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Hexosaminidase A in Amniotic Fluid of Tay-Sachs Fetuses

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Hexosaminidase A is present in relatively low concentrations in cell-free amniotic fluids from pregnancies with Tay-Sachs fetuses. This isoenzyme was determined by an immunological procedure, radial immunodiffusion, by which hexosaminidase A can be directly and specifically detected, even in the presence of excess amounts of hexosaminidase B. No hexosaminidase A could be detected by the same procedure in Tay-Sachs fetal tissues, implying that this isoenzyme in the amniotic fluid originates from the mother.

Additional Keyphrases: *inherited disorders · isoenzymes · immunochemistry · normal values · prenatal diagnosis · fetal status*

Amniotic fluid is being used currently for prenatal diagnosis of the status of the fetus in several cases of fetal disorders, including various genetic diseases, such as Tay-Sachs disease. This disease is usually expressed in accumulation of the ganglioside GM_2, mainly in the central nervous system. The enzymatic background for this effect is probably the complete absence of the acidic isoenzyme of hexosaminidase,[3] namely, hexosaminidase A in Tay-Sachs patients (*1*). The determination of hexosaminidase A in various tissues and body fluids serves, therefore, as the basis for the detection of the disease in the afflicted individual as well as for prenatal diagnosis. Because this disease is fatal and incurable, utmost emphasis is put on a reliable prenatal test for which amniotic fluid is used.

Conceptually, either amniotic cells or cell-free amniotic fluid could be used as the specimen for such a test. However, several attempts to detect hexosaminidase A, by the method of heat inactivation, in either cell-free amniotic fluid or uncultured cells did not lead to a trustworthy determination, owing to the relatively small amounts of the A isoenzyme that they contain. The most reliable assays available today are those done on cultured amniotic cells, using either heat inactivation or electrophoresis for the differential determination of hexosaminidases A and B (*2–4*). The main disadvantage in the use of cultured cells is the long time required to obtain a sufficient amount of the cells to perform the test and the inherent danger of contamination. Accordingly, several attempts were made to develop a prenatal test in which cell-free amniotic fluid is used (*5, 6*). One such test was recently developed in our laboratory, involving a combination of ion-exchange chromatography and heat inactivation.[4]

An interesting finding in most of the above-mentioned tests is the presence of a small but significant amount of material that behaves like hexosaminidase A in amniotic fluids of Tay-Sachs cases. This raises the question of whether the low values after heat inactivation are attributable only to fluctuations in the baseline of the assay method (i.e., to experimental error) or whether they represent valid figures for hexosaminidase A in Tay-Sachs amniotic fluids. To answer this question, we used in the present study an immunological assay procedure developed in this laboratory (*7*), by which even low amounts of hexosaminidase A can be determined, *directly* and specifically, in the presence of excess hexosaminidase B.

Methods

Enzyme. Hexosaminidase isoenzymes A and B were purified to apparent homogeneity from human placentas by affinity chromatography as described previously (*7, 8*). The enzymes migrated as single peaks in the analytical ultracentrifuge and on gel filtration with Sephadex G-200, and each isoenzyme appeared as a single band on polyacrylamide gel electrophoresis. Enzymic activity was determined as described elsewhere (*9*).

Immunochemical methods. Antisera against pure hexosaminidases A and B were evoked in goats. The two antisera were highly cross reactive, but specific antibodies that reacted only with hexosaminidase A were prepared by selective adsorption of anti-hexosaminidase A on Sepharose-bound hexosaminidase B (*7*). Radial immunodiffusion in agarose gels containing either the cross reactive anti-hexosaminidase B or specific anti-hexosaminidase A was performed as described previously (*7, 10*). The ratio between hexosaminidases A and B in each sample was estimated from the dilutions required to give precipitin rings of identical size with the two types of antisera.

Amniotic fluids. Amniotic fluids obtained by transabdominal puncture (*2*) were centrifuged and stored at −20 °C until used. The fluids were from mothers heterozygotic for the Tay-Sachs gene and from normal pregnant women, as controls.

Separation of isoenzymes A and B of hexosaminidase from the amniotic fluids was carried out on microcolumns of DEAE-cellulose (*10*). The hexosaminidases of the amniotic fluid were first concentrated by passing the fluid at pH 6.0 through an affinity column [Sepharose-bound 2-acetamido-*N*-(ε-aminocaproyl)-2-deoxy-β-D-glucopyranosylamine] and subsequent elution at pH 8.2 (*8*).

The prenatal diagnosis was performed by differential thermal inactivation of cultured amniotic fluid cells and confirmed by post-abortion examination of fetal tissues.

Results

Cell-free amniotic fluids were passed through the affinity column, eluted, and concentrated by dialysis under reduced

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[3] Hexosaminidase is 2-acetamido-2-deoxy-β-D-glucoside-acetamido deoxygluco-hydrolase,

[4] Navon, R., unpublished data.

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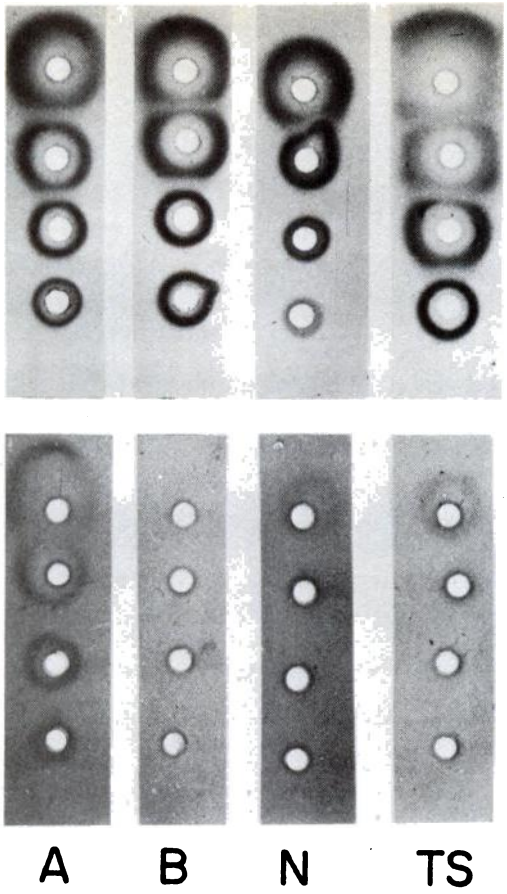

Fig. 1. Radial immunodiffusion for determination of hexosaminidase A in amniotic fluids

Top part shows 500-fold dilution of antiserum reactive with both hexosaminidases (A and B); *bottom* part shows hexosaminidase A-specific antiserum (100-fold dilution). *A*, purified hexosaminidase A; *B*, purified hexosaminidase B; *N*, normal amniotic fluid; *TS*, Tay-Sachs amniotic fluid. Four serial fourfold dilutions of each sample were applied to each plate. Ten microliters of the sample was allowed to diffuse into the agar, and the plates, after thorough rinsing, were stained with naphthol AS-BI *N*-acetyl β-D-glucosaminide plus Fast Garnet GBC

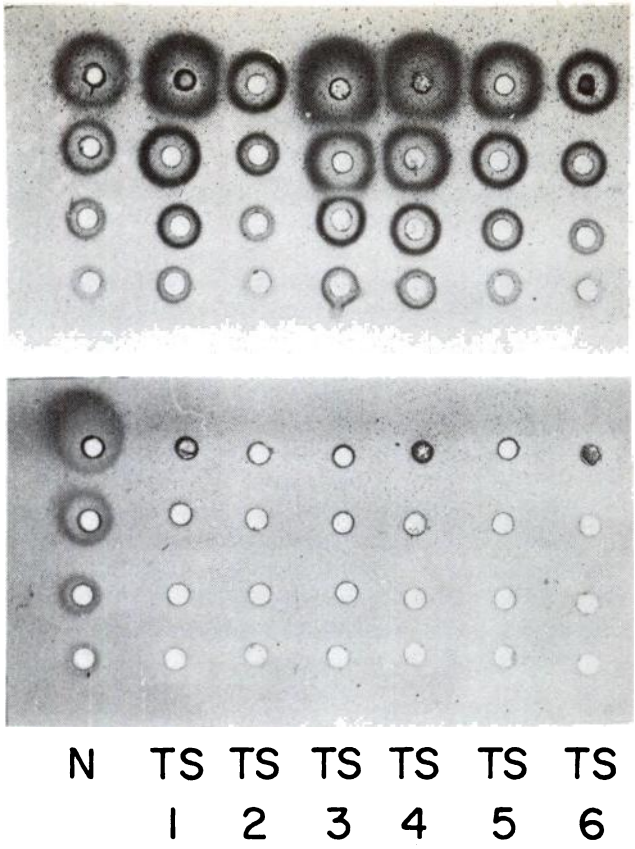

Fig. 2. Radial immunodiffusion of tissue extracts with plates containing antisera as described in Fig. 1

At *extreme left* is a sample of normal aborted fetus (*N*); all the other contain samples from tissues of several Tay-Sachs fetuses (*TS*, 1–6). Each sample was applied in four serial fourfold dilutions

pressure to yield an enzyme concentration of about 0.5 U/ml.[5] The yield of enzymic activity in the eluate was 95–100% of the starting value.

Several serial dilutions of the various samples were applied to the two kinds of radial immunodiffusion plates mentioned above, as shown in Figure 1. The results demonstrate that hexosaminidase A is present in Tay-Sachs amniotic fluid. By comparing the size of the rings obtained with the serum reacting with both isoenzymes and the specific anti-hexosaminidase A serum, the percentage of hexosaminidase A in this amniotic fluid sample was estimated; it amounted to about 2%.

The same results were observed with five additional amniotic fluids obtained from Tay-Sachs cases, the percentage of hexosaminidase A ranging from 0.4–2%.

When the assay was carried out on the acidic fraction eluted from the DEAE-cellulose column, which should contain only the A isoenzyme, samples containing equivalent enzymic activity gave rings of comparable size on the plate containing both types of antisera, regardless of whether or not they originated from a healthy fetus or from pregnancies with Tay-Sachs fetuses. Similar results were obtained for all cases tested.

To test whether or not the low amounts of hexosaminidase A found in the amniotic fluids are of fetal origin, the same assay procedure was applied to tissue homogenates of the Tay-Sachs aborted fetuses. The results (Figure 2) demonstrate that even in extreme concentrations no rings are observed in the plates containing specific anti-hexosaminidase A, and thus that Tay-Sachs fetuses do not contain any hexosaminidase A. The implication is that the A isoenzyme is of maternal origin.

Discussion

Our findings lead to the conclusion that amniotic fluid of pregnancies with Tay-Sachs fetuses indeed contain a material that is antigenically, as well as enzymatically, identical to hexosaminidase A—even though this isoenzyme is not detectable in various tissues of the afflicted fetuses. These results imply that the origin of the isoenzyme in the amniotic fluid is maternal.

No detailed information is available as yet for the mechanism involved in the transport of maternal proteins into the amniotic fluid and the factors regulating it. That such transport may occur was suggested by the passage of radioactive proteins from the circulating blood of the mother to the amniotic fluid (*11*). Another explanation for the reported results is a non-physiological contamination of the amniotic fluid with maternal blood during the amniocentesis procedure; however, this could hardly account for the observed amounts of hexosaminidase A in the samples.

The possibility that maternal proteins such as hexosaminidase A may be found in the amniotic fluid should serve as a caution in the use of cell-free amniotic fluid for prenatal diagnoses of various disorders. However, in the case of Tay-

[5] 1 unit (U) is defined as the amount of enzyme liberating 1 μmol of methylumbelliferone per minute under the assay conditions.

Sachs disease, although hexosaminidase A is definitely present in amniotic fluids, its relative concentration is low, as measured by the immunological procedure (less than 2%), and is distinctly different from the concentration present in amniotic fluids of healthy individuals, which ranges between 10 and 20%.

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