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G_{M2}-Gangliosidosis, AB Variant

Clinico-Pathological Study of a Case

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Abstract. Clinical and neuropathological studies of a case of AB variant G_{M2}-gangliosidosis have been presented. The patient was a 14 months old black female infant who had "black cherry spot" in the retinas. The total activities of β-galactosidase and N-acetyl-β-hexosaminidase, as well as the proportion of hexosaminidase A and B components in her serum and leukocytes were normal when the assays were carried out with artificial fluorogenic substrate. Diagnosis of G_{M2}-gangliosidosis AB variant was established by an abnormal increase of G_{M2}-ganglioside in the biopsied brain tissue, similar to classical Tay-Sachs disease.

Her clinical manifestation appeared to be similar but somewhat milder than those of classical Tay-Sachs disease. Light microscopic features of the cerebral biopsy were also closely similar to Tay-Sachs disease and Sandhoff disease but gliosis and neuronal loss were less pronounced. Electron microscopic study revealed numerous membranous cytoplasmic bodies (MCB) and zebra bodies in neurons. In addition, varieties of large intracytoplasmic inclusions in astrocytes, a feature distinctly different from classical Tay-Sachs disease, were observed. Numerous cytoplasmic inclusions were also present in oligodendroglia, pericytes and microglial cells.

Key words: G_{M2}-Gangliosidosis, AB Variant — Hexosaminidase A and B — Electron microscopy — Membranous cytoplasmic inclusions — Zebra bodies.

Introduction

Tay-Sachs disease was the first G_{M2}-gangliosidosis to be recognized. In 1969, it was discovered that while total hexosaminidase activity was normal or increased, there existed a profound deficiency in the activity of the heat labile A fraction of hexosaminidase (Okada and O'Brien, 1969; Sandhoff, 1969; Hultberg, 1969). Sandhoff also described a second variant of G_{M2}-gangliosidosis (1968), with a clinical course similar to Tay-Sachs disease but without predilection for Jewish children (Sandhoff's disease), in which both total hexosaminidase and hexosaminidase A were drastically deficient. In addition to storing G_{M2}-ganglioside in their nervous system, and to a small extent in their viscera, these infants also stored the asialo-derivative of G_{M2}-ganglioside in their brains and globoside in their viscera, especially in the kidneys. This variant was called the O variant of G_{M2}-gangliosidosis because both the heat labile A fraction and the heat stable B fraction of hexosaminidase were markedly deficient. Tay-Sachs disease was called the B variant, since only hexosaminidase A was absent.

Other variants of G_{M2}-gangliosidosis have since been discovered. Suzuki *et al.* (1970) described a 15 year old Puerto Rican child whose illness started at 5 years and who died at 15 years with a profound dementia, seizures, signs of corticospinal, basal ganglia, cerebellar, and anterior horn cell dysfunction, but whose eye grounds were normal. She was found to have a partial deficiency of hexosaminidase A in brain, liver, and spleen, when tested against artificial substrates (Suzuki and Suzuki, 1970). Enzyme activity in her liver was found to be profoundly deficient when tested against the natural substrate G_{M2}-ganglioside (Zerfowski and Sandhoff, 1974). Other non-infantile illnesses with G_{M2} storage and a variety of clinical findings have occurred in children (Bernheimer and Seitelberger, 1968; Klibansky *et al.*, 1970; Young *et al.*, 1970; Menkes *et al.*, 1971; Brett *et al.*, 1973). Details of enzymatic deficiencies have yet to be worked out, but it appears that more than one genetic variant may be involved. The oldest patients with G_{M2}-gangliosidosis are two Ashkenazi Jewish siblings, aged 28 and 31 years, who presented in early childhood with a very slowly progressive illness resembling a spinocerebellar degeneration (Rapin *et al.*, 1975). They too appear to be suffering from a partial deficiency of hexosaminidase A when tested on artificial substrates, yet their illness does not resemble any of the other variants reported thus far: they are not demented, have normal eye grounds, and no seizures.

Another enzymatically distinct G_{M2}-gangliosidosis is the so-called AB variant. A family of two infants with a picture almost identical to Tay-Sachs disease was described by Sandhoff (1969), Sandhoff *et al.* (1971), and by Kolodny *et al.* (1973). The analytical abnormalities of the brain of these patients were essentially indistinguishable from those with classical Tay-Sachs disease (B variant) and yet the activities of both hexosaminidase A and B were normal when assayed with artificial chromogenic substrates. When G_{M2}-ganglioside was used as the natural substrate, however, the patients tissue was deficient in the activity to cleave the terminal N-acetyl-β-galactosamine (Sandhoff and Jatzkewitz, 1972). To our knowledge, no additional patients with G_{M2}-gangliosidosis AB variant have been recorded since their original family.

In this report we are describing a third child with the AB variant of G_{M2}-gangliosidosis and the results of ultrastructural and chemical findings in a diagnostic cerebral biopsy that the patient underwent at 14 months.

Case Report

This black infant was the product of a full term uncomplicated pregnancy and delivery. There was no parental consanguinity, her mother was well, her father unavailable. She had no siblings. Early development was normal. She smiled at 2 months, played with objects by 6 months, walked in a walker at 7 months, sat unsupported for indefinite periods at 8 months, could dial a toy telephone at 12 months. The mother noted that muscle tone seemed to decrease somewhat after 9 months and that she had more difficulty getting around in her walker. She startled easily to sound but remained sociable and playful. At 12 months she had a prolonged generalized seizure and was admitted to St. Luke's Hospital. Physical examination was normal except for startle to sound, hypotonia with preserved reflexes and a white halo around each macula which appeared as a dark central spot ("black cherry spot"). The remainder of the retina was normal. The liver and spleen were just palpable, there was no skin, joint, or bony anomaly. Head circumference was normal for the age. Blood count, urinalysis, chest and skull X-rays, serum electrolytes, sugar, urea nitrogen, calcium, phosphorus, thyroxin, transaminases, bilirubin and uric acid levels were normal. An electroencephalogram showed

high voltage slow activity with diffuse spike discharges. There were no storage cells in the bone marrow. Serum 4-methylumbelliferyl β-galactosidase activity, total hexosaminidase activity, and hexosaminidase A activity were normal. The patient was transferred to the Bronx Municipal Hospital Center at 14 months of age. She could no longer sit unless propped on her hands, did not roll over or support her weight when held upright. She was hypotonic with increased tendon stretch reflexes. Spinal fluid examination disclosed a protein of 11 mg-% and no cells. An electroencephalogram and electroretinogram were normal. The amplitude of cortical evoked responses to light flashes was not increased. She underwent a right frontal cortical biopsy for diagnosis without complications. She was discharged and has been seen in another hospital on several occasions because of repeated seizures, despite anticonvulsant medications.

Materials and Methods

The biopsy specimen was processed for light and electron microscopic, histochemical and biochemical studies.

A. Light Microscopy

Some tissue was fixed in 10% formalin, dehydrated in alcohol, embedded in paraffin and the sections were stained with hematoxylin and eosin (H.-E.), periodic acid Schiff (PAS), luxol fast blue (LFB) and Bodian stains. Formol-calcium fixed frozen sections were stained with oil red 0, Sudan IV, Sudan black B and PAS. Fresh frozen tissue was used for cryostat sections. These were fixed in formol-calcium and stained with oil red 0 and PAS. Others were fixed in alcoholformalin acetic acid and stained with methyl green pyronin, methylene blue eosin (pH 5.0) acid PAS. Some PAS stains were performed on cryostat sections after diastase or alcohol treatment.

B. Histochemistry

For enzyme histochemistry, tissue was fixed in cold 10% formol-calcium for 23 hrs or in cold cacodylate buffered, 3% glutaraldehyde for 2½ hrs. The Gomori acid phosphatase procedure was performed on formalin- and glutaraldehyde-fixed frozen sections for light microscopy and on glutaraldehyde fixed non-frozen "chopper" sections for electron microscopy. The latter were then fixed in Veronal acetate-buffered osmic acid (Palade's fixative) and embedded in Epon. The sections for electron microscopy were studied either unstained or after staining with uranyl acetate only.

C. Electron Microscopy

The tissue was fixed in 5% glutaraldehyde in 0.1 M phosphate buffer, post-fixed in Dalton's fixative and dehydrated through graded alcohols, cleared in propylene oxide and embedded in Epon. One micron sections were stained with toluidine blue and areas were selected for electron microscopy. Thin sections were stained with uranyl acetate and lead citrate and studied with Philips 200 or Siemens Elmiskop 101 electron microscopes.

D. Biochemistry

Enzymatic Assays: N-acetyl-β-glucosaminidase and β-galactosidase were assayed on serum of the patient and on peripheral leukocytes isolated from the patient and her mother by differential sedimentation in 6% Dextran essentially according to Snyder and Brady (1969) with minor modifications (Suzuki *et al.*, 1971). Appropriate 4-methylumbelliferyl glycosides were used for substrates. They were purchased from Koch Light Laboratories, Colnbrook, England. The assay procedure for 4-methylumbelliferyl β-galactosidase was essentially as described by Öckerman (1968). The pH optima of β-galactosidase were 4.0 for serum and 5.0 for leukocytes (Suzuki and Suzuki, 1971). The incubation mixture for N-acetyl-β-glucosaminidase contained 1 mM substrate in 0.3 ml of 0.15 M citrate-phosphate buffer, pH 4.5, and 0.1 ml of the enzyme source. After incubation at 37° for 60 min, the reaction was terminated by the addition of 2.0 ml of 0.2 M glycine buffer, pH 10.7. The released 4-methylumbelliferone was determined by fluorometry. The differential determination of N-acetyl-β-glucosaminidase A and B components was by heat inactivation procedure (O'Brien *et al.*, 1970). The results were

semiquantitatively verified by the cellulose acetate electrophoretic procedure (Suzuki and Suzuki, 1970). The protein determination was by the method of Lowry *et al.* (1951).

Brain Ganglioside Analysis: Biopsied brain tissue weighing 102 mg was available for chemical analysis. The procedures for tissue extraction, fractionation of major components, and purification of the ganglioside fraction have been described in detail previously (Suzuki *et al.*, 1969). The total lipidbound sialic acid was determined by the resorcinol HCl procedure (Svennerholm, 1957; Miettinen and Takki-Luukkainen, 1959), on the dialyzed upper phase fraction containing all gangliosides. Thin-layer chromatography of gangliosides was carried out with precoated silica gel G plates, 250 μm-thick (Analtech, Inc., Newark, Del.), in the solvent system of chloroform-methanol-2.5 N ammonia (60:40:9, v/v/v). The plate was developed twice in the solvent with complete drying between the runs. Sialic acid-containing spots were visualized by the resorcinol spray and heating (Svennerholm, 1957).

Results

A. Light Microscopy

The biopsy specimen was grossly normal. Microscopically, the leptomeninges were not thickened and the cortical architecture was well preserved. Throughout the cerebral cortex as seen in H.-E., the neurons were markedly distended with finely granular unstained storage materials (Fig. 1). Neuronal swelling was most pronounced in layer 3. The neuronal nuclei were often displaced to the periphery, usually toward the apical dendrite. Marked axonal swellings (torpedoes) were frequent. On Golgi preparation, many abnormal synaptic spines were observed in these "torpedoes" (Purpura and Suzuki, 1975).

Methyl green pyronin and methylene blue-eosin did not stain the neuronal inclusions, but revealed the positive substance as a fine network distributed throughout the cytoplasm between and around the inclusions.

The neuronal inclusions stained pale blue with LFB but only faintly with oil red 0, Sudan IV, and Sudan black B. In PAS stains, these inclusions were darkly stained in all sections not pretreated with alcohol, but were unstained in alcohol-treated tissue (Fig. 1). The staining was unaffected by diastase. The glia, especially the astrocytes, also contained granular material which stained dark blue with LFB and, in sections not pretreated with alcohol, dark red with PAS. This staining was also not affected by diastase. In alcohol-treated sections, many glia contained PAS-positive granules, but these were not present after diastase treatment.

Myelination was normal in the white matter and axons were normal.

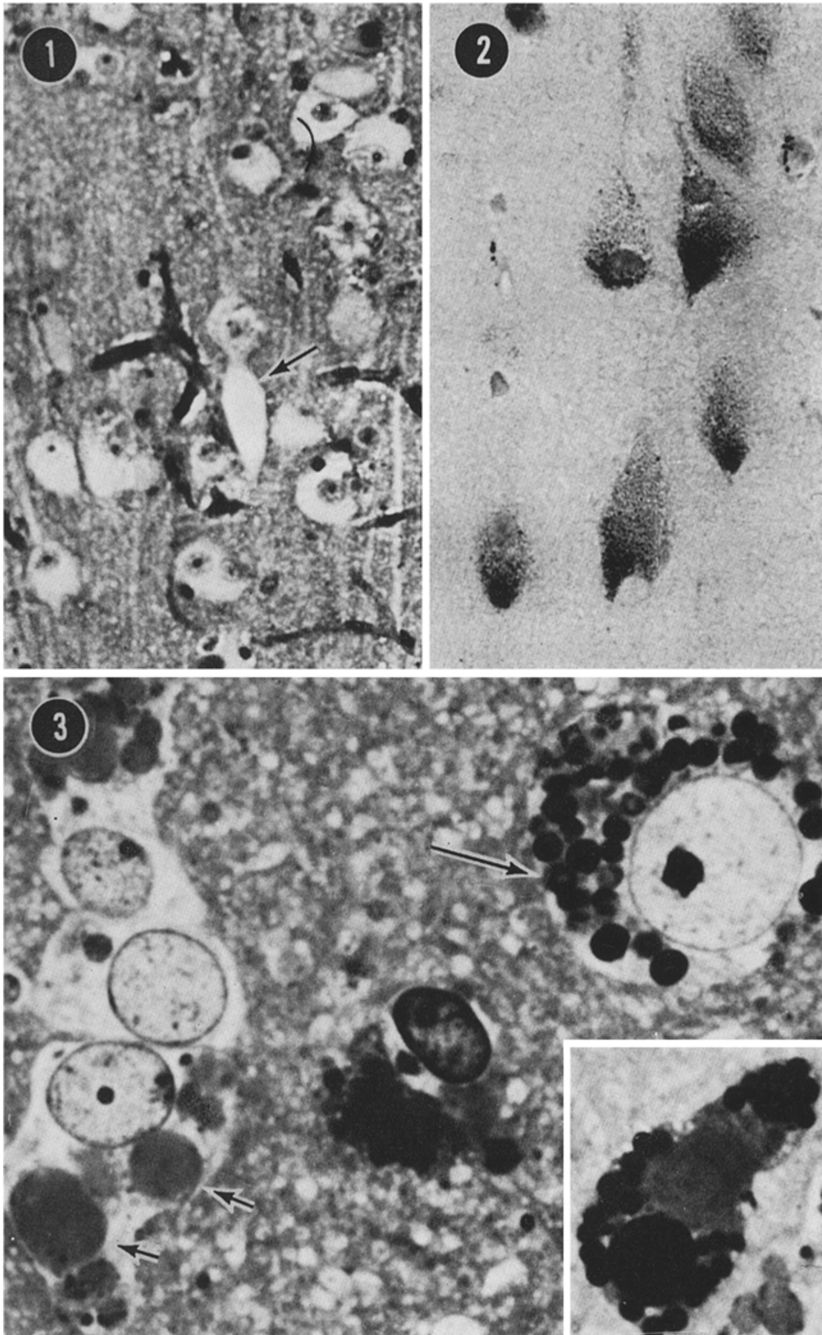
B. Enzyme Histochemistry

Acid phosphatase activity was strong and was greatest in the pyramidal cell layers. Adjacent neurons varied considerably in staining intensity, and the neuronal staining was unusual in that it was usually greatest near the axon hillock (Fig. 2) or in the distended segment of the axon. The staining appeared to be associated with the inclusions, but those perikaryal inclusions away from the axon hillock often were only lightly if at all stained. Few of the

Fig. 1. Cerebral cortical neurons showing greatly distended perikarya. An arrow indicates a swollen axonal "torpedo". The cytoplasmic storage material does not stain while capillary walls stain strongly positive. Paraffin embedded tissue stained with PAS. Magnification ×250

Fig. 2. Fine granules with acid phosphatase activity are seen in neuronal perikarya. The strongest activity is observed at or near the axonal hillock. Glutaraldehyde fixed frozen section, incubated for 45 min in Gomori medium. Magnification ×430

Fig. 3. Dark stained spherical inclusions of uniform size with an occasional clear center filled the neuronal perikarya (long arrow). Astrocytic inclusions are paler and larger than neuronal inclusions (short arrows). Insert shows neuronal inclusions as large as the nucleus. 1 μ thick sections stained with toluidine blue. Magnification ×1700



Figs. 1—3

normally dark, punctate neuronal lysosomes were identified. Acid phosphatase activity was also very strong in pericytes, and was conspicuous in the subpial astrocytes. Other scattered cortical glia, probably astrocytes, also were reactive. No staining was present in the white matter. Electron microscopic study revealed that acid phosphatase activity was localized in some of the MCB in neurons.

C. Light Microscopy of Epon Embedded Tissue

One micron sections stained with toluidine blue showed all neurons to be packed with dark blue round inclusions of rather uniform size with an occasional clear center (Fig. 3). In pyramidal neurons, the inclusions usually were more abundant at the axon hillock. They also extended into the axons and dendrites. Axonal torpedoes of the large pyramidal neurons were filled with these inclusions. Rare neurons contained very large round inclusions about the size of the nucleus (Fig. 3 inset). Small neurons contained fewer round inclusions, but dark, small, irregular inclusions were observed on occasion. Astrocytes contained numerous round pale or dark blue inclusions which were irregular in size and were sometimes as large as the nucleus (Fig. 3). These had an irregular contour with frequent dark semicircular profiles at their periphery. Such profiles were also present in the inclusions within the dilated astrocytic processes. Rare small cells, probably astrocytes, containing very smal and dark inclusions were occasionally seen. Oligodendroglia usually contained several round pale inclusions. All these inclusions were also scattered throughout the neuropil and in the white matter.

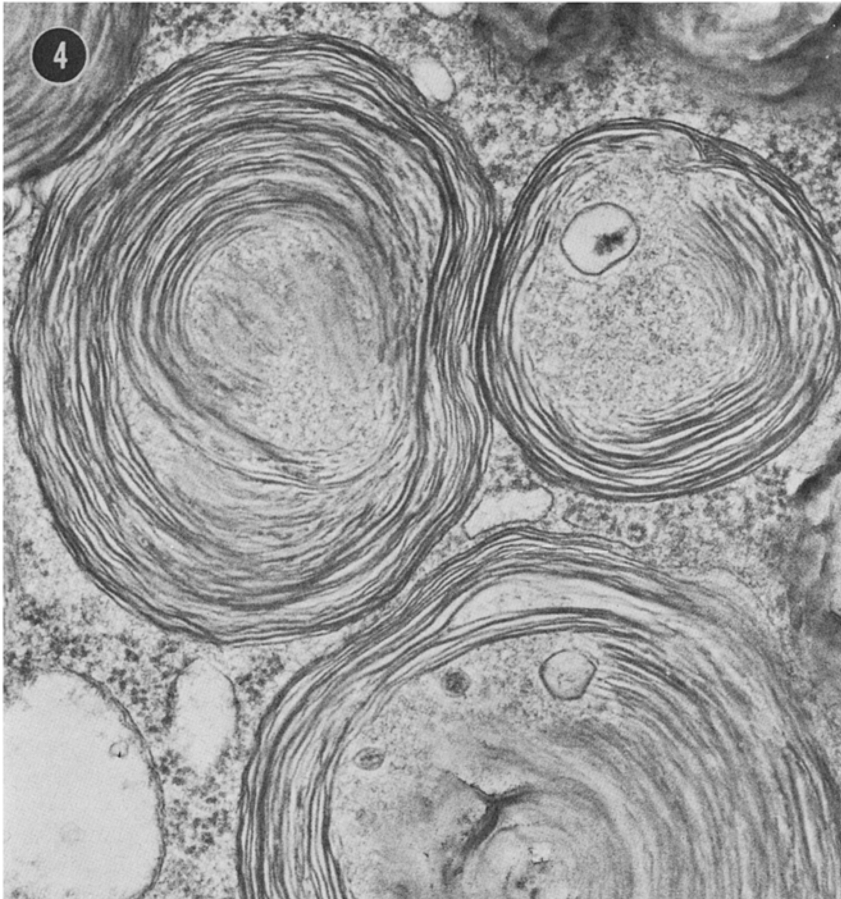
D. Electron Microscopy

Neurons: The neuronal nuclei were unchanged although they were often displaced to the apical dendrite of the cells. The nucleus had the usual dense appearance. The nuclear membrane was normal.

All normal cytoplasmic constituents were present but were reduced in proportion due to the abundance of abnormal cytoplasmic organelles. The mitochondria were usually oval and appeared normal. However, some appeared to be swollen, in which case normal cristae could not be identified, and their matrix was then pale and granular. Large numbers of ribosomes were present throughout the cytoplasm, either randomly, in clusters or rosettes or on the external aspect of the endoplasmic reticulum. The endoplasmic reticulum showed frequent dilatation due to fixation. The Golgi apparatus, neurotubules and neurofilaments did not reveal any abnormalities.

Several types of inclusions were observed in neuronal cytoplasm.

Membranous cytoplasmic bodies (MCB): The cytoplasm of the majority of neurons was filled with numerous round or oval membranous cytoplasmic bodies, which were very similar to those described in classical Tay-Sachs disease (Fig. 4) (Terry *et al.*, 1963). They measured 0.5 to 2.0 μ in diameter, most frequently about 1 μ. They were composed of concentrically arranged groups of alternate layers of electron dense and lucent lines with periodicity of 50 Å (Fig. 5). The outer lines of each group were paler than the inner lines. Groups often approached each other and the two outer pale lines merged to form a single dense line. In the center of many of the circular forms there was a homogenous or finely granular zone. The inner zone of occasional bodies was filled with straight or curved lamellae (compound bodies). Occasionally, ovoid elements bound by a double membrane and containing finely granular material were present in the middle of the MCB.



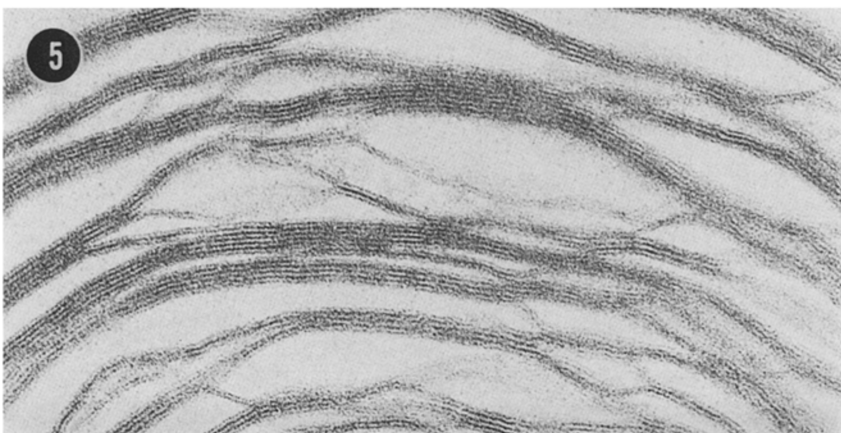


Fig. 4. Neuronal inclusions composed of concentrically arranged lamellae, which are identical to membranous cytoplasmic bodies (MCB) of Tay-Sachs disease. Magnification ×44000

Fig. 5. Higher magnification of the lamellae of MCBs. They consisted of alternate electron dense and lucent lines. Magnification ×260000

All types of MCB usually appeared together in a single neuron. However the concentric type was the most frequent.

Dendrites as well as axons contained occasional MCB. The dendrites contained the usual organelles. Only the axon close to the perikaryon showed marked ballooning and contained large numbers of MCB. Synaptic junctions appeared normal.

Zebra bodies: Smaller neurons mainly in the deeper cortical layers, often contained irregular, lobulated, partially membrane bound ovoid bodies which were similar to the zebra bodies described in Hurler's syndrome (Fig. 6) (Aleu *et al.*, 1965). They consisted of stacked alternate electron dense and lucent parallel lamellae with a periodicity of 50 Å. The outer lines of each lamellae were paler than the inner lines and fusion was frequently seen as in MCB (Fig. 6 inset). Occasionally, more complex bodies consisting of confluent zebra bodies, either partially or entirely surrounded by several layers of wavy membranes which were similar to pleomorphic lipid bodies (PLB) (Volk *et al.*, 1969) were observed (Fig. 7). The, consisted partially of flat arrays of electron dense or lucent bands and of finely granular material containing scattered membrane-bound vesicles.

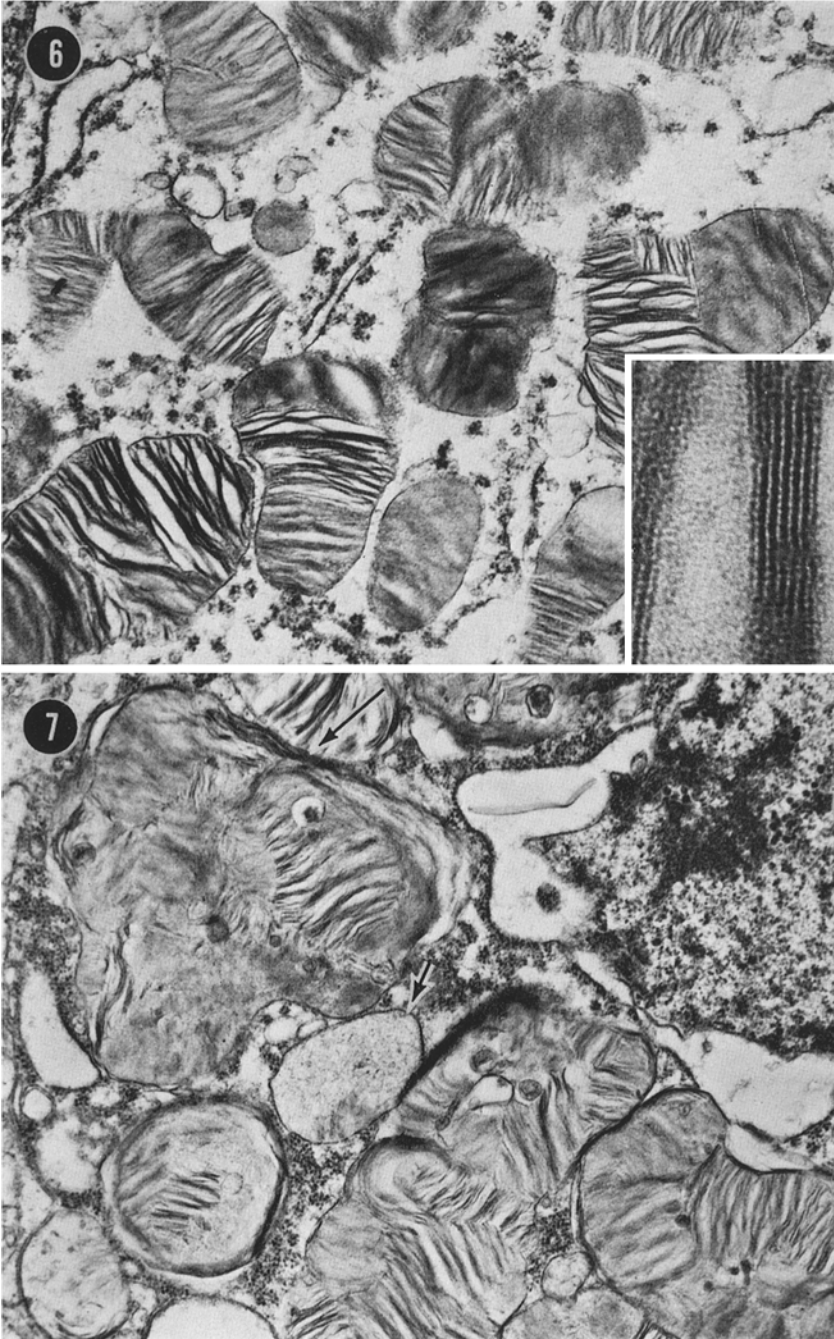
Pale amorphous, membrane-bound inclusions: These were smaller than MCBs, zebra bodies, or PLBs and occasionally were mixed or fused with them (Fig. 7). Conglomerates of all the above bodies were occasionally seen.

Astrocytes: Astrocytes in the cortex were identified by their electron lucent cytoplasm. The nuclei were homogenous in appearance and tended to be round or oval. The cytoplasm contained occasional bundles of fibrils that extended as parallel arrays into the processes. The usual organelles were present in the cytoplasm but were frequently displaced by large inclusions described below (Fig. 8). Large mitochondria up to 1.5 μ in size with dense, granular matrix and a few peripherally located cristae were frequently seen in the perikarya and foot processes of astrocytes. Astrocytic processes frequently appeared club shaped due to the increased amounts of closely packed large inclusions (Fig. 9).

Inclusions: Two types of inclusions were observed in these astrocytes. The first type was more frequently seen. The inclusions varied from 4 to 7 μ in size and while they were usually separated from each other in the perikarya, (Fig. 8) they closely interdigitated and frequently merged within the cell processes (Fig. 9). They were often seen in the foot processes around capillaries and were usually partially bound by curved or wavy stacks of electron dense and lucent lamellae with a periodicity of 50 Å (Fig. 10). These inclusions were round or oval in shape but frequently had an irregular contour. They contained loose or dense aggregates of straight or curved short membranes arranged in all directions and

Fig. 6. Zebra bodies which are almost exclusively observed in small neurons of deeper cortical layers. Magnification ×22500. Inset shows higher magnification of a portion of a zebra body. Alternate electron dense and lucent lines are clearly seen. Magnification ×290000

Fig. 7. Pleomorphic lipid bodies (PLB) within neuronal cytoplasm, which are partially surrounded by a wavy membrane (long arrow), composed of electron dense and lucent lamellae, finely granular material and small vesicles. Pale amorphous, membrane bond inclusions are seen in the vicinity of PLB (short arrow). Magnification ×20000



Figs. 6 and 7

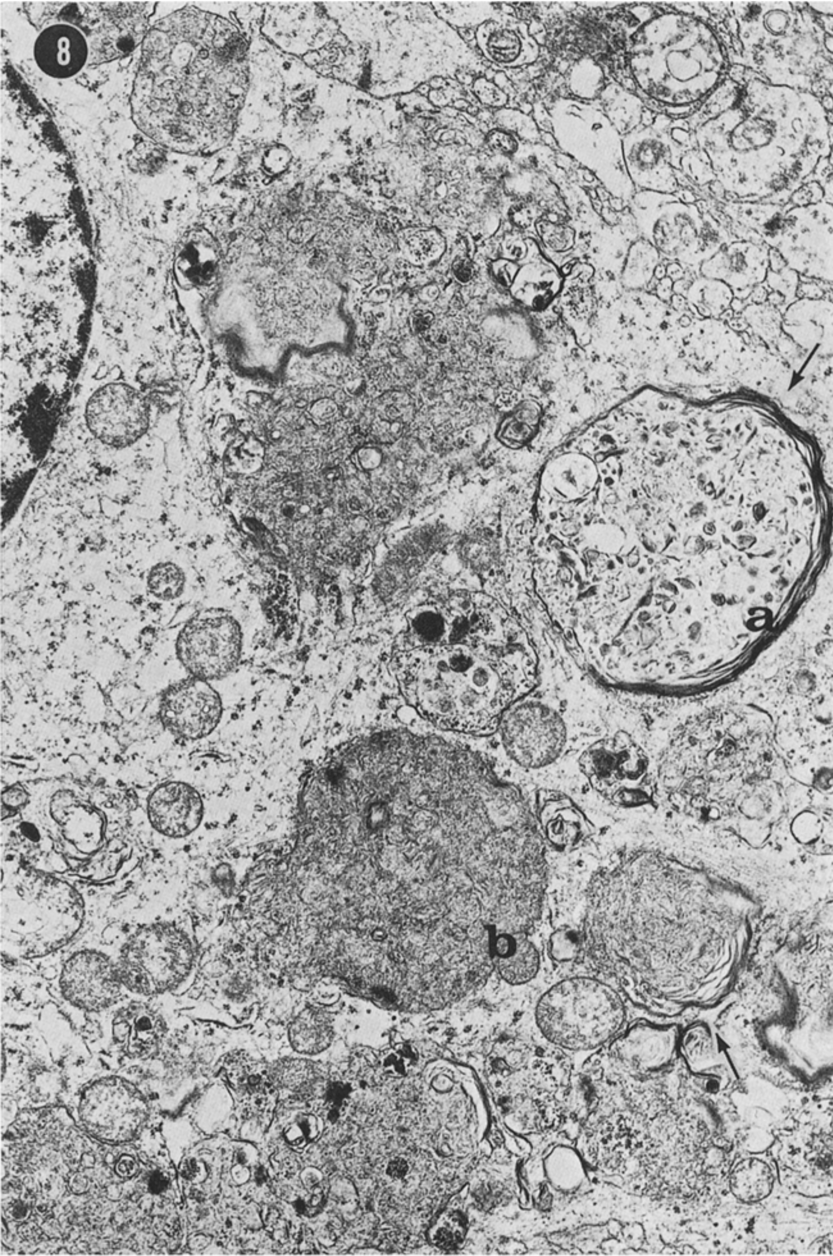


Fig. 8. An astrocyte containing varieties of inclusions. Some are partially bound by wavy membranes (long arrow). Letters *a* and *b* correspond to areas enlarged in pictures 10 and 11. Magnification ×13200

Fig. 12. Group of glycogen granules similar to those indicated by arrow on Fig. 9 are trapped in astrocytic inclusions. Magnification ×146000

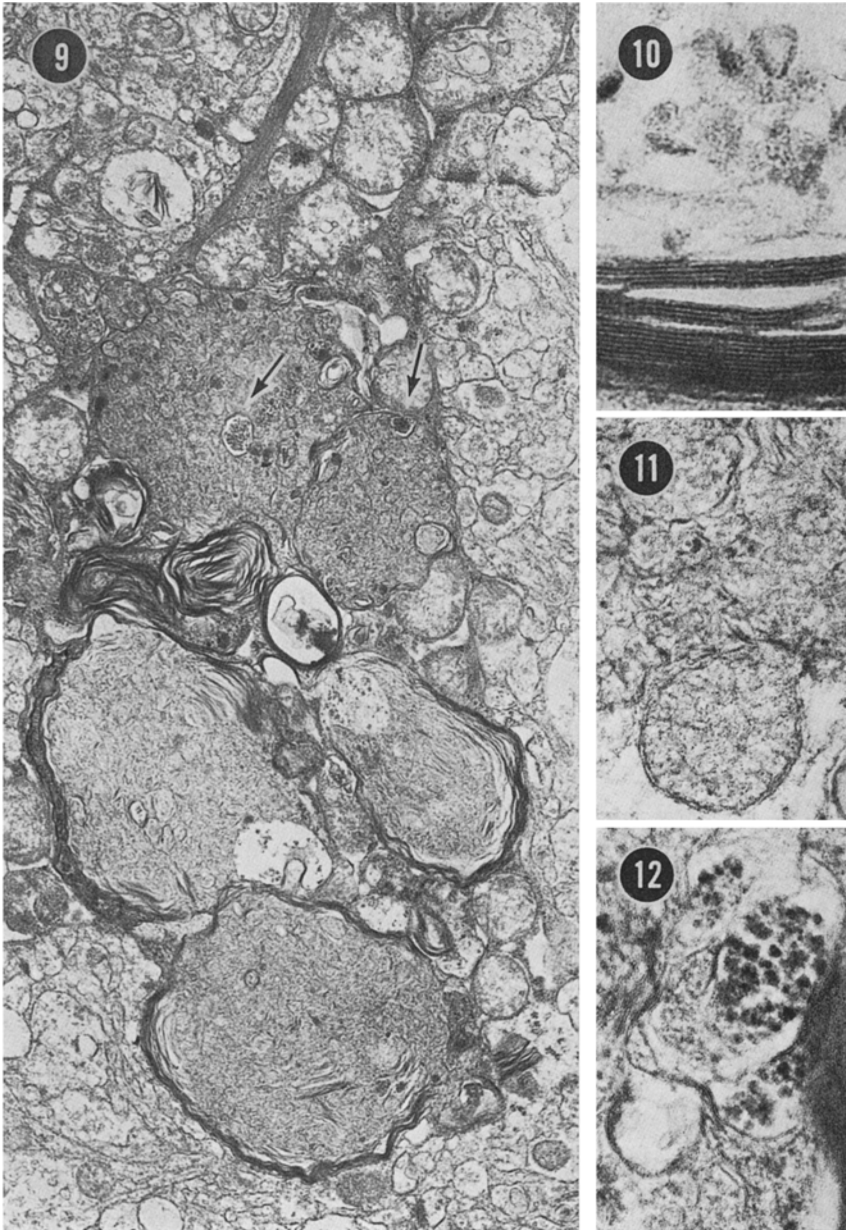


Fig. 9. Club shaped astrocytic process distended by closely interdigitated irregular inclusions. Magnification ×15400

Fig. 10. Higher magnification of area corresponding to letter *a* from an astrocytic inclusion on Fig. 8. Note the stacks of electron dense and lucent lamellae at the edge of the inclusions and the vesicle. Magnification ×60000

Fig. 11. Details of astrocytic inclusion shown in Fig. 8 composed of straight or curved short membranes. A mitochondrion merges with the inclusion. Magnification ×20000

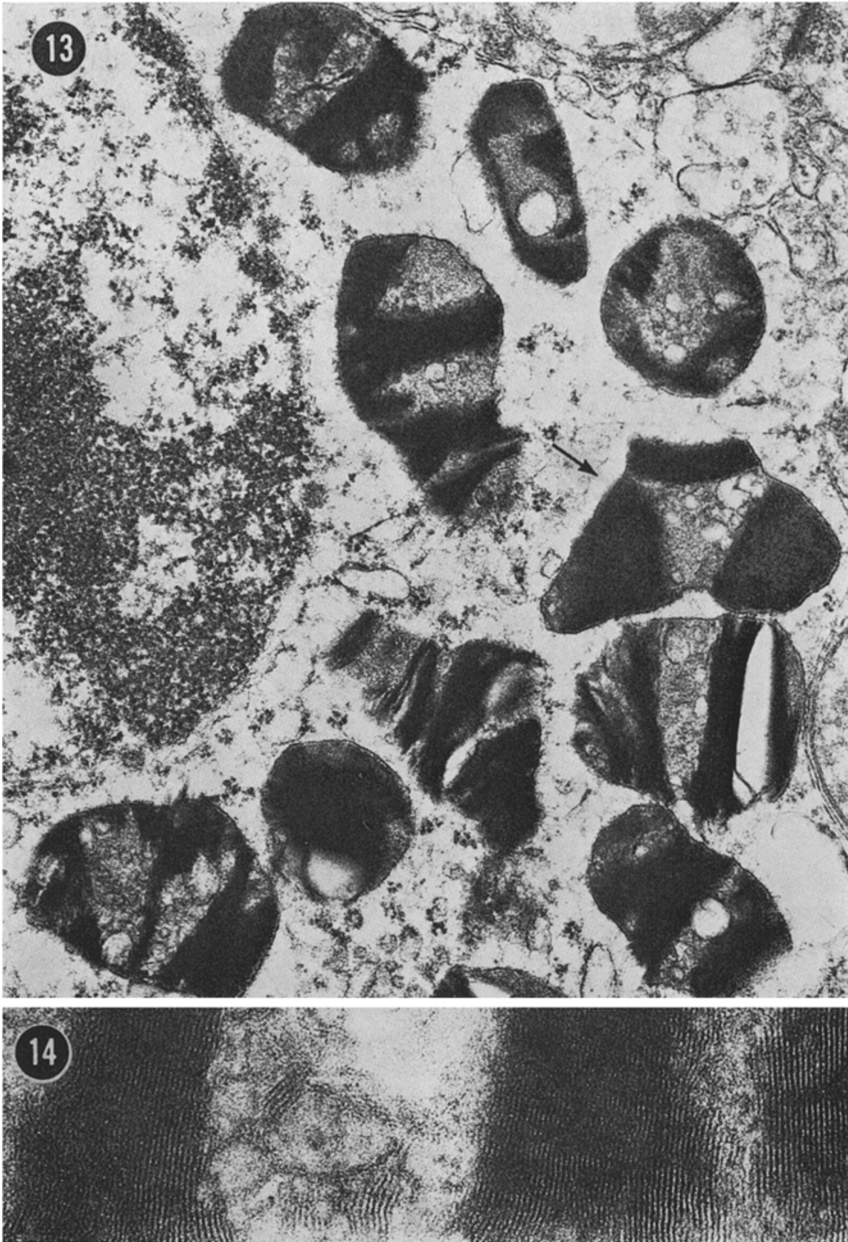


Fig. 13. Second type of astrocytic inclusions which are composed of alternating dense zones and pale zones containing vesicles giving a striated appearance. An arrow indicates a trapezoidal inclusion with a core consisting of vesicles. Magnification ×37 500

Fig. 14. Higher magnification of the dense zone of an inclusion as seen in Fig. 13 composed of alternate electron dense and lucent lamellae and a clear zone composed of vesicles. Magnification ×148 000

circular membranous profiles, sometimes forming small vesicles (Fig. 10). Large stacks of wavy or concentrically arranged membranes were occasionally seen within these inclusions (Fig. 10). Mitochondria often seemed to be entrapped at the periphery of these inclusions (Fig. 11). Frequently collections of numerous glycogen granules were trapped in these inclusions and were either free or surrounded by one or more concentrically arranged stacks of lamellae (Fig. 12). The second type of inclusions was observed more frequently in the astrocytes closely related to blood vessels or neurons. They consisted of much smaller inclusions of 0.4 to 1 μ in size. The polymorphism of their shape was marked; round, oval, triangular rounded angles, hourglass-like, and bean-like (Fig. 13). They consisted of zones containing dense and pale bands alternating with zones containing small vesicles, giving them a striated appearance. When the inclusion was elongated the lamellae were usually arranged perpendicular to the long axis. When they were triangular or trapezoidal the lamellae were usually arranged in 3 stacks with a central core consisting of vesicles. Under higher magnification the dense bands consisted of a varying number of alternating pale and dense lines with a periodicity of 50 Å (Fig. 14). Most of these inclusions were bound by a pair of membranes.

The astrocytes in the white matter contained fibrils. Nuclei and cytoplasmic organelles had a normal configuration. They contained inclusions differing in appearance form those in astrocytes in the cortex. On occasion these two types of inclusions were mixed, especially in the transition zone from grey to white matter.

The inclusions were smaller than those of the cortical astrocytes, round in shape, rather regular in size, averaging 0.2 to 0.5 μ. They were scattered throughout the cytoplasm and cell processes and did not merge. At low magnification they appeared to be electron dense and to contain round clear vesicles of smaller size. At higher magnification they consisted of closely packed straight or slightly curved stacks of lamellae with a periodicity of 50 Å, and were found lying in an electron dense granular cytoplasm. The clear vesicles were formed by circular lamellae. Occasionally the lamellae were arranged in a fingerprint-like fashion.

Oligodendroglia: These were similar in both gray and white matter. The nuclei were round and were of normal appearance. The rough endoplasmic reticulum was well developed. Large numbers of free ribosomes occurred in the cytoplasm. The Golgi apparatus was well developed. Microtubules were preserved.

Inclusions: They were round or slightly oval and rather regular in size ranging from 0.5 to 2 μ. They were scattered throughout the cell cytoplasm and processes, never merged and rarely touched each other. They occasionaly had irregular outlines and appeared as dense bodies with closely packed clear round vesicles (Fig. 15). At higher magnification, these bodies were made of stacked layers of membranes with a periodicity of 50 Å, frequently forming vesicles. The inclusions were often partially bound by a double membrane. They somewhat resembled astrocytic inclusions in the white matter.

Microglial Cells: Smaller cells with an irregular contour and elongated dark nucleus, thought to be microglial cells, also contained inclusions. These cells were frequently seen around blood vessels. The cytoplasm contained rough endoplasmic reticulum. Their inclusions consisted of conglomerates of stacks of lamellae randomly

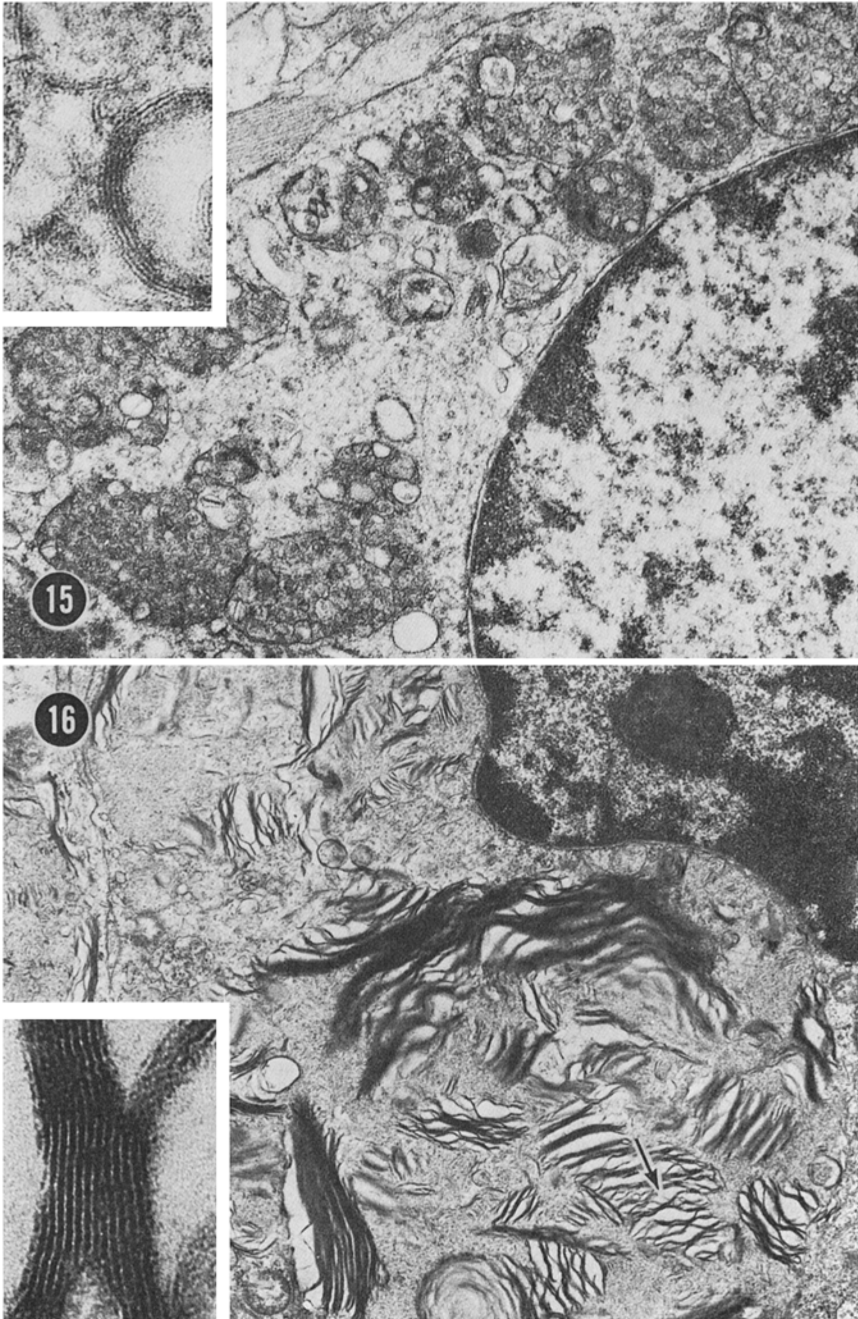


Fig. 15. An oligodendroglial cell containing round or oval regular inclusions composed of many clear round vesicles. Magnification ×20000. Inset shows higher magnification of one of these inclusions. Magnification ×172000

Fig. 16. Microglial cell: Conglomerates of randomly distributed stacks of lamellae and structure resembling MCB (arrow) filling the entire cytoplasm. Magnification ×10000. Inset shows the higher magnification of the inclusion indicated by arrow. Magnification ×360000

distributed in all directions within the cell cytoplasm, round or oval bodies resembling MCB and made of arrays of electron dense and lucent lines with a periodicity of 50 Å (Fig. 16). The outer lines of the stack were paler than the inner lines and would merge with pale lines from other stacks to form a single dense line as has been described in MCB. Cellular organelles were difficult to identify in these cells since these conglomerates filled up the entire cytoplasm and cell processes.

Vessels: The endothelial cells contained an abundance of ribosomes, endoplasmic reticulum and Golgi apparatus. Occasional dense lipid bodies were present. The mitochondria were normal.

The pericytes contained two different types of inclusions. The most abundant inclusions were ovoid lipid bodies measuring 0.2 to 0.5 μ. They were of even density but occasionally contained a clear round vacuole. These bodies were surrounded by a single membrane. Others had a round, dark and clear component. Both components were separated by several layers of membranes. The second type of inclusion, similar to zebra bodies, consisted of alternating dense and pale lines with a periodicity of 50 Å.

Myelin: Myelinated axons were generally of normal appearance although occasional degenerating myelin sheats were seen in the white matter.

D. Biochemistry

Enzyme Activities: Both serum and leukocytes of the patient had normal activities of β-galactosidase and N-acetyl-β-hexosaminidase, as well as of the proportions of hexosaminidase A and B components (Table 1). In view of the morphological and analytical findings, it should be stressed that there was no indication of N-acetyl-β-hexosaminidase abnormality in the patient's specimens when assays were carried out with artificial fluorogenic substrate. Leukocytes of the mother likewise showed no abnormality of the two lysosomal enzymes examined.

Brain Gangliosides: The water content of the biopsied brain tissue used for chemical analysis was 86% and the chloroform-methanol-insoluble residue constituted 57% of the total dry weight. These finding and the gross appearance

Table 1. β-Galactosidase and N-Acetyl-β-Glucosaminidase in Serum and Leukocytes

	4-methylumbelliferyl β-galactosidase	4-methylumbelliferyl N-acetyl-β-glucosaminidase	
		Total activity	Component A (%)
	(nmoles/hr/ml)		
Serum			
Patient	9.38	1488	52
Control 1	21.4	1125	54
Control 2	16.6	738	64
Leukocytes	(nmoles/hr/mg protein)		
Patient	91.4	988	64
Mother	119	850	51
Control	128	668	66

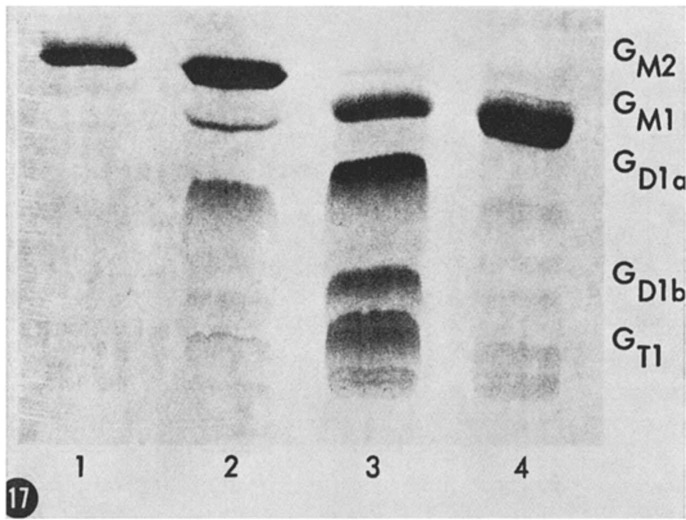


Fig. 17. Thin-layer chromatogram of brain gangliosides. Technical details are described in the text. Lane 1, G_{M2}-ganglioside standard from the brain of a classical Tay-Sachs disease patient (G_{M2}-gangliosidosis, variant B); Lane 2, brain gangliosides of this patient; Lane 3, normal brain gangliosides; Lane 4, gangliosides from the brain of a G_{M1}-gangliosidosis patient. The normal brain contains only a trace of G_{M2}-ganglioside while it is the predominant component in the patient's brain. The ganglioside nomenclature is that of Svennerholm (1963)

of the tissue suggested that this particular piece was mostly gray matter. The total ganglioside sialic acid was 2.63 mg/g wet weight. This is approximately 3—4 times that of normal control gray matter of similar age (Suzuki, 1966). Examination of the thin-layer chromatogram indicated unequivocally that a ganglioside, corresponding to G_{M2}-ganglioside from classical Tay-Sachs disease, was the predominant component in this specimen (Fig. 16). Therefore, the analytical data on brain gangliosides were essentially identical with those of classical Tay-Sachs disease (G_{M2}-gangliosidosis variant B). Coupled with the enzymatic findings above, this patient therefore has, by definition, G_{M2}-gangliosidosis, variant AB.

Discussion

From a clinical standpoint, the present case and the infants with the AB variant described by Sandhoff *et al.* (1971) and Kolodny *et al.* (1973) have similarities to and differences from children with classical Tay-Sachs disease. While Tay-Sachs disease occurs predominantly in the Ashkenazi Jews, all of these 3 cases of the AB variant were non-Jewish. The cases reported by Sandhoff *et al.* (1971) and Kolodny *et al.* (1973) were siblings in a family of Scottish-Irish-English-American Indian descent and the present case is a black infant. Seizures, myoclonus to sound, and a combination of signs of cortico-spinal and anterior horn cell involvements are seen in both Tay-Sachs disease (B variant) and AB variant, but clinical manifestations of the AB variant appear to be milder than those of classical Tay-Sachs disease. Children with classical Tay-Sachs disease

usually have severely regressed by 12 months of age, and they do not achieve the milestones noted in our patient such as walking in a walker and sitting without support for indefinite periods of time. While clinical details concerning the two siblings who also had the AB variant are not yet available, they had cherry red spots without optic atrophy and did not develop macrocephaly (Kolodny *et al.*, 1973). The boy was said to have visual fixation up to the age of two and the severity of the dementia was evidently in doubt (Sandhoff *et al.*, 1971a). Nevertheless, the siblings died aged 32 and 39 months respectively.

The light microscopic features of our case were closely similar to Tay-Sachs disease (Volk, 1974) and Sandhoff disease (variant 0) (Pilz *et al.*, 1968; Suzuki *et al.*, 1971) although cortical gliosis and neuronal loss were less pronounced. The previous report also indicated that in the AB variant, the cortical neuronal cell population was not significantly altered (Kolodny *et al.*, 1973). The storage material in both neurons and glia was strongly PAS positive, but only when the tissue had not been exposed to alcohol. The staining was most likely due to stored G_{M2}-ganglioside, as gangliosides contain carbohydrate groups and are known to be soluble in alcohol.

The acid phosphatase distribution in our case also differed from that reported for Tay-Sachs disease (Lazarus *et al.*, 1962). We observed a concentration of reactivity in the axon hillock rather than staining throughout the perikarya.

The electron microscopic features of the majority of neuronal inclusions in our case were identical to membranous cytoplasmic bodies (MCB) of Tay-Sachs (Terry and Weiss, 1963) or Sandhoff disease (Pilz *et al.*, 1968; Suzuki *et al.*, 1971; Fontaine *et al.*, 1973; Juif *et al.*, 1973; Vidailhet *et al.*, 1973), but some neurons in particular smaller ones, contained numerous, almost exclusively zebra bodies. In the electron microscopic studies of retinal ganglion cells, Kolodny *et al.* (1973) found MCB, membrane-bound vesicles and parallel arrays of lamellae. The most prominent feature in our case is the presence of varieties of inclusions in glial cells. Astrocytic inclusions have been described in Tay-Sachs disease (Terry and Weiss, 1963; Adachi *et al.*, 1971) but they were small and mostly resembled MCB. Fontaine *et al.* (1973) in their report of Sandhoff's disease described large astrocytic inclusions similar to those present in this case but varieties of large inclusions as seen in Figs. 8 and 9 have never been reported in classical Tay-Sachs disease or any other diseases characterized by an accumulation of MCB in neurons (Gonatas and Gonatas, 1965; Suzuki *et al.*, 1968b; Pilz *et al.*, 1968; Suzuki *et al.*, 1970; Volk *et al.*, 1969; Juif *et al.*, 1973). Such large inclusions were, interestingly enough, found almost exclusively in the strocytes in the cerebral cortex. The inclusions of astrocytes in the white matter were generally small and were similar to those found in G_{M1}-gangliosidosis (Gonatas *et al.*, 1965; Suzuki *et al.*, 1968b) and Sandhoff disease (Fontaine *et al.*, 1974). The difference in morphology of the inclusions in the cortical and white matter astrocytes has been also noted in other neuronal storage diseases (Suzuki *et al.*, 1968a). These differences may be a manifestation of the functional or metabolic differences of these two types of astrocytes.

From the enzymatic viewpoint, the AB variant of G_{M2}-gangliosidosis presents a very intriguing problem regarding hexosaminidase activities toward natural substrates. This is one area currently under intensive studies in several laboratories, and definitive conclusions have not been established. The most widely held view

perhaps is that both hexosaminidase A and B components can hydrolyze asialo G_{M2}-ganglioside and globoside, but that only the A component is active toward G_{M2}-ganglioside (Sandhoff and Wässle, 1971b). This will explain well the enzymatic and analytical findings in the classical Tay-Sachs disease (B variant) and Sandhoff's disease (0 variant). However, a recent report by Tallman *et al.* (1974) indicated that both hexosaminidase A and B can hydrolyze G_{M2}-ganglioside. This makes it difficult, although not impossible to explain these classical forms of G_{M2}-gangliosidosis. On the other hand, Bach and Suzuki (1975) reported that only the most acidic subfraction of hexosaminidase A component is active toward G_{M2}-ganglioside. If one postulates that only a small subfraction of hexosaminidase has G_{M2}-ganglioside-cleaving activity, one can explain the findings in juvenile G_{M2}-gangliosidosis (partial deficiency of hexosaminidase A and almost complete deficiency of G_{M2}-ganglioside hydrolysis) and in the AB variant (normal activity of hexosaminidase A and deficient G_{M2}-ganglioside-cleaving activity). Furthermore, this will also explain reported cases of healthy individuals with abnormal hexosaminidase activities. A few completely healthy relatives of patients with the B variant and the 0 variant of G_{M2}-gangliosidosis (presumed heterozygotes) have been found to lack hexosaminidase A (Vigdoff *et al.*, 1973; Navon *et al.*, 1973), and hexosaminidases A and B activity (Dreyfus *et al.*, 1975) against artificial substrates.

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