Fine structure of cutaneous nerves in ganglioside storage disease

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SUMMARY Skin punch biopsies of six children suffering from infantile or late onset Tay-Sachs disease, juvenile Sandhoff disease, or GM gangliosidosis type I, contained axons which, when viewed with the electron microscope, were distended by large amorphous black deposits. These are nonspecific residual bodies. Their large numbers indicate severe disturbance of the nerve cell and may be part of the dying back process. The three cases with Tay-Sachs disease had also axonal zebra or complex membranous bodies which appeared to be specific. Cytoplasmic vacuolation of other cells was a feature in the patient with GM₁ gangliosidosis. Biopsies of three parents were negative.

The usefulness of skin biopsies in the diagnosis of the neurovisceral ceroid lipofuscinoses was pointed out by Carpenter et al., in 1972. This method was later investigated in other storage diseases (Dolman et al., 1975, O'Brien et al., 1975). Andermann et al. (1975) reported negative skin and nerve biopsies in two Lebanese brothers with late onset GM₂ gangliosidosis. O'Brien et al. (1975) found sparse vacuoles in the epithelium of sweat glands and in endothelial cells in a biopsy specimen of skin of a child with GM₁ gangliosidosis, type I, but no changes in the axons. In a brain biopsy, on the other hand, Buxton et al. (1972) described membranous cytoplasmic bodies and dense granular material in myelinated axons in a 9 year old boy suffering from GM₂ gangliosidosis of late onset, and Patel et al. (1974) saw axons filled with amorphous granular debris and dense lamellar bodies in the cerebral cortex, and membranous cytoplasmic bodies in one myelinated axon in a spinal ganglion of a 6 year old boy who had died of GM₁ gangliosidosis, type 2. We shall describe the abnormal axonal deposits encountered in skin biopsy samples of six children with various gangliosidoses.

Methods

The skin of the deltoide region was infiltrated with local anaesthetic and biopsy samples taken with dermatological skin punches. One was used for fibroblast culture, the other was fixed in cold 2.5% buffered glutaraldehyde, cut into small pieces, washed in buffer, and embedded in plastic. Sections were cut 1 μm thick, stained with toluidine blue, and blocks containing myelinated and unmyelinated nerves prepared for electron microscopy.

Observations

CASE 1 INFANTILE GM₁ GANGLIOSIDOSIS (CLASSICAL TAY-SACHS DISEASE)

A boy developed psychomotor retardation, convulsions, and cherry-red spots at the maculae at 10 months of age. Hexosaminidase A activity was nearly absent in leucocytes and cultured fibroblasts. In a skin biopsy specimen, taken at 2 years, several axons contained abnormal inclusions. Some of these were 'zebra bodies', composed of parallel laminae of triple membranes (Fig. 1). More common were membrane-bound, electron-dense deposits, exhibiting little internal structure. These were especially prominent in nerve twigs in smooth muscle. Zebra bodies sometimes lay adjacent to dense bodies and numerous mitochondria were packed between them. A skin biopsy of the mother was negative.
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Fig. 1 Case 1. Infantile Tay-Sachs disease. Zebra bodies composed of triple layered membranes in cutaneous axon. ×219000.
CASE 2  LATE INFANTILE GM₂ GANGLIOSIDOSIS
A skin biopsy was obtained from a 4 year old boy with clinical and biochemical findings identical to
the first boy, only with onset at 2 years. Abnormal
inclusions were found in myelinated and un-
myelinated axons. Complex membrane-bound
zebra bodies, with individual bundles of parallel
lamellae arranged at angles to each other, lay
interspersed with electron-dense round bodies or
membranous cytoplasmic bodies with a dense
centre (Figs. 2, 3, 4). Mitochondria were closely
associated. Nerve fibres within smooth muscle
were again particularly affected and were striking
by their big black inclusions.
This child has recently died and portions of his
formalin-fixed brain were received for study.
Microscopically, the nerve cells throughout the
brain were distended by material which stained
blue in paraffin sections by Luxol fast blue and
deep pink in frozen sections by the periodic acid-
Schiff reagent. Under the electron microscope, the
stored material consisted of typical membranous
cytoplasmic bodies, zebra bodies, or mixtures of
the two. An occasional myelinated axon contained
similar material intermingled with amorphous
debri.

CASE 3  LATE INFANTILE GM₂ GANGLIOSIDOSIS
A 2 year old girl developed symptoms and signs
identical to the first two cases, and her fibroblasts
exhibited negligible hexosaminidase A activity. In
a skin biopsy taken at the age of 6 years, several
axons contained membrane-bound complex zebra
bodies and electron-dense, usually homogeneous,
only occasionally somewhat laminated structures
intermingled with a multitude of mitochondria.

CASE 4  JUVENILE SANDHOFF DISEASE
A skin biopsy sample was obtained from an 11
year old boy whose history dated back to age 4
years, when he was found to be retarded and from
then on developed increasing cerebellar deficit.
Hexosaminidase A and B levels were severely
reduced. Several cutaneous axons contained
numerous amorphous osmiophilic deposits, par-
ticularly in nerve twigs in smooth muscle (Fig. 5).
Occasionally, typical membrane-bound zebra
bodies were present in the cytoplasm of Schwann
cells (Fig. 6).

CASE 5  JUVENILE SANDHOFF DISEASE
A boy developed cerebellar symptoms at 4 years
and mental retardation at 6 years. Fibroblast cul-
ture showed nearly complete total hexosaminidase
deficiency. In a skin biopsy at age 10 years, the
axons of many unmyelinated nerve fascicles were
distended by multiple membrane-bound osmi-
philic inclusions. Most of these were unstructured
dense bodies (Fig. 7) and only occasionally had
some concentric lamellae which, however, did not
resemble true membranous cytoplasmic bodies. No
abnormalities were seen in Schwann cells, smooth
muscle, fibroblasts, or endothelial cells. A white
cell pellet prepared from theuffy coat of venous
blood fixed in glutaraldehyde failed to show any
abnormal inclusions.

CASE 6  GM₁, gangliosidosis, TYPE I
A 6 month old girl with psychomotor retardation,
visceromegaly, and cherry-red spots at the
maculae, was found to have a markedly reduced
blood level of beta galactosidase. The most striking
feature in a skin biopsy specimen was tremendous
vacuolation of endothelial and perithelial cells
(Fig. 8), fibroblasts, and smooth muscle. These
vacuoles were empty or contained only small
Fig. 3  Case 2. Late onset Tay-Sachs disease. Lipid body with triple membranes in unmyelinated cutaneous axon. ×80 000.
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Fig. 4  Case 2. Late onset Tay-Sachs disease. Complex lipid and small dense bodies in cutaneous axon. ×31100.

shreds of membrane. Marked vacuolation was also present in lymphocytes from the peripheral blood (Fig. 9). Only unmyelinated nerves were seen in the skin. Many of these were distended by osmiophilic dense bodies identical with those seen in the other children. Skin and blood samples of both parents were normal.

Discussion

All six children had abnormal skin. Vacuolation of cells is a well known feature in various tissues in GM1 gangliosidosis and renders the diagnosis easy, even with the light microscope. These vacuoles may represent the dissolved residuum of water soluble glucosaminagalactan (Lowden et al., 1973).

The inclusions in the axons could only be seen with the electron microscope, and were not visible in 1.0 µm section stained with toluidine blue. There were two types. In infantile and late onset Tay-Sachs disease, the zebra bodies and complex laminar lipid deposits encountered may be the ganglioside. Intermingled with these, much more prominent, and as the only inclusion in the cases of generalised gangliosidosis type 1 and juvenile Sandhoff disease, were osmiophilic dense bodies, usually without internal structure, but occasionally showing partial concentric lamination. Buxton et al. (1972) described in late onset GM2 gangliosidosis type 1, dense osmiophilic bodies in axons of the central nervous system and also dense osmiophilic granular debris. These authors considered these deposits to be part of the disease process. Adachi et al. (1974) in a study of eight fetuses afflicted with Tay-Sachs disease found in the ganglion cells of the retina electron-dense granular material which showed a transition to membrane-bound cytosomes and eventually to zebra bodies. Suzuki et al. (1970) found in the neurones of necropsy specimens of a girl suffering from juvenile GM2 gangliosidosis, a mixture of membranous cytoplasmic bodies, zebra bodies, irregular...
loose lamellar structures, dense granular material, membranovesicular bodies, and lipochrome. They felt that the huge conglomerates were quite unique in this condition.

They seem, however, to be identical with the amorphous deposits seen by us in the cutaneous axons of all our patients, and were much more prominent than the typical lipid inclusions. These lysosomal dense bodies are nonspecific. Some, but not all, resembled degenerating mitochondria. They were quite striking and were definitely abnormal. They probably indicate severe disease of the nerve cell. Terminal portions of the axons, such as intramuscular twigs, appear most prone to be laden with such debris, swept there by peripheral axoplasmic flow. These deposits may be an expression of the ‘dying back’ process.

Only a few specific lipid inclusions were seen in Schwann cells in one of the two patients with juvenile Sandhoff disease. This contrasts with the very generalised storage prevalent in the infantile type (Dolman et al., 1973) and thus conforms to the general pattern of inborn errors of metabolism, in which the disease, and with it any storage, tends to be milder, the later the age of onset. In keeping with this concept are also the negative findings in parents of the patients.

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References


