were obtained 10 to 28 days after amniocentesis. Assays of amniotic fluid or uncultured amniotic cells may be unreliable if contamination with maternal blood occurs. Assays of cultured amniotic cells are unreliable if bacterial contamination occurs, since some bacteria contain a heat-labile β-D-*N*-acetylglucosaminidase with an acid *p*H optimum and an electrophoretic migration very similar to hexosaminidase A *(10)*.

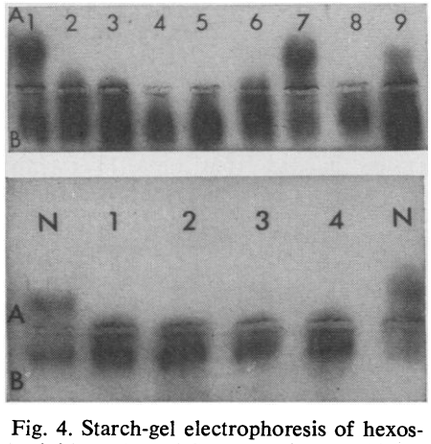

Fig. 4. Starch-gel electrophoresis of hexosaminidase A and B, according to Okada and O'Brien *(1)*, demonstrating (top) absence of hexosaminidase A in liver (lane 2), kidney (lane 3), spleen (lane 4), lung (lane 6), and heart muscle (lane 8) of case 8 compared to liver from Tay-Sachs child (lane 5), liver from two control fetuses of 14 and 16 weeks gestation (lanes 1 and 7), and placenta from case 8 (lane 9). (Bottom) Livers from cases 8, 12, 14, and 15 (lanes 1–4) compared to control fetal livers (*N*) demonstrating absence of hexosaminidase A in each fetus.

Enzyme assays of amniotic fluid, uncultured amniotic cells, and cultured amniotic cells should provide a sufficient cross-check of methodology, so that artefacts can be avoided. Thus far, agreement in our laboratory has been excellent regarding presence or absence of Tay-Sachs disease in the fetus when data from amniotic fluid, uncultured amniotic cells, and cultured amniotic cells are compared. It is important to emphasize here that Tay-Sachs disease must be confirmed in each kindred by enzyme assay before amniocentesis, since clinical and pathological diagnoses are not sufficiently precise to distinguish Tay-Sachs disease from related neuronal lipidoses.

In one of the high-risk pregnancies (case 2) an intermediate reduction of hexosaminidase A was found in uncultured amniotic cells, suggesting heterozygosity, but cultured amniotic cells were not obtained. Serum hexosaminidase assay in the baby at 7 weeks demonstrated a partial reduction of hexosaminidase A, indicating heterozygosity. In seven fetuses, prenatal enzyme assays of cultured cells indicated that they were not heterozygous. This was confirmed in one (case 4) by enzyme assays of cultured skin fibroblasts postnatally. Serum assays were carried out in the early postnatal period in four babies; substantial hexosaminidase A activity was found in each. Unfortunately, the very large variations of serum hexosaminidase A activity shortly after birth makes it impossible to detect heterozygotes reliably by this method. It will be necessary to repeat serum assays of these children later in life to determine whether prenatal detection of heterozygotes is possible by this method. Fortunately, this determination is of no immediate clinical importance since heterozygotes are free of neurological symptoms.

The prenatal diagnosis of Tay-Sachs disease is limited to kindreds in which an affected child has been diagnosed. In a study of 88 kindreds in which Tay-Sachs disease had occurred *(11)*, 82 percent of all cases were found to be first-affected children.

First-affected children with Tay-Sachs disease can only be detected prenatally if their parents are identified as heterozygous before they reproduce. It has been estimated *(12)* that 4,050 siblings and 54,445 first cousins of Tay-Sachs patients have been born over the past 30 years in the United States; the risk of these individuals for being heterozygous is 0.66 and 0.25, respectively. If these individuals marry and reproduce within their own ethnic group, they will produce 44 children with Tay-Sachs disease. Heterozygote detection by serum hexosaminidase assay *(3)* is obviously worthwhile in these individuals since they have a tenfold higher risk of heterozygosity than the highest risk group [Ashkenazi Jews *(13)*]. In order to prenatally detect all cases of Tay-Sachs disease, mass population screening, now feasible by automation of the serum hexosaminidase A assay *(3)*, will be necessary.

Tay-Sachs disease is incurable and likely to remain so. The immediate practical importance of prenatal diagnosis is that families in which the disease has appeared can now prevent the recurrence of this fatal untreatable hereditary neurological disease in their subsequent children.

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