Morphologic Characteristics and Chemical Composition of Christmas Tree Cataract

G. Adrien Shun-Shin,* Gijs F. J. M. Vrensen,† Nicholas P. Brown,* Ben Willekens,† Marianne H. Smeets,† and Anthony J. Bron*

Purpose. Christmas tree cataract consists of highly refractile multicolored “needles” crisscrossing the lens fibers of the deep cortex. The fact that the colors vary according to the angle of the incident light, and that in retroillumination only a dim outline of the cataract is seen, would suggest that Christmas tree cataract is a diffractive phenomenon. This study was performed to unravel the ultrastructure and chemical composition of the Christmas tree needles.

Methods. Eight lenses from donor eyes and four extracapsularly extracted lenses with Christmas tree cataract were investigated by scanning and transmission electron microscopy. The chemical composition was studied with energy-dispersive x-ray microanalysis and Raman microspectroscopy.

Results. Scanning electron microscope examination showed that the needles are smooth, rectangular, plate-like elements bordered by membranes and amorphous material and running crisscross through the lens. In the specimens for transmission electron microscopic examination, the needles proved to be largely dissolved, but the remains showed regular spacings of approximately 5 nm. Material identical in spacing and electron density was found in neighboring cells bound to a reticular membranous network originating from the fiber-limiting membranes. Energy-dispersive x-ray and Raman microanalysis showed that the needles have a high sulfur content and pronounced S-S, CS-SC, and C-S vibrations. The cytoplasm adjacent to the needles and reticular meshwork had an elevated Ca++ content.

Conclusions. It is concluded that cystine is the most likely candidate for the Christmas tree needles and that the needles probably are formed as the result of an age-related aberrant breakdown of crystallins induced by elevated Ca++ levels. Invest Ophthalmol Vis Sci. 1993;34:3489-3496.
Patients admitted for routine cataract extraction in Oxford were examined at the slit-lamp after full pupillary dilation. Four lenses were found to have Christmas tree cataracts situated within the deep perinuclear cortex. The patients had no other notable ocular or systemic disorders. Informed consent was obtained.

At routine extracapsular cataract extraction, with the use of sodium hyaluronate, the nucleus and some of the surrounding cortex were expressed carefully after capsulotomy. The nucleus–cortex was examined at the slit-lamp, and, in all four cases, the Christmas tree cataract still could be identified readily. The four nuclei with their surrounding deep cortex then were placed in 5% phosphate-buffered glutaraldehyde (pH 7.3).

Donor eyes were examined in Amsterdam within 24 hours of enucleation. Eight lenses with Christmas tree cataract were identified and then fixed in a 0.08 M cacodylate-buffered solution containing 1% glutaraldehyde and 1.25% paraformaldehyde (pH 7.3).

At both centers, the tenets of the Declaration of Helsinki were followed, and appropriate approval was obtained from the institutional human experimentation committee.

After fixation for several days to weeks, all lenses were dissected, and the pieces were screened biomicroscopically for Christmas tree cataract elements. Some pieces were dehydrated with ethanol, critical point–dried with CO₂ as the intermediate, and coated with a few nanometers of platinum. The specimens were inspected and micrographed in a Philips SEM 505 scanning electron microscope (Philips Industries, Eindhoven, The Netherlands) with a secondary emission detector. Some pieces were treated identically except they were not coated. On these pieces, elemental microanalysis was performed with an EDAX PV 9800 system and windowless detector (EDAX PV 9760/26) (EDAX-Int., Mahwah, NY).

For transmission electron microscopic examination, pieces of four lenses containing Christmas tree elements were postfixed in OsO₄, dehydrated in a graded series of ethanol, and embedded in epon. Ultrathin sections were cut, stained with uranyl acetate and lead citrate, and inspected and photographed in a Philips EM 400 electron microscope (Philips Industries, Eindhoven, The Netherlands). Some unstained sections were used for elemental analysis with a Philips CM 12 electron microscope (Philips Industries) equipped with an EDAX PV 9800 system and an ultrathin window detector (EDAX PV 9760/53). Pieces containing Christmas tree cataract elements were placed in phosphate-buffered saline for Raman analysis. Raman spectra were recorded with a confocal Raman spectrometer. The laser excitation wavelength was 660 nm, and an objective of 63× was used. Raman analysis was restricted to the 650 to 1750 cm⁻¹ spectral region.

RESULTS

Although the number of needles varied from a few isolated needles to an innumerable number, as shown in Figure 1, the ultrastructure by scanning electron microscopic examination was fully consistent. In all lenses, the needles were in nonopaque regions. The lens fibers in the area appeared normal. At low-power examination with a scanning electron microscope (Fig. 2A), stacks of interrupted lens fibers were found to be covered with smooth-surfaced material. At medium power (Fig. 2B), this smooth-surfaced material proved to consist of plate-like elements that, upon fracturing, exhibited rectangular substructures. The plates bordered apparently cut lens fibers that proved to have the normal membrane ultrastructure of lens fibers in the deep cortex and core, with grooves and ridges. In some instances, the surface of the plate-like elements was less smooth (Fig. 2C), most likely representing the face of the needles directly neighboring the lens fibers.

A problem with transmission electron microscopic examination is that—either because of dissolution during preparation for transmission electron microscopic examination or during ultrathin sectioning—most of the content of the needles disappeared, leaving only ghosts of the needles. In low-power micrographs obtained during transmission electron microscopic examination (Figs. 3A, B), the ghosts proved to run crisscross through the lens, dissecting the lens fibers, transversely or obliquely to their long axis. In some instances, however, part of the needle content at the borders of the empty spaces remained in the sections (Fig. 4A). This material proved to have a regular spacing with a periodicity of approximately 5 nm. Similarly spaced material also was observed some-
FIGURE 2. Scanning electron microscopic views of the aberrant regions in Christmas tree cataractous lenses. (A) Low-power view illustrating stacks of interrupted lens fibers (arrowheads) covered with smooth-surfaced material (asterisks). (B) Medium-power view of the smooth-surfaced material showing the plate-like appearance and rectangular substructures. Notice the normal appearance of neighboring lens fibers with grooves and ridges (arrowheads) characteristic of deep cortical and nuclear lens fibers. (C) This is similar to (B) except the plate-like elements exhibit imprints of adjacent fibers that have been fractured away during preparation for scanning electron microscopic examination.

A most curious observation was made in one lens, which, in addition to the needles, exhibited regionally broadened lens fibers containing an electron-dense reticular meshwork, surrounding a myelin-like body partly filled with amorphous material and partly empty (Fig. 5A). At a high magnification, the myelin-like body proved to be continuous with the meshwork, which was filled with electron-dense material with a spacing of approximately 5 nm (Figs. 5B, C). The origin of the reticular meshwork, as illustrated in Figure 6, is most likely the fiber-limiting membrane.

More or less similar deformations of the fiber-limiting membranes have been observed in the other three lenses treated for transmission electron microscopic examination.

Finally, it was observed that in some micrographs obtained by examination with a scanning electron microscope, the plate-like elements were covered by cobblestone-like elements (Fig. 7A), which are most likely identical to the endfeet of fibers seen on some of the needles during transmission electron microscopic examination (Fig. 7B).

The uncoated specimens of the four lenses ana-

FIGURE 3. Transmission electron microscopic views of Christmas tree crystals completely (A) or partly (B) dissolved during the transmission electron microscopic examination or ultrathin sectioning. (A) Medium-power micrograph illustrating that the course of the crystals is unrelated to the course of the fibers that are cut randomly (asterisks). Notice that the cut fibers surrounding the crystals have a normal ultrastructural appearance. (B) High-power view of a thin crystal-like element running straight through different fibers (F-F) without interruptions at the fiber membranes (arrowheads).
Figure 4. High-power transmission electron microscopic views of remains of Christmas tree crystals with spacing of approximately 5 nm. (A) Part of a large crystal from which most is dissolved. (B) Small crystal running through the cytoplasm of a normal fiber and surrounded by an apparent membrane (small arrows). Notice that the adjacent cytoplasm (cyt) has a normal appearance. FLM: fiber-limiting membrane.

Figure 5. Transmission electron microscopic views of aberrant lenticular elements in the region of Christmas tree crystals. (A) At low magnification, the apparently broadened lens fibers consist of a reticular meshwork (RM), myelin body (MB), and electron-opaque core. Notice that the adjacent lens fibers have a normal fine granular cytoplasm. The membranes of these fibers are stained more densely compared with a region more remote from the crystals. (B) High-power view of the reticular meshwork (RM) continuous (arrowhead) with the myelin body (MB). Cyt: cytoplasm. (C) High-power view of the reticular meshwork showing the membranes of this meshwork (arrows) and fine spacing of its contents (approximately 5 nm) when cut longitudinally.

Labeled for elemental composition of the Christmas tree elements by scanning electron microscopy all showed very pronounced sulfur peaks and slightly enhanced Ca++ peaks as compared with adjacent normal fibers (Fig. 8). No phosphorous peaks were observed in any of the Christmas tree elements. In addition, radiographic microanalysis showed an increased Ca++ signal in the regions of the reticular meshwork (Fig. 5) as compared with closely adjacent normal fiber regions.

Raman microspectroscopic examination of Christmas tree elements detected enhanced Raman shifts for CS-CS, S-S, and CS bonds as compared with normal regions of the lens (Fig. 9). In the aberrant Christmas tree regions, Raman signals of normal protein contents (phenylalanine, tyrosine, tryptophan, amide III, and CH2-backbone) also were registered. This is not surprising because the sample size in Raman microspectroscopic analysis is small.
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FIGURE 6. Survey transmission electron microscopic view of a reticular meshwork closely associated with a fiber-limiting membrane (FLM, arrowheads). Notice that the cytoplasm of the adjacent fibers has a normal fine granular appearance.

FIGURE 7. Low-power scanning (A) and transmission (B) electron microscopic views of Christmas tree crystals studied by amorphous cobblestones (arrows [A]) or endfeet (asterisks [B]).

FIGURE 8. Radiographic spectrum of a Christmas tree crystal (B) and neighboring normal fiber region (A). Notice the dramatic increase in the sulfur peak (S) and the slightly higher calcium peak (Ca) in the crystals.

DISCUSSION

Highly refractile, multicolored, needle-shaped lenticular inclusions have been described extensively in the clinical literature.\(^1\)\(^-\)\(^8\) It has been suggested, on indirect evidence, that the needles are cholesterol crystals\(^1\)\(^\text{5-8}\) or proteinic in nature and probably represent tyrosine or cystine crystals.\(^5\)\(^-\)\(^7\) In a study on the phospholipid and cholesterol content of distinct types of human cataracts, Murawski and Koch\(^8\) observed a significant increase in cholesterol per 100 mg wet weight in Christmas tree and coronary cataracts. When expressed as the total amount per lens, however, no differences were evident, indicating that there is no specific accumulation of cholesterol in Christmas tree cataracts. The relative increase in cholesterol is the result of a decrease in lens wet weight possibly resulting from
from the phospholipid moiety of the membranes, would be expected. No such peak was observed in any of the Christmas tree elements.

In the current study, performed on 12 lenses, lens fibers neighboring the Christmas tree crystals proved to have a normal ultrastructure. This is to be expected because, clinically, Christmas tree cataract can be found in otherwise clear lenses. The striking rectilinear crystal-like structures, constituting the Christmas tree cataract, traverse the lens in all directions, in keeping with clinical observations. When the scanning and transmission electron microscopic observations are reviewed, it can be concluded that they are made up of a varying number of plate-like elements stacked together with a periodicity of approximately 5 nm, and thus are identical to the structures observed by Hayes and Fisher.2,3

All four specimens studied by transmission electron microscopy showed sites of abnormal membrane ultrastructure more or less comparable to that illustrated in Figure 6. An extensive reticular meshwork within neighboring structures was found only in one specimen. It might be argued that this is a spurious finding with no relevance for the origin of the plate-like elements; however, on clinical grounds, it would appear that Christmas tree cataract formation is a slow process and, thus, one might expect the sites of formation to be few and far apart. Moreover, no other ultrastructural abnormalities were found in the sections that could explain the origin of the plates. On the basis of the findings shown in Figures 5 and 6, it can be postulated that the initial formation site of the plate-like elements is the reticular meshwork, which originates from the fiber-limiting membrane.

The assumption that the reticular meshwork is the origin of the crystals leaves questions to be answered. For instance, what is the underlying biochemical abnormality leading to the deformation of the fiber-limiting membrane and, subsequently, formation of the reticular meshwork, and what is the nature of the electron-dense, 5-nm-spaced material enclosed within the meshwork? What is the process that leads to a plate-like linear propagation of material across the fibers? As indicated above, Raman microspectroscopic examination showed an age-related decrease in the protein content of the lens core,3 generalizing the suggestion of Murawski and Koch9 of a membrane-associated breakdown of proteins in old cataractous lenses. There is ample evidence for the presence of proteolytic enzymes—endopeptidases and exopeptidases—also in the core of the mature lens,12-14 and it has been suggested by Van Heyningen and Waley15 that “lens proteins are broken down to amino acids in vivo” by these enzymes. The main reason for this breakdown tentatively has been suggested to be related to the de-
The two main endopeptidases (ie, the trypsin-like protease and multicatalytic endopeptidase complex) preferentially degrade α-crystallins. Recently, Fleschner and Cenedella have shown, in bovine lenses, that the main extrinsic proteins of "native" plasma membrane fractions are αA-crystallin and modified αA-crystallin. There is also ample evidence that the proteolytic enzymes are activated by Ca++. Azuma and Shearer have demonstrated that calpain-induced proteolysis is the mechanism for diamide cataract. The current radiographic microanalytic observations show an enhanced Ca++ content adjacent to the crystals and in the reticular meshwork. Because of this evidence, it can be postulated that either the enhanced accumulation of denatured crystallins or the local increase in Ca++ leads to increased protein breakdown and segregation of some of the breakdown products in the lumen of the reticular meshwork.

Because the current radiographic microanalytic observations show a high sulfur content of the Christmas tree crystals and because crystals are formed in concentrated solutions of one molecular or macromolecular species, only few candidates for the molecular nature of the crystals remain (ie, methionine or its reduced form methionine sulfoxide, cysteine, cystine, and oxidized or reduced glutathione). Because Raman microspectroscopic examination also showed increased CS-SC and S-S vibrations, methionine, methionine sulfoxide, cysteine, and oxidized glutathione can be excluded.

Glutathione is present in the lens in high concentrations. In the rabbit lens epithelium, for example, the concentration of glutathione, reduced and oxidized, is 64 μmol/g, but the level is five times less in the cortex. Truscott and Augustyn showed that non-protein sulfhydryl, of which more than 90% is glutathione, is present at levels of 127 nmol and 364 nmol in the nucleus and cortex, respectively, of clear lenses, decreasing to 13 nmol and 364 nmol, respectively, in Pirie type IV cataracts. It is not known whether the concentration of glutathione would reach crystallization levels in the human lens because reduced and oxidized glutathione are soluble compounds. In addition, it is difficult to conceive why reduced glutathione, physiologically present in high concentrations, should be sequestered in the lumen of the reticular meshwork. Cystine is the least soluble of the naturally occurring amino acids, with a solubility coefficient of 0.003 mg/100 g water at 20°C for the DL cystine and 0.011 mg/100 g water at 25°C for L cystine. In contrast, cysteine is highly soluble. Thus, it seems to be the most likely candidate. Circumstantial evidence in favor of the cystinic nature of the crystals is reported in the article of De Jong, Bleeker-Wagemakers, Vrensen et al, describing a case of inherited juvenile crystalline cataract and uncombable hair. The extracellular crystals, enwrapped by membranes, proved to have a spacing of approximately 5 nm and to be most probably of a cystinic nature. Studies on inherited cystinosis have shown that, in the benign variant of this disease, birefringent brick-shaped and needle-shaped crystals of a cystinic nature are present in numerous cell types. As in our study, these crystals tend to be dissolved on treatment for light and electron microscopic examination.

In conclusion, it can be postulated that Christmas tree cataract may result from the accelerated breakdown of membrane-associated denatured proteins induced by a stimulation of proteolytic enzymes triggered by high levels of Ca++. The peptides and amino acids are accumulated in the lumen of the reticular meshwork, and cystine is concentrated beyond the level of crystallization and forms growing crystals.

Key Words
- human lens, cataract, electron microscopy, energy-dispersive x-ray analysis, Raman spectroscopy

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References


