Article abstract—Enzyme replacement therapy was attempted with two Tay-Sachs-diseased individuals—a 14-month-old child and a 7-week-old infant. Treatment consisted of repeated weekly intrathecal injections of pure hexosaminidase A. Injection of this enzyme resulted in almost complete disappearance of GM\(_2\) from the serum, but did not bring about dissolution of the GM\(_2\) membranous cytoplasmic bodies in the brain, as detected by electronmicroscopy. Both patients tolerated the treatment without apparent clinical complications, but no clear-cut improvement was noted as a result of prolonged injections of hexosaminidase A. Since this treatment was initiated in both an advanced stage and a very early stage of the disease, we conclude that enzyme replacement therapy by this route is not beneficial for patients with Tay-Sachs disease.

NEUROLOGY 29: 848-854, June 1979

Enzyme replacement in Tay-Sachs disease


The use of exogenous enzymes for replacement therapy in human enzyme deficiencies has been considered feasible in recent years.\(^1\)\(^-\)\(^4\) This approach has been proposed particularly for inherited storage diseases, which are characterized by the accumulation of a catabolite, usually within the lysosomes of the affected organs. In Gaucher disease, Brady and associates\(^5\)\(^-\)\(^6\) reported that even a single injection of purified glucocerebrosidase effected a remarkable decrease in the levels of glucocerebroside in the liver and erythrocytes. They also treated patients with Fabry disease by replacement of ceramide trihexosidase.\(^7\)\(^-\)\(^8\) Enzyme replacement therapy has also been attempted in Tay-Sachs disease (GM\(_2\) gangliosidosisis type B), which is characterized by absence of the A isozyme of \(\beta\)-N-acetylhexosaminidase and accumulation of ganglioside GM\(_2\) in the central nervous system.\(^9\)\(^-\)\(^10\) The disease is inherited as an autosomal recessive trait; detection of heterozygotes and prenatal diagnosis are now reliable,\(^11\) but universal screening of susceptible populations has not been achieved. We are still frequently confronted with affected children.

Isolated attempts at enzyme substitution in Tay-Sachs disease have not been of therapeutic value\(^12\)\(^-\)\(^13\) because the blood-brain barrier may prevent the penetration of sufficient amounts of active enzyme to the target cells, and also because of the rapid clearance and degradation of hexosaminidase by the liver after intravenous administration of this enzyme. We developed a procedure for purifying hexosaminidase A in large quantities,\(^14\)\(^-\)\(^15\) and, by binding of the enzyme to the soluble carrier poly-N-vinylpyrrolidone, decreased the rate of the proteolytic degradation of the enzyme without affecting its enzymatic activity. These two new developments seemed to justify a further trial of enzyme replacement therapy in Tay-Sachs disease.

Case 1. This girl was admitted for treatment at the age of 14 months. She was the first child of unrelated Ashkenazic Jewish parents. At 5 months of age, an arrest of psychomotor development was noticed by the parents. Tay-Sachs disease was confirmed by the presence of "cherry red spots" in both ocular fundi, and by differential determination of hexosaminidase A and B in serum and leukocytes, with total absence of hexosaminidase A. Following diagnosis, progressive deterioration took place, and at the time of treatment she was aphasic and expressionless, had mild decerebrate posturing, regurgitated when fed, and showed a marked startle reflex. There was virtually no spontaneous limb movement; her muscle tone was flaccid, and tendon reflexes were not elicited. The pupils were equal in size and reacted to light directly and consensually. Fundoscopy showed bilateral normal-colored optic disks and gray-white areas (about three times the diameter of the disk), in both maculae, with a central red spot. Sensory response to pain, although difficult to assess, was present. She could not hold her head upright, never smiled, and did not grasp objects. Her heart and lungs were normal, and no hepatosplenomegaly was found. Laboratory studies indicated normal blood urea nitrogen, glucose, serum creatinine, sodium, potassium,

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Accepted for publication November 2, 1978.

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Before the clinical trial, safety of the enzyme preparations was evaluated by intrathecal injection of 3.5 mg of pure hexosaminidase A and 4.0 mg of PVP-hexosaminidase A into vervet monkeys weighing 1.5 kg each. The monkeys did not show any immediate or delayed toxic effect or pyrogenic reaction.

Enzymatic assay. Hexosaminidase activity was measured with 4-methylumbelliferyl-N-acetyl-β-D glucosaminide (Pierce, USA) as a substrate.17 Differential determination of hexosaminidases A and B was carried out by three different methods: heat inactivation (3 hours at 50°C) in 0.05-M Na-citrate buffer, pH 5.0 (hexosaminidase A is heat-labile); radial immunodiffusion; and an enzyme binding immunoassay. In the immunoassays, we used an antiserum that cross-reacts with hexosaminidases A and B, as well as the IgG fraction of a specific antihexosaminidase A serum.18

Radioiodination of PVP-hexosaminidase A. PVP-bound hexosaminidase A was iodinated with 

Materials and methods. Hexosaminidase A and PVP-hexosaminidase A. Hexosaminidase A was extracted from human placenta and purified to homogeneity, as described previously.14,15 The preparations of pure hexosaminidase A used here had a specific activity of 28 to 31 units per milligram protein. (One enzyme unit is defined as the amount of enzyme that liberates 1 μmole of product per minute under the assay conditions.)

Polyvinylpyrrolidone (PVP) was covalently conjugated to pure hexosaminidase A, as described previously.16 Separation of the modified enzyme from unreacted PVP was carried out on a Biogel A 1.5-meter column. In the different preparations, 8 to 10 moles of PVP were bound per mole of hexosaminidase. The enzyme retained full activity after binding to PVP, and the final specific activity of the preparation was approximately 15 units per milligram of conjugated enzyme.

Enzyme preparations were filtered through a 0.45-μm Millipore filter, and their sterility was verified. The enzyme solutions were kept frozen at -20°C until used. No loss of activity was observed during storage for 6 months.
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Figure 1. Case 1. Clearance of free (●) and of PVP-bound (○) hexosaminidase A from the circulation after intravenous injection. The level of enzyme activity found in the serum 2 minutes after injection was taken as 100 percent activity value.

to 1.5 mg in 2 ml), was injected initially into the left lateral ventricle through a fine catheter inserted into the ventricle under aseptic precautions. Four such injections were given within 2 weeks. These were followed by eight lumbar puncture (LP) injections at 3-day intervals. At this stage, the patient was discharged from the hospital and subsequently received seven additional LP injections at weekly intervals, so that a total of 19 CSF doses were administered.

Case 2. Treatment was initiated at age 7 weeks and consisted of LP injections only. Each injection contained 1 to 1.5 mg of hexosaminidase A as a mixture of the free and PVP-bound enzymes. The first two weekly injections were given in the hospital, and she subsequently received 40 weekly LP injections, of the same dose, as an outpatient.

Side effects of enzyme administration. In case 1, a marked pyrogenic response characterized by rigors, peripheral cyanosis, tachycardia, and pyrexia of 39.5° C occurred 30 minutes after the first intravenous injection of enzyme. This dramatic event was controlled by chlorpromazine and prothiazine. Subsequent blood cultures were sterile. Premedication with prothiazine was used for the next four treatments and then discontinued. The only untoward effect of the injections was fever, usually not more than 38° C, which occurred an hour after injection and returned to normal within 4 hours. Case 2 had no pyrogenic reaction to the enzyme injections. Blood chemistry analyses throughout the trial showed no indication of toxicity. CSF was examined frequently, with no change in CSF chemistry, but with an increase of cell count usually not exceeding 100 cells per cubic millimeter, and the cultures were sterile.

Results. Chemical determination of enzyme and substrate. Case 1 only. (1) The initial serum level of hexosaminidase was 4.6 milliunits per milliliter with no hexosaminidase A, as concluded from the complete heat stability of the enzyme and total incapacity to react with specific antibodies to hexosaminidase A. Activity of hexosaminidase in the CSF was 0.61 milliunits per milliliter, 89 percent of which was heat-stable. The 11 percent decrease in enzyme activity during heat treatment was probably due to inactivated B isozyme, as it showed no binding to specific antihexosaminidase A antiserum. (2) Two serum samples taken before treatment contained 25 to 29 μg per milliliter of GM2 (normal value, less than 1 μg per milliliter).

Clearance of enzyme after injection. Case 1 only. Intravenous injection of hexosaminidase A and of PVP-hexosaminidase A. Hexosaminidase A (0.7 mg in 1 ml) was injected intravenously, and serial venous blood samples were taken. The same procedure was repeated 2 days later with 2 ml of 0.5 mg per milliliter of the PVP-bound enzyme.

Immediately after the injections, a more than tenfold increase in serum hexosaminidase was observed, followed by a rapid decrease (figure 1). The decline was much more rapid with free enzyme than with the PVP-bound enzyme. With free en-

Figure 2. Case 1. Clearance of free (●) and PVP-bound (○) hexosaminidase from the cerebrospinal fluid. Administration of the enzyme and withdrawal of samples were performed via an intraventricular cannula. The enzymatic activity found in CSF 2 minutes after injection was taken as 100 percent activity value.
enzyme, no exogenous hexosaminidase could be detected after 20 to 40 minutes, whereas after injection of PVP-bound enzyme, 30 percent of the original activity was retained in the blood 4 hours later; after 24 hours, enzyme activity in the serum was still above normal values.

Intraventricular injection of free and PVP-bound hexosaminidase A. Administration of enzyme preparations (0.5 ml each) and sampling of CSF were performed through a catheter inserted into the left lateral ventricle. The cannula was rinsed with sterile saline, and the first drops of CSF were discarded. Marked increases (1300-fold for the free and 400-fold for the PVP-bound enzyme) were observed in the CSF level of hexosaminidase immediately after the injections, with subsequent decreases (figure 2). Enzyme activity reached a plateau (two to three times the base level) after 5 hours and was maintained for 48 hours. Free and bound enzyme exhibited similar clearance patterns from the CSF.

Serum levels of hexosaminidase after intraventricular injection of free enzyme also increased by 30 percent over the original value, and decreased to basal levels within 48 hours. A similar but somewhat lower effect was observed after the injection of PVP-conjugated enzyme.

Distribution of radiolabeled enzyme after lumbar injection. PVP-bound hexosaminidase A was labeled with $^{131}$I (6 µCi per µg of conjugated enzyme). Thirty micrograms of labeled enzyme was mixed with 0.5 mg of unlabeled enzyme and injected by lumbar puncture. Cisternography was performed at intervals up to 72 hours after the injection. Brain scans 2, 4, and 6 hours after injection showed a normal distribution of radioactivity. After 24 hours, only small amounts of radioactivity were detected, and after 72 hours, it was too low to be recorded. In a sample of CSF taken at 24 hours, most of the radioactivity (72 percent) was insoluble in trichloroacetic acid (TCA).

Enzyme replacement treatment. Clinical observations. In case 1, about 2 weeks after initiation of the replacement trial, signs of clinical improvement were observed, with increased limb movement, disappearance of decerebrate posturing,
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Table. GM₂ in sera before and after enzyme replacement treatment

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<tr>
<th>Treatment</th>
<th>Concentration of GM₂ (µg/ml)</th>
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<tr>
<td></td>
<td>Normal serum</td>
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<tr>
<td>None</td>
<td>1.29</td>
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<td>Intravenous injection</td>
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<tr>
<td>Intraventricular injection</td>
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* Replacement treatment was by injection of free hexosaminidase A, as described in the text, and the values of GM₂ were determined in samples taken 2 or 6 hours after injection.

† An untreated child with Tay-Sachs disease.

ability to lift her head from a prone position, disappearance of regurgitation on feeding, decrease in the startle response, and smiling or laughing after appropriate stimuli. There was no additional improvement after the first month, and treatment was therefore discontinued after 10 weeks. One month later deterioration was evident, and 2 months after therapy ended she died of respiratory failure. At postmortem examination, no complications arising from the treatment could be detected.

Electroencephalographic measurements before and during the first 10 days of treatment showed irregular low-voltage background activity, which was replaced at frequent intervals by runs of sinusoidal 4- to 5-Hz rhythms of rather high amplitude (50 to 200 µV). Twenty days after initiation of treatment, the record was dominated by high-voltage rhythmic 4- to 5-Hz activity with very few intervening low-amplitude dysrhythmic periods. The record taken after termination of treatment demonstrated partial reversion to the original pattern, and, for the first time, spike-and-wave complexes were apparent. No consistent effect could be noted as a result of the treatment. Repeated fundoscopic examinations showed no change in the “cherry red spot,” nor were there changes in the visual evoked potential (VEP). The electroretinogram (ERG) after full dark adaptation presented responses of higher amplitudes than normal, but without any delay in the implicit time (figure 3). This pattern of the retinal response was preserved during the entire process of dark adaptation. The VEP pattern was almost totally extinguished: Neither the ERG nor the VEP changed during treatment.

In case 2, treatment was initiated while the patient still exhibited normal clinical psychomotor development. EEG recordings showed a normal pattern for her age until age 10 months. At age 11 months, deterioration was first observed, and at age 14 months, total blindness was noted, with a marked startle reaction. At 17 months, she was floppy, apathetic, and did not respond to external stimuli. Treatment was discontinued at age 12 months.

Chemical, histologic, and immunologic examinations. The levels of GM₂ in the serum of case I decreased after administration of the enzyme to the blood or the CSF (table). However, it was not clear whether the entire effect took place in vivo or the residual injected hexosaminidase degraded part of the ganglioside in vitro. Intravenous injection caused more drastic decreases of serum GM₂ levels (down to normal values) than did the intrathecal injections.

A frontal lobe brain biopsy of case 1 was performed 3 months after initiation of treatment, and immediately fixed for both light and electron microscopy. Light microscopy showed that all neurons had a pale, swollen cytoplasm. Ultrastructural analysis (figure 4) revealed massive accumulation of membranous cytoplasmic bodies with concentric and some parallel lamellae. The changes were typical of GM₂ gangliosidosis.

The possibility of development of an immune response against hexosaminidase A in the patients was excluded by enzyme-binding experiments: Serum samples, taken after the last injection, were incubated with pure hexosaminidase A and PVP-hexosaminidase A for 15 minutes at 37°C. Subsequently, the IgG fraction of the patients’ serum was precipitated by rabbit anti-human IgG, and enzyme activities were measured in both the supernatant and the precipitate. No activity was
lysosomes presents several difficulties. An inherent obstacle is the requirement that the enzyme come into direct contact with the intracellular deposits of GM₂ in the brain. To circumvent the blood-brain barrier, we administered the enzyme in milligram amounts, intraventricularly or into the spinal canal. This route of injection seemed advantageous because penetration of macromolecules from the CSF to the intercellular space of the brain has been reported to be feasible. ⁰⁻²⁵ GM₂ serum levels decreased after administration of free and PVP-bound enzyme by either intravenous or intraventricular routes. The presence of hexosaminidase A in serum after intraventricular or intrathecal injection indicated that the enzyme crossed the CSF-blood barrier. However, there was no indication that the enzyme actually entered brain cells.

A second difficulty in enzyme replacement is the rapid clearance of intravenously injected enzyme by the liver and by proteolytic degradation. In the clearance experiments, PVP-bound enzyme was active in the circulation much longer than free hexosaminidase A. This accords with our previous observations in rabbits, and with the resistance of the enzyme conjugate to proteolytic inactivation. ¹⁶

In the present study, we used a mixture of both free enzyme and the PVP-conjugate to ensure both immediate and long-duration activity of the enzyme.

In case 1, treatment was initiated late in the disease; clinical blindness and severe motor retardation were evident. Suspecting that this hampered the chances of success, we treated case 2 early, before any clinical symptoms developed. We therefore tested replacement treatment on both established GM₂ deposits and on the active progressive process of GM₂ accumulation.

Clinical follow-up and pathologic examination of case 1 did not indicate any serious harmful effects of the treatment. Pyrogenic reactions were easily controlled. As for its beneficial effects, some clinical improvement of case 1 followed the initial injections, but there was only limited arrest of deterioration, and improvement of some functions, and both mental and physical retardation were still severe.

In case 2 it was not possible to determine the immediate effect of the treatment because there were no clinical symptoms at first. However, treatment did not eliminate the "cherry red spot"; the visual evoked response remained extinguished, and a progressive increase in the amplitude of the ERG, typical of Tay-Sachs disease, was present. The pattern of psychomotor deterioration did not differ from that in the typical course of the disease. Therefore, enzyme replacement treatment was not beneficial in this case.

We conclude that administration of enzyme into the subarachnoid space is not effective either for

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**Figure 4.** Case 1. Electronmicrographs of brain cells in a biopsy taken 3 months after the initiation of treatment. The massive accumulation of GM₂ is manifested by the presence of a large number of membranous cytoplasmic bodies.

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found in the precipitates, indicating that no immune response to the enzyme, at least at the humoral level, was elicited.

**Discussion.** Since the symptoms of Tay-Sachs disease are caused by accumulation of GM₂ in brain cells, and since there is no indication that the lesions are irreversible, the desired effect of treatment would be elimination of the ganglioside deposits, with functional recovery of the brain.

The delivery of effective enzyme to brain cell
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removal of the massive deposits of GM2 already present at the initiation of treatment, or even in preventing progressive postnatal accumulation of GM2. Investigations of an experimental form of Tay-Sachs disease in animals are required before further clinical enzyme trials.

Acknowledgments

We thank Dr. J. Braham for interpretation of the electroencephalogram, Dr. V. Godel for the electrophysiologic eye examinations, and Dr. J. Bubis for the pathologic examinations.

References