Early Evolution Of Cytoplasmic Inclusion Bodies in Tay-Sachs Disease[1] (35154)

B. W. VOLK, M. ADACHI, J. FRIEDLAND, L. SCHNECK, AND C. VALENTI

Isaac Albert Research Institute, Birth Defects Center and Department of Pediatrics, Kingsbrook Jewish Medical Center, and the Department of Obstetrics and Gynecology, State University of New York, Downstate Medical Center, Brooklyn, New York 11203

Tay-Sachs disease (TSD) is an autosomal recessive disorder. These children manifest clinical symptoms at the age of 6–8 months, with progressive psychomotor regression and blindness, terminating in death usually by 3–4 years of life (1). The disease is an inborn error of glycolipid metabolism resulting in the massive accumulation of Tay-Sachs or G_{M2} ganglioside in brain (2) and viscera (3, 4). The main pathological feature is the accumulation of the lipid material within the nerve cells of the central nervous system, as well as of the autonomic and somatic nervous systems causing ballooning of the cytoplasm which contains numerous fine vesicles. Ultrastructurally, the cells contain round or oval lipid cytosomes, called membranous cytoplasmic bodies (MCB) (5), consisting of concentrically arranged membranes which measure on the average 1 μ in diameter.

The present report is concerned with the prenatal and early postnatal evolution of these MCB. The study was performed on brain and liver of a 21-week-old aborted embryo prenatally diagnosed as TSD (6) and that of a 14-week-old Tay-Sachs infant. Amniocentesis was done on a woman who previously had a Tay-Sachs child. The diagnosis of fetal TSD was made because only trace amounts of hexosaminidase A, shown to be absent in TSD (7), were present in uncultured cells and fluid obtained by amniocentesis. The pregnancy was, therefore, terminated. The biochemical studies on the fetus confirmed the in utero diagnosis of TSD (6). The same organs of a normal fetus aborted at 15 weeks served as controls.

[1] Supported by a grant from the National Tay-Sachs and Allied Diseases Association, Inc.

The enzyme determination of hexosaminidase A and B activities in the brain, and liver amniotic cells and fluid was performed by acrylamide gel electrophoresis using 4-methylumbelliferyl substrate (8). The ganglioside extraction and chromatographic isolation procedures were carried out according to the methods of Folch *et al.* (9) and Rouser *et al.* (10). The total sialic acid concentration and percentage distribution was determined by the methods of Svennerholm (11) and Suzuki (12).

The biochemical studies showed a lack of hexosaminidase A in the fetal and infant brain and liver (6) and both brains showed increased total ganglioside concentration as compared with controls. Thin-layer chromatography displayed an apparent increase of G_{M2} (Tay-Sachs) ganglioside in both cases.

Electron microscopic studies were performed on brain and liver of the Tay-Sachs fetus as well as on the brain of the Tay-Sachs infant and also on brain and liver of the normal control embyo and of a 12-week-old normal infant. The tissues were minced in cold 3% buffered glutaraldehyde, fixed for 3 hr and post-fixed in cold 1% buffered osmium tetroxide for 1 hr. After fixation, the tissues were dehydrated in a graded series of ethanol and embedded in epoxy resin. Ultrathin sections were obtained with glass or diamond knives and viewed with electron microscopes (RCA EMU 3E and 3G). Additional portions of tissue were fixed in 10% formaldehyde solution and embedded in paraffin for histological studies and stained in hematoxylin and eosin, periodic acid–Schiff (PAS), Luxol fast blue (LFB), toluidine blue, alcian blue, phosphotungstic acid and hematoxylin, trichrome, and Romanes prepa-

rations. Frozen sections were stained with Sudan black B, Sudan IV, and oil red O techniques.

Histologic sections from the brain of the fetus showed immature neurons and absence of cytoplasmic swelling. With various histochemical preparations, no visible stored material was demonstrated in the neuronal cytoplasm. In the spinal cord of the fetus, however, the anterior horns showed well-developed motor neurons without swelling of the cytoplasm which contained innumerable fine vacuoles exhibiting positive reactions in PAS and LFB preparations. The liver showed absence of histological or histochemical abnormalities. The infant with TSD displayed well-developed neurons in brain without swelling of the cytoplasm. There were, however, fine cytoplasmic vacuoles present which showed positive reactions with PAS and LFB stains. A liver biopsy from the Tay-Sachs infant contained a strongly LFB-positive material in Kupffer cells, while the hepatocytes were unchanged.

Electron microscopically, the fetal brain showed occasional dense bodies in the neuronal cytoplasm consisting of fine granules mixed with a slightly dense amorphous material (Fig. 1). Very occasional concentric circular membranes were observed in these bodies. Typical MCB were not observed. The endoplasmic reticulum was frequently distended and contained also a granular material which was similar to that seen within the cytoplasm. The mitochondria showed no abnormalities. In the liver, the hepatocytes contained very occasional small dense bodies.

The neurons of the TSD child contained a small number of intracytoplasmic inclusion bodies exhibiting different features, which could roughly be divided into three groups; the first group was composed of a membrane-bound dense amorphous material (Fig. 2) which was similar to that observed in the fetal brain. The second group consisted of membrane-bound, round bodies containing dense amorphous material and fine granules as well as circular membranous structures (Fig. 3). The third group was made up of membrane-bound bodies containing well-developed concentric membranes (Fig. 4) which were similar to the MCB seen in the later stages of TSD. However, these structures were rarely seen. In the liver, the hepatocytes were unchanged, while the Kupffer cells contained membrane-bound, round bodies, filled with a finely granular and amorphous material. Control studies of the organs of the normal fetus and of the 12-week-old normal infant failed to show any comparable alterations.

The present study supports the previously advanced hypothesis that neuronal and astrocytic lipid bodies originate in endoplasmic reticulum (13), since in the neurons of the fetal brain this organelle was frequently dilated and contained a dense fine granular amorphous material (Fig. 1). This assumption is based upon the observation that the neuronal cytoplasmic dense bodies are in contiguity with the endoplasmic reticulum.

It is not surprising that the characteristic MCB were absent in the embryo and were only observed in the infant in the early stage of TSD. It has been shown that in the later stage of TSD these cytosomes are biochemically composed of 90% lipid of a characteristic molar composition and 10% protein (14). These cytosomes gradually evolve as visual expressions of the maturing lipid pattern which forms during the progression of the disease. In the fetal Tay-Sachs brain there are relatively low molar concentrations of cholesterol, phospholipid, and proteolipid compared to infant Tay-Sachs brain (15). This could account for the absence of characteristic MCB.

Finally, this study demonstrates that the ultrastructural and biochemical alterations in TSD have already occurred at a time when amniocentsis becomes feasible (12–14 weeks of gestation). Preliminary observations on cultured tissue of an 8-week-old embryo from a mother who had a previous child with TSD, indicates that the relationship between absent hexosaminidase A and increased G_{M2} ganglioside is already apparent by 8 weeks of gestation (16).

Summary. Ultrastructural and biochemical studies were performed on the brain and liver of a 21-week-old embryo obtained by a therapeutic abortion from a woman who had

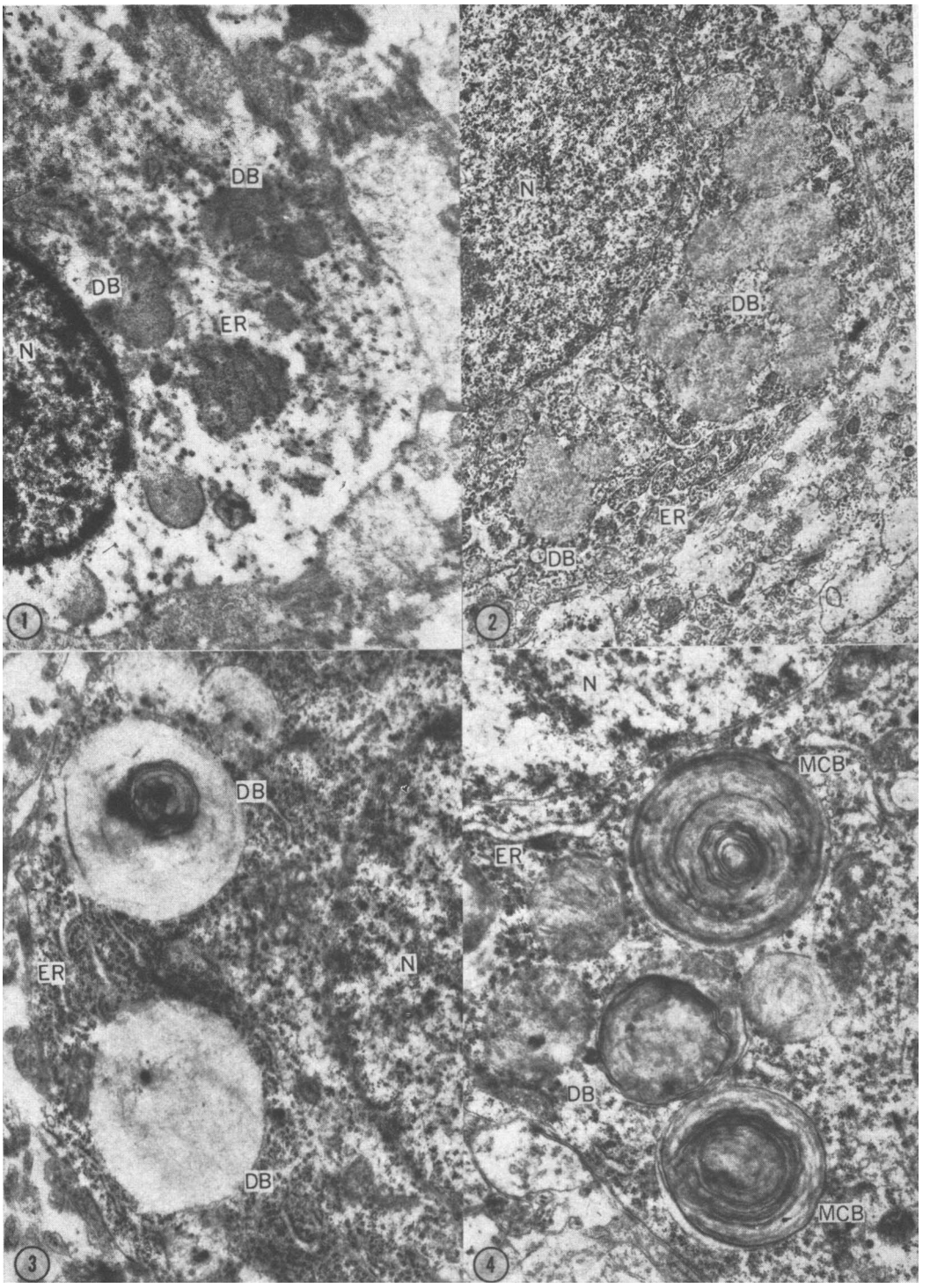

FIG. 1. Neuronal cytoplasm of brain of a 21-week-old embryo with biochemical characteristics of Tay-Sachs disease showing amorphous electron-dense bodies (DB) and prominent endoplasmic reticulum (ER) containing a finely granular material. Nucleus (N). ×10,900.

FIG. 2. Neuronal cytoplasm of a 14-week-old infant with Tay-Sachs disease filled with pleomorphic membrane-bound bodies containing electron-dense material (DB). Endoplasmic reticulum (ER). Nucleus (N). ×9000.

FIG. 3. Portion of neuronal cytoplasm of same child as in Fig. 2, showing round, membrane-bound bodies filled with a finely granular material. One of them contains a structure consisting of concentric membranes. Endoplasmic reticulum (ER). Nucleus (N). ×16,250.

FIG. 4. Portion of neuron of the same child as in Figs. 2 and 3. Only occasionally well-developed membranous cytoplasmic bodies (MCB) are seen. Dense bodies (DB). Endoplasmic reticulum. Nucleus (N). ×23,350.

previously a child with Tay-Sachs disease. Similar studies were carried out on brain and liver of a 14-week-old infant afflicted with this disease. In both cases there was absence of hexosaminidase A activity in liver and brain, and increased amounts of G_{M2} ganglioside were found in cerebral tissue. Electron microscopically, it was possible to trace the evolution of the membranous cytoplasmic bodies from prenatal ill-defined finely granular cytoplasmic bodies to the membranous cytoplasmic bodies seen in the early postnatal stage of the disease. The study, furthermore, indicates that the in utero diagnosis of Tay-Sachs disease can be confirmed by morphological and biochemical studies on the fetus.

1. Volk, B. W., ed., "Tay-Sachs Disease." Grune & Stratton, New York (1964).

2. Svennerholm, L., Biochem. Biophys. Res. Commun. **9**, 436 (1962).

3. Svennerholm, L., *in* "Inborn Disorders of Sphingolipid Metabolism" (S. M. Aronson and B. W. Volk, eds.), p. 169. Pergamon, New York (1967).

4. Schneck, L., Kleinberg, W. and Volk, B. W., Proc. Soc. Exp. Biol. Med. **130**, 404 (1969).

5. Terry, R. D., and Korey, S. R., Nature (London) **188**, 1000 (1960).

6. Schneck, L., Friedland, J., Valenti, C., Adachi, M., Amsterdam, D., and Volk, B. W., Lancet **1**, 582 (1970).

7. Okada, S., and O'Brien, J. S., Science **160**, 1002 (1968).

8. Friedland, J., Schneck, L., Saifer, A., Pourfar, M., and Volk, B. W., Clin. Chim. Acta **28**, 397 (1970).

9. Folch, J., Lees, M., and Sloane-Stanley, G. H., J. Biol. Chem. **226**, 497 (1957).

10. Rouser, G., Kritchevsky, G., and Yamamoto, A., *in* "Lipid Chromatographic Analysis" (G. Marinetti, ed.), p. 99. Dekker, New York (1967).

11. Svennerholm, L., Biochim. Biophys. Acta **24**, 604 (1957).

12. Suzuki, K., J. Neurochem. **12**, 629 (1965).

13. Adachi, M., Torii, J., Volk, B. W., and Schneck, L., J. Neuropathol. Exp. Neurol. **24**, 138 (1970).

14. Samuels, S., Korey, S. R., Gonatas, J., Terry, R., and Weiss, M., *in* "Cerebral Sphingolipidoses" (S. M. Aronson and B. W. Volk, eds.), p. 309. Academic Press, New York (1962).

15. Schneck, L., Friedland, J., Adachi, M., and Volk, B. W., unpublished data.

16. Schneck, L., Amsterdam, D., Valenti, C., Adachi, M., and Volk, B. W., unpublished data.

Received July 13, 1970. P.S.E.B.M., 1970, Vol. 135.