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Corneal Opacity in LCAT Disease

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Deficiency of lecithin:cholesterol acyltransferase, LCAT disease, is one of the dyslipoproteinemias with characteristic lipid deposits in the cornea. The present report documents the clinicopathologic abnormalities of one case in which a full-thickness corneal specimen was obtained at the time of corneal transplantation. The characteristic clinical abnormality was a progressive corneal opacification with a peripheral arcus that extended into the sclera. The pathologic abnormality consisted of vacuoles prevalent in the anterior corneal stroma by light microscopy and containing extracellular, membranous deposits by electron microscopy. These observations confirm and supplement the previous six pathologic reports of corneal changes in LCAT disease and demonstrate, for the first time, histopathologic evidence of unesterified cholesterol in the corneal stroma of LCAT disease.

Key Words: Lecithin:cholesterol acyltransferase deficiency—Progressive corneal opacity—Dyslipoproteinemia—Glare effect—Anemia—Renal disease—Unesterified cholesterol in tissue—Free lecithin in tissue.

Lecithin:cholesterol acyltransferase (LCAT) is the enzyme responsible for esterifying plasma cholesterol. Its absence is characteristic of a genetic syndrome, first described by Norum and Gjone (1) and now variously known as: classical LCAT deficiency, familial LCAT deficiency, or simply LCAT disease. It has been described in approximately 50 patients, predominantly from Scandinavia. The pedigrees, illustrated by that in an American cohort (2), point to an autosomal recessive form of transmission.

Progressive deposition of lipids in the cornea is one of the cognate signs of the syndrome. A resultant glare effect may be an incapacitating symptom, but visual acuity is impaired to only a slight degree. Other findings are anemia, renal disease, and a predominance of unesterified cholesterol in blood and an accumulation of unesterified cholesterol and lecithin in tissues.

The corneal opacity begins at an early age and is characterized by a diffuse cloudiness of the corneal stroma. It is most marked in the periphery, thus simulating an age-related arcus (senilis), but unlike this arcus, it tends to extend to the limbus without the sharply demarcated clear interval characteristic of the usual arcus. Chlorioretinal lesions were described in two patients (3).

The literature is replete wth analysis of the biochemical and physiologic properties of the enzyme and its association with high-density lipoprotein (HDL). The literature also contains clinical reports documenting the corneal opacity (1–6) and reports of the pathologic changes in the corneas of five additional patients (7–10). We are also aware of two unpublished cases (K. Kenyon, unpublished observations; H. Perry, unpublished observations). These pathology studies have shown prominent vacuoles and membranous particles in the corneal stroma.

The present article documents the clinicopathologic observations on an additional patient (the eighth) whose vision was sufficiently reduced to warrant surgery. This is the third reported patient to have had a full-thickness graft. Our findings add to that of others by emphasizing the preferential localization of vacuoles in the anterior one-half of the cornea and, for the first time in LCAT disease, by demonstrating histologically the presence of unesterified cholesterol throughout the corneal stroma.

CASE REPORT

The patient was a 55-year-old woman, of Scottish–Irish descent, who was first noted to have cloudiness of the corneas at 23 years of age, when

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she complained of difficulty in night driving (presumably due to glare). Corneal opacities increased with time. Ophthalmic examination while in her late 30s suggested a dystrophy of undetermined type. Later a medical examination disclosed a hypocholesterolemia (113 mg/dl), a cholesterol/cholesteryl ester ratio of 4.8 (nl, 0.7), and an Apo A-1 level one-third the normal value, leading to the diagnosis of LCAT deficiency. Lipoprotein cholesterol levels were as follows: HDL, 9 (nl, 37); low-density lipoprotein, 70 (nl, 125); very-low-density lipoprotein, 10 (nl, 37); and triglycerides 170 (nl, <190). She was also found to have a sideroblastic anemia with a moderate number of target cells but no evidence of renal disease. In recent years the visual difficulty consisting of severe glare had increased to an incapacitating extent.

Except for a thyroidectomy at age 32 years and the anemia at age 34 years, she had had no significant illness or surgery. Her three children, now in their late 20s and early 30s, were thought to have normal corneas but were not available for examination. Her 1 sibling (male), her parents, and 10 additional adults in the pedigree were also thought to have normal corneas. The parents were not consanguineous. Her father had died at age 85 years with coronary artery disease and her mother at age 67 with colon cancer. Medication taken by the patient included Pyribenzamine for hay fever, occasional Tedrol for asthma, and propranolol for "stress."

Ophthalmic examination at age 55 years showed diffuse haziness of the corneal stroma (Fig. 1) with conspicuous accentuation in the paralimbal (arcus) region. The appearance was distinctly different from that of the usual arcus senilis. The entire corneal periphery contained a white ring abutting the sclera and partially traversed by superficial limbal vessels. Unlike the usual arcus that has a clear and sharply demarcated area in the corneal periphery, the corneal opacity in the present case (and in reported cases) extended into this peripheral zone and, in places, reached the sclera. Moreover, the opacity had an irregular, whitish character in contrast to the more homogeneous white appearance of the usual arcus. Like the usual arcus, however, the opacity was stromal and gradually diminished toward the axial part of the cornea. The surface of the cornea was smooth and reflective. Pachymetric measurements indicated a thickness at the upper limits of normal (OD 0.588 mm; OS 0.597 mm). The patient was myopic in the range of −6 to −7 D. The corrected vision was 20/30 OU but deteriorated to 20/200 in bright light. The eyes were otherwise normal.

A full-thickness corneal transplantation was performed by one of us (N.M.), without complications.

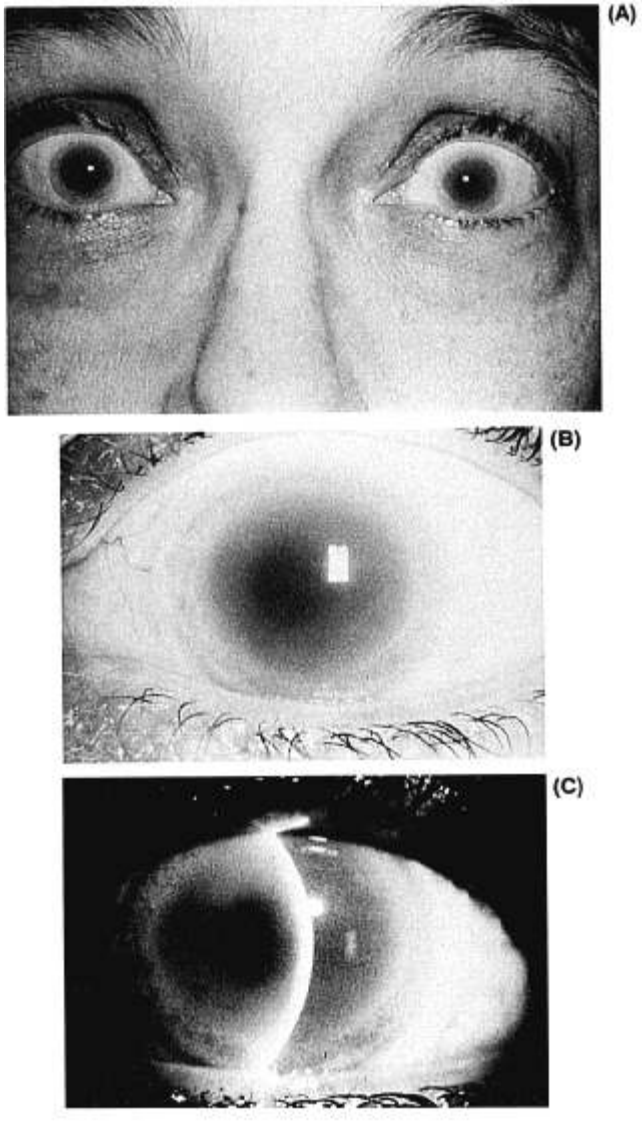

FIG. 1. Cloudy corneas as seen in **(A)** full view, **(B)** close-up, and **(C)** slit lamp. Despite the cloudiness, the visual acuity was 20/30 but the glare effect was severe.

The corneal disc removed was divided into four quadrants, which were separately treated as follows: (a) fixed in 4% glutaraldehyde in 0.15 *M* phosphate buffer (pH 7.2) for 1 h. Then one portion was transferred to 10% buffered formalin, embedded in glycol methacrylate, and stained with toluidine blue for light microscopy. The other portion was postfixed in 1% osmic acid and embedded in Epon for electron microscopy; (b) fresh frozen and frozen sectioned (unfixed) for Oil-Red-O and Sudan Black staining; (c) fixed in formalin and frozen sectioned for Oil-Red-O, Sudan Black, and filipin staining (11); and (d) embedded in paraffin for hematoxylin and eosin staining.

In the 8 months after the transplantation, the grafted cornea has remained clear.

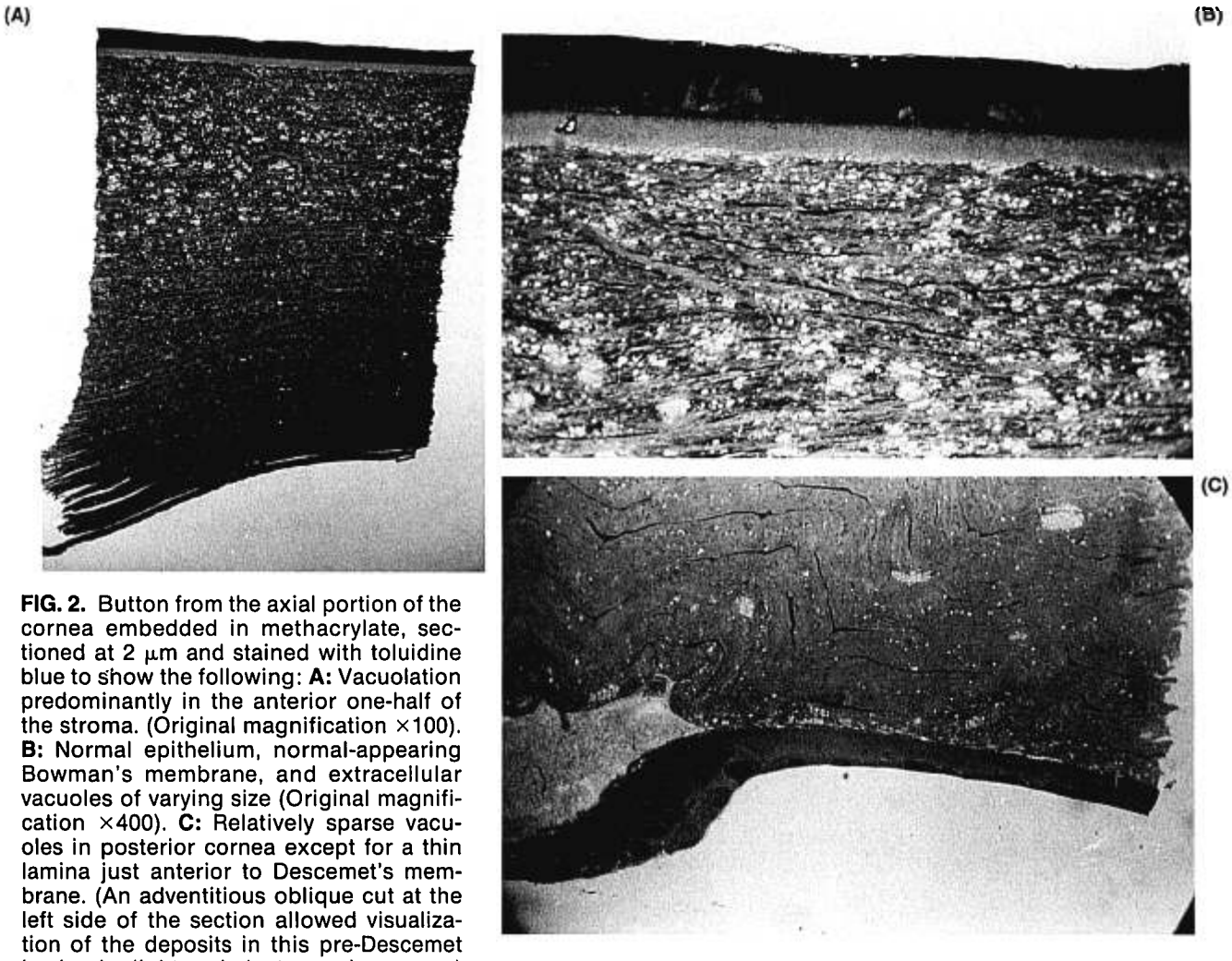

FIG. 2. Button from the axial portion of the cornea embedded in methacrylate, sectioned at 2 μm and stained with toluidine blue to show the following: **A:** Vacuolation predominantly in the anterior one-half of the stroma. (Original magnification ×100). **B:** Normal epithelium, normal-appearing Bowman's membrane, and extracellular vacuoles of varying size (Original magnification ×400). **C:** Relatively sparse vacuoles in posterior cornea except for a thin lamina just anterior to Descemet's membrane. (An adventitious oblique cut at the left side of the section allowed visualization of the deposits in this pre-Descemet lamina by light and electron microscopy.) (Original magnification ×400.)

Pathology

Vacuolation of the anterior one-half of the corneal stroma was the most conspicuous abnormality seen by light microscopy. Although detectable in routine, paraffin-embedded tissue cut at 7–10 μm and stained with hematoxylin and eosin, the vacuoles were better visualized in methacrylate-embedded tissue cut at 1–2 μm and stained with toluidine blue (Fig. 2). The vacuoles varied in size (<3 μm), contained an amorphous substance, and were situated in the extracellular compartment between the collagenous fibers. Although much less abundant in the posterior one-half of the stroma, a compact layer of vacuoles was present separating Descemet's membrane from the stroma.

By electron microscopy the vacuoles contained myriad membranous deposits (Fig. 3) and, in places, showed a suggestive proximity to granular ground substance. Bowman's membrane showed much finer vacuoles (~0.5 μm) than the rest of the stroma did. An oblique section through the pre-Descemet lamina displayed the morphology of the membranous deposits especially well (Fig. 3C).

The other anatomic structures of the cornea, stained by hematoxylin and eosin and toluidine blue, appeared essentially normal. Specifically, the epithelium showed only spotty thickening of its basement membrane. Descemet's membrane and the endothelium were normal. The keratocytes were also normal.

Filipin fluorescence (Fig. 4) indicated unesterified cholesterol in Bowman's membrane and on the collagenous framework throughout the corneal stroma, whereas the epithelium, Descemet's membrane, and the endothelium did not stain. Oil-Red-O and Sudan Black showed no consistent stain of any part of the corneal button.

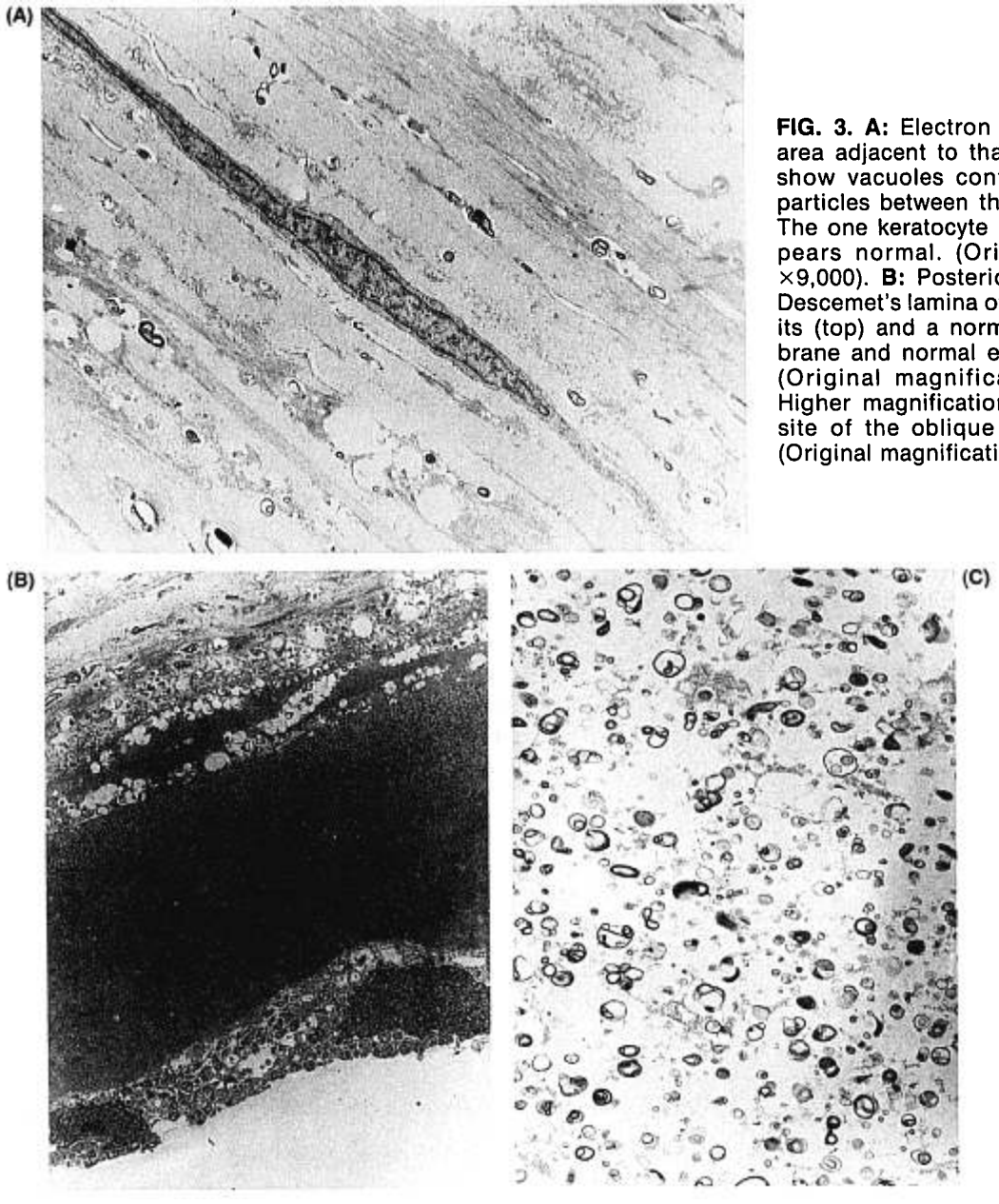

FIG. 3. A: Electron microscopy from an area adjacent to that shown in Fig. 2 to show vacuoles containing membranous particles between the collagenous fibers. The one keratocyte bridging the field appears normal. (Original magnification ×9,000). **B:** Posterior layers with a pre-Descemet's lamina of vacuoles and deposits (top) and a normal Descemet's membrane and normal endothelium (bottom). (Original magnification × 30,000). **C:** Higher magnification of the particles (at site of the oblique section in Fig. 2C). (Original magnification ×30,000).

COMMENT

The prime abnormality was the presence of vacuoles containing membranous particles between, and partially replacing, the collagenous framework. The origin of these particles is not apparent. One would expect much of it to be unesterified cholesterol and phospholipid (i.e., lecithin) because these constituents increase in the cornea in LCAT disease (10). The lack of staining of the cornea with Oil-Red-O and Sudan Black decreases the possibility of significant amounts of triglyceride and cholesteryl ester deposition. Filipin fluorescence, however, indicated the diffuse presence of unesterified cholesterol superimposed on the collagenous framework and Bowman's membrane (Fig. 4). This has been previously demonstrated in Schnyder's dystrophy (12) and in other corneal lipid dystrophies in which HDL is lacking (H.S. Kruth, unpublished observations). No such staining occurred in normal control corneas. However, the relationship of the filipin-stained lipid deposits to the vacuolar particles in the present case is uncertain. Despite the obvious accumulation of cholesterol, there were no crystals nor abnormal birefringence in this (central) button of the cornea.

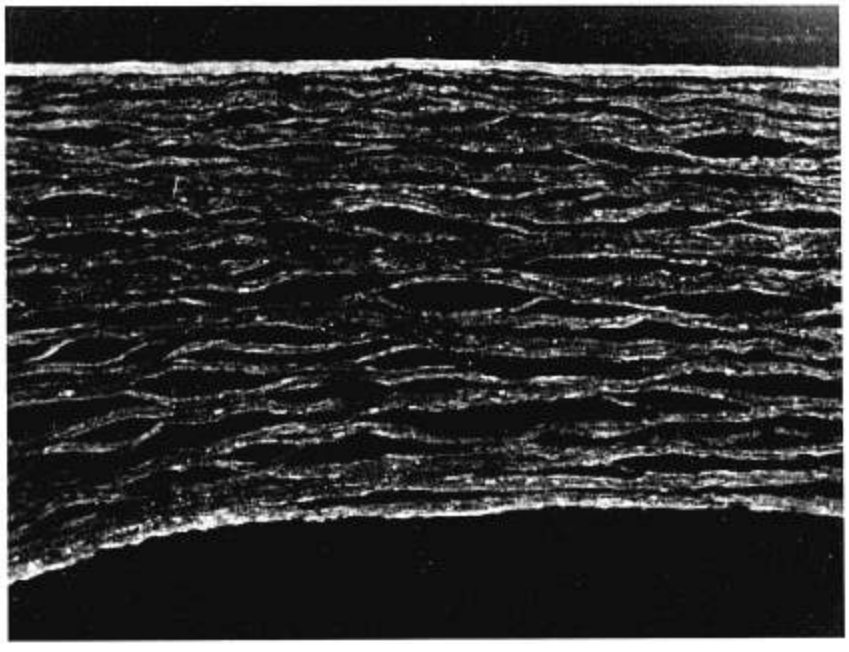

FIG. 4. Filipin fluorescence shows unesterified cholesterol superimposed on the collagenous framework (including Bowman's membrane and the pre-Descemet lamina). The epithelium, Descemet's membrane, and endothelium showed no significant staining and are not evident in the present photograph (Original magnification ×320).

The abnormal deposits were entirely extracellular which, together with the normal morphology of the keratocytes and other cells of the cornea, suggests that the deposition was not a manifest result of faulty metabolic processes within the cellular components of the cornea. Possibly the low levels of HDL simply impaired removal of lipids from the cornea as has been shown to be the case in certain other tissues (13).

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