Development of Abnormal Lipid Bodies in Various Sphingolipidoses[1] (35860)

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The sphingolipidoses are hereditary disorders (1, 2) in which lipids accumulate in brain and other organs. The patients show often progressive psychomotor deterioration in infancy. The nature of the metabolic defects in the more common sphingolipidoses has been demonstrated in the past few years (2), and the abnormality has been shown to be due to absence or significant decrease of catabolic enzymes (3). Despite our knowledge as to biochemical abnormalities in these disorders, the subcellular organelles concerned with the enzyme defect and the primary site for accumulation of the abnormal lipid cytosomes are unknown.

The present study indicates that the abnormal lipid cytosomes are derived from the smooth portion of the endoplasmic reticulum and that the mitochondria appear to be the organelle which is primarily associated with the enzyme defects.

Materials and Methods. Ultrastructural studies were carried out on brain biopsies of six patients with Tay–Sachs disease, two children with Niemann–Pick disease, one infant with G_{M1}-gangliosidosis, one child with systemic G_{M2}-gangliosidosis and one patient with infantile Gaucher's disease, ranging in age from 4 months to 3 years.

The brain biopsies were immediately diced in cold (4°) 3% buffered glutaraldehyde solution, then fixed for 3 hr and postfixed in cold (4°) 1% buffered osmium tetroxide solution for 1 hr. Portions of the specimens were fixed only in cold (4°) 2% buffered osmium tetroxide solution for 90 min. After fixation, the tissue was rinsed briefly in distilled water, dehydrated in graded series of ethanol, and embedded in epoxy resin. Ultrathin sections were obtained with glass or diamond knives. Some of the blocks were studied by serial ultrathin sections. The sections were stained with uranyl acetate and lead hydroxide and viewed with electron microscopes (RCA EMU 3E and 3G).

Results. The abnormal cytosomes are alike in neurons and astrocytes in all sphingolipidoses studied (4). However, due to the fact that they are less densely distributed in the cytoplasm of astrocytes the details of the altered subcellular organelles could be observed more clearly in these cells.

In the early phase of Tay–Sachs and Niemann–Pick disease, when the lipid bodies were relatively scarce many mitochondria were observed exhibiting frequently close proximity to the smooth portion of the endoplasmic reticulum (Fig. 1). In the later stage of the disorder, they declined significantly in number and large amounts of the abnormal cytosomes became apparent, which then exhibited close proximity to the smooth portion of this organelle (Figs. 2 and 3).

In the advanced phase of Tay–Sachs disease the cytosomes consisted of concentrically arranged membranes (Fig. 4), while in cases of Niemann–Pick disease the well-developed lipid bodies displayed loosely arranged parallel membranes (Fig. 3). In the other types of sphingolipidoses the picture was similar to that observed in Tay–Sachs disease and Niemann–Pick disease. These observations suggest that the smooth portion of the endoplasmic reticulum is the primary site for the accumulation of the abnormal lipids in sphingolipidoses.

Discussion. During the early stage of Tay–Sachs disease the abnormal cytosomes consist of either a finely granular or a dense

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

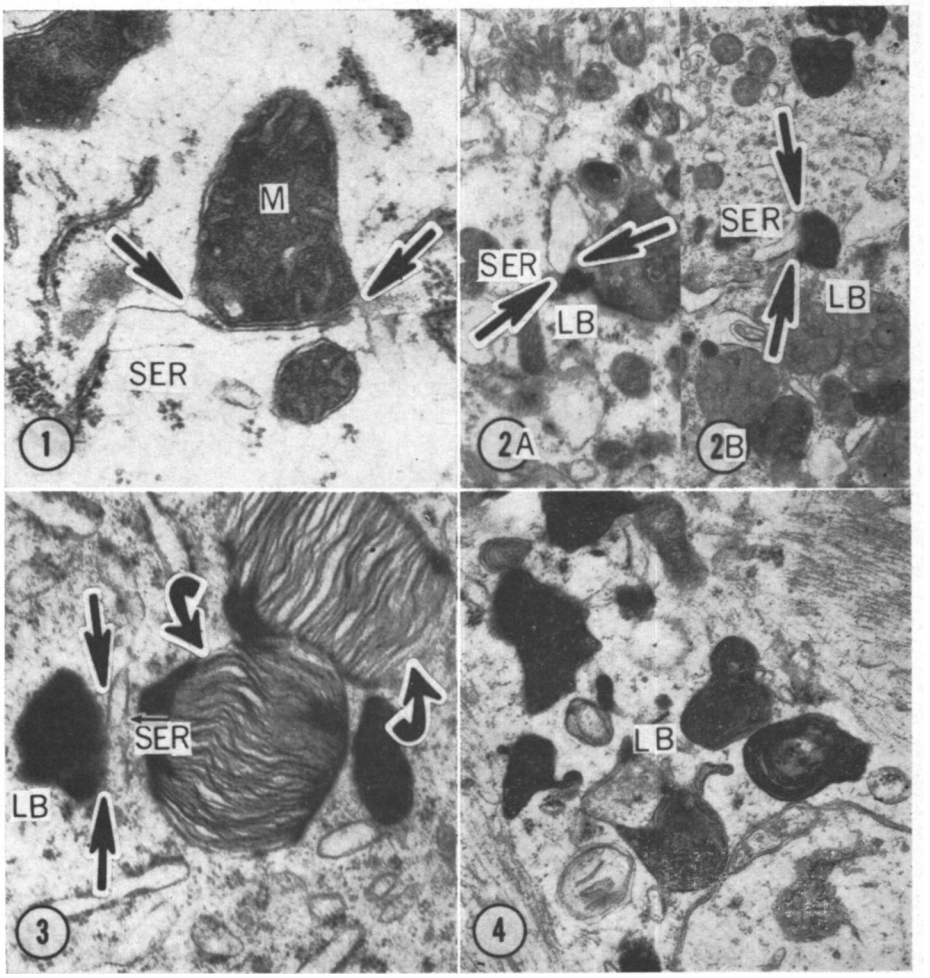

FIG. 1. Astrocytic mitochondria (M) from a patient with Tay–Sachs disease showing close proximity (arrows) to the smooth portion of the endoplasmic reticulum (SER); ×32,400.

FIG. 2A and B. Abnormal lipid bodies (LB) in astrocytes from patients with Tay–Sachs disease are contiguous (arrows) with the smooth portion of the endoplasmic reticulum (SER). (A) ×28,000; (B) ×14,000.

FIG. 3. Abnormal lipid bodies (LB) in astrocyte from an infant with Niemann–Pick disease show close proximity (arrows) to the smooth portion of the endoplasmic reticulum. Two well-developed lipid bodies (curved arrows) consist of loosely arranged parallel membranes; ×29,100.

FIG. 4. In the advanced stage of Tay–Sachs disease the well-developed lipid bodies (LB) consist of concentrically arranged lamellae. The endoplasmic reticulum and mitochondria are scarce; ×23,350.

amorphous material (Fig. 2). The picture is similar to that observed in the fetal stage of the disorder (5). In fetal Tay–Sachs disease, however, the typical membranous bodies seen in the advanced stage of this condition are not observed. This was believed to be due to the fact that some constituents of the cytosomes such as cholesterol, phospholipid, and proteolipid showed relatively low molar concentrations in fetal brain (5). In other words, the morphologic variations from a fine granular or a dense amorphous material to characteristic membranous structures at the different stages of these disorders are believed

to be related to the morphologic expression of different molar constitutents of lipids and proteolipids in the deposited material during the evolution of these diseases.

It is generally believed that the sphingolipidoses are the result of a lysosomal enzyme defect (6). However, abnormal lipid bodies were found already in fetal Tay–Sachs disease while lysosomes were not well developed at this stage (5). Previous enzyme histochemical studies (7) suggested that the cytoplasmic lipid bodies in the advanced phase of Tay–Sachs disease are secondary lysosomes, probably of the residual type, reflecting the cellular effect to eliminate the accumulated lipid material.

In an early stage of the various sphingolipidoses, the mitochondria and endoplasmic reticulum showed a close proximity before accumulation of abnormal cytosomes. The mitochondria significantly disappeared or markedly decreased in number before the lipid bodies emerged from the smooth portion of the endoplasmic reticulum. The mitochondria have been shown to play an important role in several organs during various phases of lipid metabolism (8, 9) and are known to contain catabolic enzymes (10). Therefore, it seems that a specific enzyme defect in this organelle is responsible for the accumulation of the abnormal lipids in these disorders.

This seems to be borne out in preliminary studies of mitochondrial fractions from normal and Tay–Sachs disease brains in which differences of hexosaminidase activities were observed. The mitochondrial fraction from the normal brain showed 28.6% *N*-acetyl hexosaminidase A activity, while it was absent in that from the Tay–Sachs brain.

Summary. The sphingolipidoses are hereditary disorders, secondary to enzyme defects, in which lipids accumulate in brain and other organs. The present studies indicate that the abnormal lipid cytosomes are derived from the smooth portion of the endoplasmic reticulum, and that the mitochondria appear to be the organelle that is primarily associated with the enzyme defect. Preliminary studies of mitochondrial fractions from normal and Tay–Sachs disease brains showed that the mitochondrial fraction of normal brains contained 28.6% *N*-acetyl hexosaminidase A activity, while it was absent in that from Tay–Sachs disease brain.

1. Aronson, S. M., and Volk, B. W., *in* "Medical Aspects of Mental Retardation" (C. H. Carter, ed.), p. 684. Thomas, Springfield, Ill. (1965).

2. Brady, R. O., Med. Clin. N. Amer. **53**, 827 (1969).

3. Brady, R. O., N. Engl. J. Med. **275**, 312 (1966).

4. Adachi, M., Torii, J., Karvounis, P. C., and Volk, B. W., Acta Neuropathol. **18**, 74 (1971).

5. Adachi, M., Torii, J., Schneck, L., and Volk, B. W., Arch. Pathol. **91**, 48 (1971).

6. O'Brien, J. S., Lancet **2**, 805 (1969).

7. Wallace, B. J., Volk, B. W., Schneck, L., and Kaplan, H., J. Neuropathol. Exp. Neurol. **25**, 76 (1966).

8. Bernhard W., and Rouiller, C., J. Biophys. Biochem. Cytol. Suppl. **2**, 73 (1956).

9. Lever, L. D., Amer. J. Anat. **97**, 409 (1955).

10. Penn, N. W., Biochim. Biophys. Acta **37**, 55 (1960).

Received May 6, 1971. P.S.E.B.M., 1971, Vol. 138.