Menkes' kinky hair disease: a light and electron microscopic study of the eye

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Light and electron microscopic studies of the ocular tissue of a case of Menkes' kinky hair disease are described. The copper deficiency responsible for this systemic and neurologic disease appears to cause a progressive degeneration of retinal ganglion cells, loss of nerve fibers, and optic atrophy. The pigment epithelium is also abnormal with only small and irregular melanin granules present among electron-dense inclusion bodies. Abnormal elastica is present in Bruch's membrane.

Key words: Menkes' kinky hair disease, ocular pathology, electron microscopic study
Menkes' disease, retinal ganglion cell degeneration.

In 1962, Menkes and co-workers1 described a sex-linked recessive hereditary disease characterized by early progressive psychomotor deterioration, seizures, spasticity, hypothermia, pili torti, and characteristic facies. Later studies2++ of this disease demonstrated bone changes resembling scurvy and tortuosity of cerebral arteries due to fragmentation of the internal elastic lamina. Similar vascular changes were also found widely in other arteries2 occasionally accompanied by occlusion. Menkes attributed the disease to a metabolic defect whose site he was unable to localize.

In 1966, O'Brien and Sampson2 performed lipid analyses on frozen brain tissue in two siblings with kinky hair disease and found significantly lower values in the proportions of docosahexaenoic acid, the most highly unsaturated fatty acid in the brain, in the glycerophosphatides from cerebral gray matter. This finding raised the possibility that the pathogenesis of the disease might be due to the accumulation in neurons of lipid oxidation products which may inhibit the mitochondrial and microsomal enzyme system. Additional support for this hypothesis had already come from Aguilar and co-workers3 who found sudanophilic, acid-fast, and periodic acid-Schiff positive inclusions in the cytoplasm of the Purkinje cells.

A new approach to the etiology resulted from the *Department of Neurology, Massachusetts General Hospital; Howe Laboratory of Ophthalmology, Massachusetts Eye and Ear Infirmary, Boston, Mass. and the **Laboratory of Vision Research, National Eye Institute, National Institutes of Health, Bethesda, Md.

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when Danks and co-workers\textsuperscript{5,7} noting that copper-deficient sheep have abnormal wool and similar arterial changes to the children with Menkes' kinky hair disease, investigated copper metabolism in seven affected babies. They found abnormally low levels of serum copper, copper oxidase, and ceruloplasmin, and a defect in the intracellular transport of copper in the gut epithelium and the release of copper from these cells into the blood. He attributed the defect in biogenesis of elastin and collagen to a chronic copper deficiency state reminiscent of the widespread connective tissue disease in copper-deficient animals since copper, in the enzyme amino oxidase, is needed to form the cross-linkages between lysine residues of elastin. A decreased activity of cytochrome oxidation has also been demonstrated in kinky hair disease and an inadequacy of cytochrome oxidase may result in a lack of high-energy phosphate required for nerve cell maintenance and function. Copper also has an important role in myelin formation.\textsuperscript{8} Thus, the extent to which each of these biochemical pathways contributes to the neurologic deficit and to the pathologic changes is under extensive study.

Various therapeutic regimens have been tried to correct the copper deficiency. Recently, Lott and co-workers\textsuperscript{11} have reported some success following the administration
of high-dose oral copper supplement in the presence of L-histidine.

Studies in Australia have shown that Menkes' disease is not rare and the clinical, neuropathologic, and biochemical features have already been reported extensively in the literature. Nevertheless, the eye has received little attention. Neither Menkes and co-workers nor Bray noted any abnormality of the fundus in their cases even though the infants failed to follow a light, or showed constant horizontal nystagmus. Other observers report a normal appearance of the retina apart from possible mild optic disc pallor or the suggestion of tortuosity of the vessels. In three additional cases no eye examination is described. Billings and Degnan recorded an electroretinogram (ERG) in their patient who they suspected was blind. The fundi were unremarkable but the infant, at age 13 months, failed to fix or follow objects or respond to menace. The ERG showed moderately decreased photopic beta-waves (a measure of cone function) and almost no scotopic beta-waves (a measure of rod function) bilaterally. With a flash stimulus almost no visually evoked response (VER) was recorded over the occiput. Similar results have been documented in another case and an attempt made to correlate the abnormal ERG with serum copper levels. An absence of improvement in visual function after elevation of the serum copper to normal was noted. To our knowledge the eyes in these cases have not yet been examined histologically.

Reports of the histopathologic changes in the eye in Menkes' disease have in fact been sparse and confined to light micro-
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Case Report

G. B. (NEI: LVR E208), a white boy 3.3-years-old was the 5 pound, 14 ounce product of an eight-month pregnancy. He was the youngest of seven children. He had five normal siblings, aged 4 to 14 years, and one male sibling who died 10 years earlier at the age of 16 months with Menkes' disease.

The infant's early development was slow and frequent seizures began at age two months. Initially these consisted of intermittent twitching of the left-sided limbs, rolling back of the eyes, cyanosis, and salivation. Each seizure lasted only a few minutes. An electroencephalogram (EEG) showed random high voltage 2 to 3 cycles per second activity over the entire left hemisphere and random sharp waves over the left temporal lobe. He was treated with phenobarbital. The patient had extremely light, sparsely distributed, coarse stubbly hair and eyebrows. The face was pale with puffy cheeks and micrognathia. No bony abnormalities were noted.

By age five months mental retardation was evident. A biopsy of the right cerebellum and right posterior temporal tip was performed and revealed marked loss of neurons and intense astroglisis. The serum copper level was 20 mcg. per cent (normal 75 to 160), ceruloplasmin 2.0 mg. per cent (normal 15 to 35), urinary copper 18.5 mcg. per 24 hours. Blood amino acids were normal.

The neuropathologic study together with the evaluation of the serum and urine copper levels established the diagnosis of Menkes' kinky hair disease and the patient received parenteral administration of trace elements including zinc, copper, magnesium, and iodine. After several weeks of therapy the serum copper level was 20 mcg. per cent, ceruloplasmin 20 mg. per cent. The hematocrit ranged between 35 and 41 per cent.

Fig. 4. Ganglion cells in the foveal zone. A, relatively normal cell. Mitochondria are markedly swollen. B, degenerating ganglion cell. Nucleus is karyolytic. Rough endoplasmic reticulum is swollen (*). Both x16,500.
The neurologic signs of a progressive degenerative disorder continued unchecked. Angiographic study of several arteries showed minute changes which were considered to be secondary in nature.

At age 2.10 years, five months before death, a neuro-ophthalmic examination was performed. The eyes showed the characteristic roving movements of a blind child. The patient did not blink or cry in response to bright light or to menace and failed to fix on or follow a light or toy. No nystagmus could be elicited with the optokinetic drum. The irides were blue and did not transilluminate. The cornea and lens were transparent. The pupils were equal and only just reactive to light, equal bilaterally. Roving eye movements showed a full range of ocular motility and there was normal horizontal ocular deviation on rotation. There was no nystagmus. The fundus showed a blond appearance similar to that of albinism with prominent visibility of the choroidal vessels. The macula had a normal foveal reflex but slight mottling of the pigment epithelium was present just temporal to the fovea in both eyes. The retinal blood vessels were normal. The optic nerves showed marked optic atrophy. Both the discs were normal in size and configuration, uniformly pale, and almost chalk white in color. In contrast the retina of the posterior pole looked a “healthy pink” suggesting widespread loss of nerve fibers from the nerve fiber layer.

Pathologic study. The eyes were enucleated about five hours after the death of the patient. One eye was fixed in 10 per cent neutral formalin for general histologic study. The disc areas were stained with hematoxylin-cosin, periodic acid-Schiff reaction and Bodian’s combined silver, and Luxol fast-blue staining. A small portion of the posterior retina was embedded in gelatin and the frozen sections stained with Oil red O. The other eye was opened at the pars plana with a small slit cut and fixed in 4 per cent glutaraldehyde solution at room temperature for a few days. Small pieces of the cornea, trabecular meshwork, ciliary body, optic nerve, and several locations of the retina were excised. They were postfixed in 1 per cent osmium tetroxide for one hour, dehydrated in ethyl alcohol, and embedded in an epoxy resin. Sections were cut 0.5 μ thick, stained with toluidine blue, and examined light microscopically. The ultrathin sections were stained doubly with uranyl acetate and lead citrate and examined by electron microscopy.

Histologic findings. The size of the globe and proportions of each component of the eye are normal. The cornea, sclera, and ciliary body appear to be normal. The iris epithelium is markedly vacuolated, apparently due to intercellular separation. The lens tissue at the bow and the anterior zone is moderately vacuolated. The lens epithelium and the capsule show no abnormality. The angle and the trabecular meshwork also appear to be normal except for some pigmentation in the trabecular cells. Although the retina shows normally formed layers and foveal structure, the number of ganglion cells are markedly decreased. This is strikingly evident in the macula zone where only a single cell layer of ganglion cells is preserved (Fig. 1). Numerous glia cells are present in the ganglion cell and nerve fiber layers. The surviving ganglion cells are vacuolated but no pathologic substance has accumulated. The pigment epithelial cells are irregular in size and appear less pigmented than normal. The choroidal tissue is free from infiltrating cells. Both retinal and choroidal blood vessels appear to be normal histologically. However, flat preparations of the retinal vessels, in the general area, show numerous acellular strands (Fig. 2).

The optic nerve is atrophic. The disc surface is covered with proliferated glia cells and the septal connective tissue is considerably thicker. A myelin stain reveals marked demyelination of the nerve particularly in the peripheral bundles,
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Fig. 6. Optic nerve. Number of the myelinated fibers is markedly reduced. Myelin sheaths of individual fibers are thinner than normal. Some are degenerating. Microtubules are absent in the axon. A nucleus of the astrocyte is seen in the lower left corner. The astrocytic cytoplasm is markedly increased. ×8,000.

and a Silver stain shows considerable loss of nerve axons. The remaining nerve fibers show a bead-like structure. Glia cells are increased profoundly in both the intraocular and retrobulbar zones. No fat droplets have accumulated in the cells of the optic nerve. The changes in the optic nerve are subsequent to the death of the retinal ganglion cells.

Electron microscopy. Since the material was obtained a few hours after the death of the patient and stored in the fixative for a prolonged time, preservation of the cytologic detail is not ideal. The retinal tissue shows a considerable degree of postmortem change with swollen mitochondria and vacuolation in many cells. However, several meaningful findings are observed in the eye.

As the ganglion cells have decreased, the inner layers of the retina are replaced by increased cytoplasm of Müller's cell (Fig. 3). The remaining nerve fibers contain fine neurofibrils instead of microtubules. The surviving ganglion cells in the macula zone still maintain characteristic microorganelles. However, their mitochondria, round endoplasmic reticulum, and Golgi apparatus are markedly swollen, and some cells also show degenerating changes (Fig. 4, A). The cytoplasm of these cells is edematous and the nucleus is karyolytic (Fig. 4, B). Although cell components in the inner plexiform layer are relatively well preserved, no synaptic organs are demonstrated in the sections. Also the outer synaptic organs are poorly preserved. Cone synapses have changed into large bodies containing amorphous substance. The pedicles have no interdigitating horizontal cell components, synaptic vesicles or ribbons. Nuclei of the photoreceptor cells are relatively well preserved and their number appears to be within the normal range. Many mitochondria of the inner segments are markedly swollen (Fig. 5). The rod outer segments are mostly disarrayed but their individual disc membranes appear to be normal. The cone outer segments, especially in the macula zone, show irregularly arranged membranes. The retinal capillaries seem to be normal, but some vessels have a slightly tortuous basement membrane with uneven thickness. Their endothelial cells show an increased number of rough endoplasmic reticula. The cytoplasm of the mural cells of these capillaries is considerably electron dense.

The optic nerve is atrophic. The number of myelinated nerve fibers is markedly reduced. The tissue is occupied by astrocytes which have increased their number. The remaining nerve fibers have relatively thin myelin sheaths and the micro-
Pigment epithelium in the posterior zone. The cytoplasm contains numerous electron dense inclusion bodies. Only a few melanin granules are seen in this area. Appearance of the micro-organelles is normal. ×10,000.

Fig. 7.

Bruch's membrane in the posterior zone. Elastica is small and scattered irregularly (arrows). Fine fibril substance is forming banded masses. ×18,000.

Fig. 8.

The pigment epithelial cells show reduction of melanin granules. Large spindle-shaped melanin bodies, which are commonly present in the apical portion of the cell and in the microvilli, are absent. Only small and irregular melanin granules are present among electron-dense inclusion bodies (Fig. 7). Lipofuscin particles are also abundant. Mitochondria contain small electron-dense particles within the matrix. Other micro-organelles appear to be normal. Apicolateral junctions are normal and the epithelial cells are firmly attached to each other. The finding of sparse fine microvilli may be due to postmortem change. Vesicular substance, which is present in the sub-
Fig. 9. Higher magnification of Bruch's membrane in the posterior zone. Banded masses show 1,000 Å spacing. Elastica is small and sparse. ×48,000.

retinal space, appears to be broken pieces of the fine microvilli of the pigment epithelium. The basal infoldings are somewhat widened, but the basement membrane is uninterrupted.

The structure of Bruch's membrane is abnormal. The most striking change is the scanty and irregular distribution of the elastic membrane (Fig. 8). Only small pieces of well-formed elastica are present within Bruch's membrane. The distribution and number of collagen fibers appear to be normal. Fine fibrils, which are normally seen around the elastic membrane, are irregularly distributed. They often aggregate into banded masses (Fig. 9). These bandings are identical to those of 1,000 Å collagen fibers, which are seen regularly in Bruch's membrane in senescence. The basement membrane of the choroidal endothelium appears to be normal. However, there are several locations where the basal portion of the endothelial cell extends into Bruch's membrane.

The corneal epithelium, Bowman's membrane, Descemet's membrane, and the major part of the stroma appear to be normal. However, the superficial stroma, including the central zone, show several small foci in which lamellar arrangement of the collagen fibers is irregular and fine fibrils have been increased (Fig. 10). These findings are similar to those of the normal limbus or of a scar. No deposit of abnormal substance is present in Descemet's membrane. The trabecular meshwork is normally developed. The trabeculae contain numerous well-developed elastic tissue which is surrounded by fibrils. Normal collagen fibers are scanty in the trabecula (Fig. 11). Melanin particles and other inclusion bodies are present abundantly in the trabecular endothelium. The Schlemm's canal is also well developed. The endothelial cells show their normal giant vacuoles (Fig. 12). No appreciable changes are noticed in the ciliary body.

Discussion
The present case shows all the clinical and pathologic characteristics of Menkes'
kinky hair disease. As Danks and associates have shown, this disease is believed to be caused by a generalized copper deficiency in the body and the low copper levels in cells and tissue fluid appears to seriously interfere with certain enzyme systems and the maintenance of neural cells and hair.

Light and electron microscopic studies of the ocular tissue of this case have demonstrated interesting findings. The developmental process of the eye appears to be normal, since the size of the globe, thickness of the retina, and the diameter of the dura mater of the optic nerve are normal for the age of the patient. The most striking pathologic change is a degeneration of the ganglion cells with loss of nerve fibers and atrophy of the optic nerve. The ganglion cell appears to degenerate first and signs of degeneration are seen in some remaining ganglion cells of the macula zone at the time of death. The pathologic process seems to be slowly progressive and the necrotic cell debris has been carried out from the retina tissue so that no accumulation of specific substance is noted within the retina and optic nerve. Also no phagocytic or inflammatory reaction is present in the eye. These findings are somewhat different from those of the central nervous system.

The mitochondria of the surviving ganglion cells, photoreceptor inner segments, and of other neural cells of the retina are...
markedly swollen and an electron-dense substance is present in the matrix of the mitochondria in the pigment epithelium. These mitochondrial changes may be significant in light of the demonstration of the deficiency in cytochrome oxidation in this disease but, since swelling of mitochondria is the most common postmortem change, it is difficult to definitely correlate these findings to a dysfunction of the cytochrome oxidation system.

The photoreceptors are not involved directly in this disease. Lamellar membranes of the outer segments as well as the main photoreceptor cells are well preserved. Marked swelling of the synaptic pedicles may be due to postmortem change.

The metabolic deficiency of copper appears to have a direct effect on maintenance and structure of hair since tyrosinase, a copper containing enzyme, is needed for the synthesis of melanin. Similarly, copper may play a role in melanin formation in other pigmented cells. The distribution and amount of melanin granules of the pigment epithelium are uneven. The cytoplasm contains smaller and irregularly shaped melanin particles. Numerous electron-dense inclusion bodies and lipofuscin granules are abundant in the cytoplasm. These changes seem to correlate with the pathologically pale funduscopic appearance in this child. The abnormal pigmentation in the trabecular meshwork may be due to phagocyosis of degenerated melanin pigments within the eye.

The changes in Bruch’s membrane are interesting and noteworthy. The elastic membrane of Bruch’s membrane is well developed at the time of birth. Although it is not clear whether the pigment epithelial cell or endothelial cell of the choroidal capillary is involved, the elastica formation of Bruch’s membrane of this case appears to be considerably suppressed. The amount of the elastica is significantly small and it forms small patches. The fine fibrils which are normally present around the elastica aggregate into large banded structures. Pathologic changes of the elastic tissue of the blood vessels have been reported in other organs and it seems that a similar pathogenetic process in elastica formation may be occurring in Bruch’s membrane. The retinal capillaries show slight pathologic changes and the flat preparations demonstrated several acellular strands and an uneven caliber of the vessels. These changes may however be nonspecific.

Changes in the cornea may also be nonspecific with scarring secondary to exposure. Although the lamellar arrangement is not quite normal in general, the corneal collagen fibers appear to be well developed.
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REFERENCES


