Calcium-containing Opacities in the Human Lens

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Cooperative Cataract Research Group (CCRG) photographic procedures developed by Chylack¹ ² have made it possible to localize and analyze specific lens opacities for their ultrastructural and chemical characteristics. One type of human lens opacity has been shown to have a high phosphorus/sulfur ratio (as compared to normal lens fiber cells) and an accumulation of unit membranes.³ The present paper describes another variety of human lens opacity with the following characteristics: (1) high calcium, low sulfur, undetectable phosphorus, as determined by Energy Dispersive X-ray Analysis (EDXA) of bulk specimens in the scanning electron microscope (SEM), or “thick” sections with the transmission mode of the SEM; (2) spheroidal shape; (3) up to approximately 300 µm in size; and (4) birefringence. Microchemical analysis of these opacities shows that the calcium is in the form of calcium oxalate. These calcium-containing opacities, which have been detected in 14 out of 406 human cataractous lenses, have a characteristic morphology, as seen in the CCRG stereo photographs. Therefore, the presence of these calcium opacities, if not obscured by other kinds of opacities, can be detected with a high degree of accuracy in the fresh lens from the CCRG photographs alone. Invest Ophthalmol Vis Sci 24:1194-1202, 1983

Materials and Methods

Only human lenses were used in this study and all were freshly extracted cataractous lenses. Lenses were marked with a small ink dot on the anterior surface near the equator, for the purpose of orientation during the subsequent fracture procedures.¹ ² They were photographed immediately with the standard CCRG system producing stereo 35 mm color slides.¹ In addition, the lenses were checked for areas of birefringence by placing a light source and a polarizer below the lens and another polarizing filter (analyzer) above the lens.

The lenses were then put directly into 2.5 to 4% glutaraldehyde buffered with 0.1 M Na cacodylate (pH 7.2-7.4) at room temperature for 24 hrs. They were dehydrated in a graded series of ethanol beginning with 10%. The lenses were left in 100% ethanol overnight to completely remove water. Subsequently, the ethanol was exchanged with Freon 113 and critical-point dried in Freon 13 at a maximum pressure of 900 psi at 35 C.

The dried lenses were fractured through areas of interest that had been determined by viewing the CCRG stereo slides. The fractured lens halves were mounted on carbon stubs and coated with carbon. A Philips PSEM 500 scanning electron microscope

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equipped with a Kevex 7021 energy dispersive x-ray analyzer (EDXA) was used to study the lenses.

Selected areas of lenses that contained calcium opacities as revealed by SEM-EDXA of that lens were dissected out and immersed in propylene oxide followed by infiltration with Epon and polymerization. Sections of approximately 0.5 to 1 \( \mu \text{m} \) were viewed in the SEM in the transmission mode and the EDXA used to study the elemental composition.

To determine if the calcium in the inclusions was in the form of oxalate, a test was performed on portions of the dried lens tissue containing these discrete opacities. The specimen pieces were heated on a glass slide in a 500 C oven for 20 min, cooled and covered with a coverslip. While observing the ashes microscopically a drop of concentrated sulfuric acid was placed at the edge of the coverslip. The production of gas bubbles (CO2) when the acid reached the calcium deposits indicated the presence of oxalate.

**Results**

Calcium-containing opacities as they appear in the fresh lens within minutes after cataract extraction are shown in Figure 1. The posterior view, as seen with reflected light, shows a number of these opacities, which typically show up as amber-colored, spheroidal-shaped structures. The amber color may relate indirectly to the surrounding or background yellow-to-brown nuclear color (nuclear sclerosis).

Lenses that clearly show these structures in the CCRG photographs have consistently shown a typical morphology at the SEM level, and a typical elemental spectrum, with a major peak for calcium, as determined by EDXA. These calcium-containing opacities with their characteristic morphology, have been detected in 14 out of 406 of the human cataractous lenses we have studied.

Figure 2A shows at very low magnification, a scanning electron micrograph of a large portion of the fractured surface of a lens which contained the spheroidal opacities, according to the CCRG photographs of the fresh lens. Four of the spheroidal structures are evident in this SEM field. A calcium map of this same field is shown in Figure 2B. The signal from calcium is strong enough to be detected at this low magnification. The map indicates accumulations of calcium corresponding to the four spheroidal structures plus a fifth calcium accumulation, probably corresponding to an opacity-related structure, not detected in the SEM. The calcium-containing spheroidal structures range up to approximately 300 \( \mu \text{m} \) in size among our specimens.

At a higher magnification (Fig. 3A) the surface structure of the spheroids is evident. The fracture plane passed over the surface of two spheroids, revealing a striated surface pattern. The calcium map of this same field (Fig. 3B) shows the accumulation of calcium in these structures.

In some cases the spheroids were cross-fractured,
Fig. 2. A. Low magnification SEM of human cataractous lens showing inclusions corresponding in size and shape to the spheroidal opacities seen in CCRG photographs of fresh lenses. Four inclusions are evident in this field (marker: 0.5 mm). B. Calcium map (dot pattern) of same field shown in A. Calcium accumulations occur at the sites of the four inclusions. A fifth site of calcium accumulation (arrow) probably corresponds to a spheroidal "opacity" not evident in the SEM photograph.
Fig. 3. A, Higher magnification SEM. The specimen fractured along the surfaces of two spheroids, revealing details of their surface structures (marker: 100 µm). B, Calcium map of the same field shown in A. The concentration of calcium in the spheroids is evident.
revealing something of their internal morphology (Fig. 4), which appears to consist of structures radiating out from a central location. The outermost part of the spheroid is very distinctly demarcated from the surrounding fiber cells. This corresponds to the distinct demarkation of the calcium within the spheroid, and the undetectability of it in the surrounding region, as shown by the calcium line scan. Spectral analyses at spots within the outside of a spheroid were made with thick sections (0.5–1.0 µm), using the transmission mode of the SEM. Sections of these structures for the transmission mode of the SEM made from critical-point dried material are difficult to obtain, which accounts for the appearance of the section in Figure 5. The outline of the spheroid is clear. Arrows indicate spots which were selected for analysis. The EDXA spectra indicate that the spheroid contains high calcium, low sulfur and undetectable phosphorus.

Detectability of the spheroidal opacities in the fresh lens is greatly enhanced in the CCRG photographic setup by the use of transmitted light, with the lens placed between crossed polarizers. Figure 6A shows the posterior view of a fresh cataractous lens photographed with reflected light. Spheroidal opacities, which can be detected with some difficulty in the color stereos, are not apparent in these prints. The same lens, between crossed polarizers, photographed with transmitted light, however, shows a number of light spots, which correspond to the spheroidal opacities (Fig. 6B). The birefringence of these opacities can, therefore, be used to enhance their detectability in the fresh whole lens. Human lens nuclei freshly obtained from extracapsular extractions can also show the birefringent calcium-containing opacities (Figs. 7A–C). The birefringent opacities show up particularly well in portions of fixed lenses flat-mounted in Epon.

Microchemical analyses showed the presence of oxalate within the spheroidal opacities. This strongly
suggests that the birefringence of the spheroidal opacities is due to a high concentration of calcium oxalate.

**Discussion**

Much has been written about the possible relationship between calcium and cataractogenesis.\(^7\)\(^-\)\(^1\(^1\)\(^1\) No attempt has been made here to review the literature in this field, but the references cited above refer to much of it.

In the present paper, multiple small opacities, detectable as spheroidal masses in the freshly excised lens, are described. With the use of the CCRG photographs as a guide, correlations between the appearance of these opacities, as they appear, in the fresh lens with their appearance at the SEM level has been made.

The opacities contain high concentrations of calcium according to energy dispersive x-ray analysis of SEM samples. The undetectability of a phosphorus peak in any of the spectra indicates that the bulk of the calcium cannot be in the form of a phosphate. The strong birefringence of these opacities suggests that the calcium may be in the form of oxalate (or possibly urate). The strong positive microchemical reaction for oxalate indicates that the opacities are indeed primarily in the form of calcium oxalate.

Although these opacities have not, to our knowledge, been described before, abnormal deposits of calcium have been reported in the human retina and lens at the histological and ultrastructural levels.\(^5\)\(^,\)\(^1\(^2\)\(^,\)\(^1\(^3\)\(^\) Other work has shown that naphthalene-induced formation of calcium oxalate crystals in the retina and vitreous of rabbits is accompanied by a drop in ascorbic acid levels in the vitreous and aqueous, and it has been suggested that calcium oxalate is produced by the oxidation of ascorbic acid.\(^1\(^4\)\(^,\)\(^1\(^5\)

A major conclusion of the present report is that
this unique opacity with its highly specific chemical composition (calcium oxalate) can be detected with a high degree of accuracy in the fresh lens with the use of CCRG photographic procedures, providing it is not obscured by other kinds of opacities.

All fresh lenses that were found to have these characteristic opacities were subsequently found to have the corresponding calcium-containing spheroids. It is thus possible to say that when these unique opacities are detected in a fresh lens, it contains abnormal deposits of calcium oxalate.

A search for possible correlations between the presence of these calcium oxalate-containing opacities and other aspects of the patients' medical histories, particularly relating to calcium and oxalate, has not as yet been carried out. However, such a study can be facilitated by the CCRG photographs alone, thus avoiding more lengthy histochemical procedures. If it should prove possible to detect these opacities directly in the patient, this search would be greatly enhanced, by identifying larger numbers of patients with such opacities. This is increasingly true as the percent
Fig. 7. A, Posterior view, CCRG stereo photograph of a human lens nucleus (from extracapsular surgical extraction method) containing calcium opacities. Arrows indicate the same opacities in each photograph of this series. B, Same lens nucleus viewed with transmitted light through parallel polarizers. C, Same as in B except polarizer (analyser) above lens is rotated 90° in relation to polarizer below lens. Opacities are seen to be birefringent.

of cataract operations involving extracapsular extractions is increasing.

Also, it is now possible to re-examine CCRG photographs from previous records and identify calcium oxalate spheroidal opacities. This would increase the number of patients whose medical histories could be studied in relation to possible calcium and oxalate disorders.

Key words: cooperative cataract research group (CCRG), human cataracts, calcium, oxalate, scanning electron microscopy, energy dispersive x-ray analysis (EDXA or EDS), microchemical analysis.
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