The ocular pathology of Type A Niemann-Pick disease
A light and electron microscopic study

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Niemann-Pick disease, a disorder of sphingomyelin metabolism due to lack of the enzyme sphingomyelinase, has been known for many years to cause storage of sphingomyelin and cholesterol in brain and abdominal viscera. A cherry-red spot has indicated retinal involvement, as well. The present pathologic findings in the eyes of a patient with Type A Niemann-Pick disease confirm recent clinical impressions of more widespread ocular involvement. Sphingolipid storage in corneal stroma and endothelium and lens epithelium offers an explanation for the subtle opacities seen in these structures clinically. The further involvement of iris sphincter muscle, ciliary epithelium, retinal pigment epithelium, and vascular endothelium has no recognized clinical counterpart, but is consistent with the normal wide distribution of sphingomyelin as a constituent of cytoplasm and cell membranes in animal tissues.

Key words: Niemann-Pick disease, eye pathology, pathology, lipidosis, sphingolipidosis, sphingomyelin lipidosis, electron microscopy, membranous cytoplasmic bodies, cherry-red spot.

In 1931, shortly after Niemann-Pick disease had been separated from Gaucher's disease as a distinct clinical entity,1 Goldstein and Wexler described their clinical and pathologic findings on the eyes of an infant who died of Niemann-Pick disease with neurologic involvement.2 Cherry-red spots were the only ocular abnormality recognized clinically, and the retinal ganglion cells were identified as the major site of lipid accumulation pathologically. Additional pathologic reports of ocular involvement have appeared and have conformed in most regards to this original description.2-5 In 1935, sphingomyelin was identified as the predominant phospholipid accumulating in brain and liver.6 The clinical spectrum of the disease was gradually clarified and a classification of four types of sphingomyelin lipidoses was proposed.7 This classification has now been broadened to five types to include adult patients not...
Fig. 1. Light microscopy of the retina. A, frozen section of retina adjacent to fovea, stained with hematoxylin and photographed with crossed polarizing filters. Birefringent material is evident in thick layer of ganglion cells (double arrow) and several amacrine cells (single arrow) of bipolar layer. Lesser amounts of birefringent material are in nerve fiber and inner plexiform layer and scattered sites at level of rod and cone inner segments. (x100.) B, frozen section of perifoveal retina stained with Sudan Black B, showing lipid in ganglion cells (single arrow) and to a lesser extent in inner plexiform layer, synaptic portion of outer plexiform layer, and inner segments of rods and cones. (x100.) C, paraffin-embedded section of peripheral retina stained with hematoxylin and eosin, showing distended ganglion cells (double arrow) and amacrine cells (single arrow). (x160.)

clearly belonging to the earlier categories.8 In 1966, Brady and co-workers9 identified a deficiency of sphingomyelinase activity in the Type A disease, and this was confirmed and extended to the Type B disease shortly thereafter.10 Enzyme levels in a few patients with the remaining three types of sphingomyelin lipidosis have not been markedly reduced.8

In the classical form of Niemann-Pick disease, now referred to as Type A, with hepatosplenomegaly, foam cells in the bone marrow, and central nervous system involvement, about half the reported cases have had recognized cherry-red spots.11 Beyond this, "a peculiar clouding of the cornea in some patients" has been mentioned but not further documented.12 A recent report of clinical findings in the eyes of four patients with Type A disease has pointed out a striking combination of cherry-red spots with subtle corneal and lenticular opacities, distinctive enough to allow at least tentative clinical diagnosis.13 The opportunity to examine pathologically the eye of a patient with Niemann-Pick disease offered the possibility of identifying the nature of this more widespread ocular involvement on an ultrastructural basis. No prior electron microscopic study of the eye of a patient with Niemann-Pick disease is available, although such studies of abdominal viscera and the central nervous system are well documented.14-18

Materials and methods

The eyes studied were those of a 21/2-year-old male, first noted to have hepatosplenomegaly at age six months. Biopsies of bone marrow and liver at that time were consistent with Niemann-Pick disease. The patient gradually became obtunded and eventually lost voluntary movement and developed flexion contractures of his limbs. He had bilateral cherry-red spots, but at the time of his terminal hospitalization pupillary responses to light were still present. At that time an ophthalmologic consultant did not consider his optic discs pale. No other ocular abnormalities were recognized, although examination was difficult because of the patient's depressed mental status and precarious cardiorespiratory condition. Ascites and oliguria developed shortly before the patient died. An autopsy was performed approximately four hours after death. The hepatosplenomegaly and ascites were confirmed. There was infiltration of liver, spleen, lymph nodes, adrenals, bone marrow, and lungs by foam cells. In the brain there was thinning of cortical gray matter grossly and neuronal accumulation of lipid microscopically. The eyes were removed and opened before fixation in order to excise the macular areas. One eye was then fixed in 10 per cent neutral formalin and the other in four per cent glutaraldehyde, the latter being switched to formalin after 24 hours. The eyes were recovered in this state approximately
Biochemical studies on the patient revealed virtual absence of sphingomyelinase activity in liver, spleen, urine, and cultured skin fibroblasts. Total phospholipids in the liver were 126.5 mg per gram wet weight (normal, 25 ± 2.7) of which 65 per cent was sphingomyelin (normal, 7 ± 1.4 per cent). The cholesterol in liver was 19.9 mg per gram wet weight (normal, 3.9 ± 0.8).

The formalin-fixed material was used for frozen sectioning, followed by staining with Mayer's hematoxylin, Baker's acid hematein, Oil Red O, Sudan Black B, or the periodic acid-Schiff reaction. Other portions of the same material were dehydrated in graded ethanols, embedded in paraffin, sectioned, and stained with hematoxylin and eosin for light microscopy. The glutaraldehyde-fixed material was cut into small blocks and placed in 1 per cent osmium tetroxide for two hours, then dehydrated and embedded in epoxy resin. One micron sections were examined by light microscopy after staining with 1 per cent toluidine blue. Thin
sections were cut, stained with uranyl acetate and lead citrate, and examined with the electron microscope.

**Results**

The paraffin-embedded material revealed abnormalities only in the retina. Ganglion cells were distended by a granular eosinophilic material which in many areas was partially lost in processing (Fig. 1, C).

In spite of their swollen condition the ganglion cells appeared to be present in normal numbers and the nerve fiber layer was of normal thickness (Fig. 1, A and B). Amacrine cells at the inner border of the bipolar cell layer were also distended and had vacuolated cytoplasm (Fig. 1, C). In frozen sections of the formalin-fixed material both ganglion and amacrine cells

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**Fig. 4.** Portion of retinal ganglion cell showing numerous MCB. Mitochondria (m) and dilated endoplasmic reticulum (er) are also evident. The area outlined in lower right is shown in greater magnification in Fig. 5. (×15,000.)
revealed marked granular cytoplasmic birefringence by polarization microscopy (Fig. 1, A). This same birefringence was also present to a lesser extent in inner plexiform and nerve fiber layers, and minimally in the inner segments of rods and cones.

Frozen sections of other portions of the eye revealed a remarkably wide distribution of the material giving the granular birefringence. Corneal stromal cells were markedly positive (Fig. 2) as were corneal endothelium and lens epithelium. Corneal epithelium had only the slight linear birefringent quality normally present due to its fibrillar cytoplasm. Occasional conjunctival epithelial cells had granular birefringence, but vascular endothelium, nonpigmented epithelium of ciliary body, and retinal pigment epithelium were uniformly and

Fig. 5. Enlargement of a portion of cytoplasm of ganglion cell shown in Fig. 4. MCB cut in several different planes are evident, as are mitochondria (m) and endoplasmic reticulum (er). (X42,000.)
Fig. 6. Retinal pigment epithelial cell containing numerous MCB with lamellar material cut in various planes. What appear to be single lamellae at this magnification are actually groups of several osmiophilic and osmiophobic lines (see Fig. 8). Mitochondria (m), pigment granules (pg), and phagocytized outer segment material (os) are easily distinguishable from the MCB. (x15,000.)

markedly positive, while ciliary muscle, lens fibers, and pigmented epithelium of iris were completely negative.

Various stains were used to further characterize the storage material. Sections of the retina were studied most extensively because of their abundant cytoplasm which is not masked by collagen fibers or melanin pigment. The birefringent cytoplasmic areas were mildly positive with Baker’s acid hematein, Oil Red O, Sudan Black B (Fig. 1, B), and periodic acid-Schiff. One micron sections of epon-embedded material showed the cytoplasmic vacuoles in corneal stromal cells and lens epithelium more clearly, and in retinal ganglion cells
the same finely granular material was present as had been seen in paraffin-embedded tissue. Toluidine blue did not stain the storage material differently from other cytoplasmic structures.

Thin sections examined with the electron microscope showed the storage material to be present in the form of membranous cytoplasmic bodies (MCB). These inclusions were bound by single membranes and had a predominantly lamellar architecture. Their shape was round or oval and in size they varied from approximately 0.2 to 3.0 μ. Occasionally a larger inclusion seemed to result from a coalescence of several smaller ones. Their distribution was similar to that of the birefringent material seen by light microscopy. Thus, retinal ganglion cells (Figs. 4 and 5) and retinal pigment epithelium (Fig. 6) were heavily involved. Corneal stromal cells (Fig. 3), lens epithelium (Figs. 7 and 8), and corneal endothelium (Fig. 9) had moderate numbers of MCB, as did vascular endothelium and sphincter muscle of iris. In the retina, beyond the heavy involvement of

**Fig. 7.** Lens epithelium with relatively well-preserved MCB and mitochondria (m). Lens capsule (lc) is at upper left and cortical lens fiber at lower right. Rectangular area enclosing MCB is enlarged in Fig. 8. (×17,000.)
Fig. 8. MCB of lens epithelial cell showing single trilamellar membrane encircling storage material. The alternating osmiophilic (dark) and osmiophobic (light) lines have a periodicity of 55 to 60 Å. (x75,000.)

ganglion and amacrine cells, the basal portion of Müller cells, scattered glial cells, and rod and cone inner segments (Fig. 10) all contained some MCB.

The lamellar material in the MCB varied slightly from one tissue to another. In the retinal ganglion cells it appeared more swirled and regular, whereas in glial and Müller cells and retinal pigment epithelium it was irregular, clumped, and vacuolated. Within any one cell variations in the MCB could be observed depending on the orientation of the lamellae to the plane of section (Fig. 5). In lens epithelium the lamellar arrangement was especially well preserved (Fig. 8) and the alternating electron dense and lucent lines were easily seen. The periodicity in these lamellae was 55 to 60 Å. In the somewhat less orderly MCB of retinal pigment epithelium the periodicity was about 65 Å. Despite these variations the MCB in different parts of the eye had basic similarity of structure. They could be readily distinguished from mitochondria, dilated endoplasmic reticulum, melanosomes, and phagocytized rod outer segments. There was no increase in the number of lysosomes, apart from the lipid stored in brain and viscera of other patients with Niemann-Pick disease. The periodicity of the lamellar material from an adult patient was found to be 47 Å by Lynn and Terry, somewhat smaller than our measurement of 55 to 65 Å. A similar period has been found for the membranous inclusions of Tay-Sachs disease but the fine structure is slightly different. The MCB from liver, spleen, and brain of a patient with Niemann-Pick disease have been isolated by sucrose gradient and their chemical composition agrees well with earlier data on the abnormal elevation of lipids in portions of whole liver. Morphologic similarity of these isolated MCB to those in the various ocular tissues of our patient suggests by analogy that the abnormally stored material is primarily sphingomyelin and cholesterol. It has been proposed that the alternating osmiophilic and osmiophobic lines of the MCB are related to a regular orientation of the nonpolar (ceramide) and polar (phosphoryl choline) ends of the sphingomyelin molecule. Variations in the fine structure from one tissue to another might be accounted for by known variations in the structure of the long-chain fatty acids in the ceramide portion of the molecule. Since sphingomyelins normally are widely distributed in plasma, cytoplasm, and cell membranes, it is not surprising that a disorder in the catabolism
of this group of substances would result in widespread accumulation of MCB. They have been previously reported in vascular endothelium, renal podocytes, neurons, and Schwann cells, in addition to their better known presence in histiocytes of liver, spleen, and bone marrow and ganglion cells of brain. There is nothing to suggest the presence of abnormal sphingomyelins in Niemann-Pick disease. It is known that the major constituents of myelin in the central system are in their usual proportions.

Since several disorders of lipid metabolism with ocular manifestations are now known, it is instructive to compare some pertinent aspects of their morphology and biochemistry. Tay-Sachs disease is the most common cause of a cherry-red spot in the retina, but it has not been noted either clinically or pathologically to involve extraretinal portions of the eye. Early loss of vision, disruption of ganglion cells, and optic atrophy are characteristic features of Tay-Sachs disease, whereas patients with Niemann-Pick disease usually retain some vision as well as normal numbers of ganglion cells and nerve fibers. Less intense cherry-red spots have been observed in a number of other sphingolipidoses and mucolipidoses. Presumably, the rate of accumulation of lipid in these disorders is slower than in Tay-Sachs or Niemann-Pick disease. For example, in metachromatic leukodystrophy the laminated inclusion bodies in retinal ganglion cells account for only a slight increase in perifoveal haze before optic atrophy sets in to eventually eliminate vision. In Farber's lipogranulomatosis a subtle peri-
foveal opacification has been observed and was found to be due to glycolipid accumulation in ganglion cells.\textsuperscript{26} No ultrastructural studies of the eye in this condition have been reported. In mucolipidosis I\textsuperscript{27} and lactosyl ceramidosis,\textsuperscript{28} in some patients with a juvenile form of cerebral degeneration,\textsuperscript{29-31} and in a still unclassified mucolipidosis\textsuperscript{32} cherry-red spots have been reported, but no ocular pathology is available. In the systemic mucopolysaccharidoses, on the other hand, no cherry-red spot has been observed clinically, but in Type II (Hunter’s syndrome) membranous lamellar inclusions have been observed histologically in retinal ganglion cells,\textsuperscript{33} suggesting glycolipid accumulation. The combination of both mucopolysaccharide and sphingolipid storage in the same disease is also well established in \(G_{M1}\) gangliosidosis, in which absence of a beta-galactosidase results in accumulation of a ganglioside in the central nervous system and retina and of keratan sulfate in abdominal viscera and cornea.\textsuperscript{34} Although the clinical picture of corneal haziness and a cherry-red spot in this disease is similar to that in Niemann-Pick disease, the varied nature of the storage material in different tissues is in contrast to the uniformity of Niemann-Pick deposits. Fabry’s disease is the only other sphingolipidosis in which both corneal and lens opacities have been demonstrated to be due to accumulation of lipid material. In Fabry’s disease, however, the corneal opacities are superficial clinically and MCB are found histologically only in corneal epithelial cells.\textsuperscript{35, 36} The clinical appearance of the lens in the two disorders is quite different.\textsuperscript{17, 37} Retinal
involvement is not usually a part of Fabry’s disease. Finally, no primary ocular accumulation of lipid is known to occur in either Gaucher’s disease or Krabbe’s disease, two sphingolipidoses in which the storage material takes on a tubular rather than lamellar configuration. Patients with Krabbe’s disease develop optic atrophy, but this is thought to be due to a defect in myelination of the optic nerve. The ultrastructure of the eye in Gaucher’s disease has not yet been reported.

We presume that the corneal stromal and endothelial involvement in Niemann-Pick lipidosis accounts for the corneal haziness seen clinically and that the lens epithelial involvement gives rise to the granular opacity seen on the anterior lens surface. This would be in the same fashion that retinal, ganglion cell involvement causes opacification of the normally transparent perifoveal retina. No functional disturbance appears to result from retinal pigment epithelial storage of lipid or from lipid accumulation in the scattered ocular sites herein described.

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REFERENCES