Crystals in the Lens

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Crystal formation in the lens in both the young and the aged has been reported by various observers from time to time. The morphology and the chemical nature of the crystals are also of varied nature; for example, Goulden [12] and other observers have described crystalline opacities in the lens showing green and blue iridescence in morbid conditions such as thyroid dysfunction, postoperative tetany, mongolian idiocy, and myotonia atrophica. Vogt [26] as mentioned by Parker [22] has described a "spear cataract" consisting of shiny crystalline needles. Riad [24] has reported a pedigree with lens crystals which looked like tiny broken glass fragments, rectangular in shape, with a notch at one corner. Verhoeff reported a case in which protein crystals produced the picture of coralliform cataract.[30] Among the varied chemical constituents of the crystals, cholesterol is frequently found in different types of senile, traumatic, and complicated cataracts. The "spear cataract" of Vogt consisted mainly of cystine. In Gifford and Puntenney's case,[11] the chemical substance was thought to be calcium sulfate. The crystals described by Riad were indistinguishable from cholesterol crystals. Discrete spheroidal crystalline deposits have been observed within the sclerotic nucleus of Morgagnian cataract by Zimmerman and Johnson [29]; these deposits have been identified as calcium oxalate by histochemical and other tests.

A case with crystals of definite morphological characteristics is described in this paper, and detailed histochemical analysis of the crystals is presented.

Report of Case

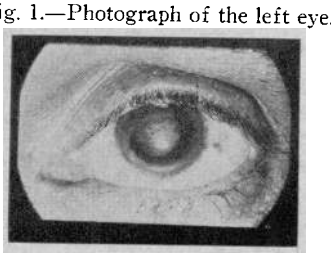
A Hindu male, aged 50 years, attended the ophthalmic department of the N. R. S. Medical College, reporting defective vision in each eye for the last 3 years, and was examined by one of us on Jan. 31, 1961. There was nothing significant in his history except myopia. There was nothing unusual in the family history regarding his eye condition.

Examination.—Left Eye: Vision was finger counting at 1 foot which improved to finger counting at 2 feet under homatropine. The lens on oblique illumination showed punctate and irregular flakes of opacities, mostly in the central part (Fig. 1). On slit-lamp examination, the flakes of opacities appeared to be grayish-white crystals, more or less polyhedral prismoids, varying in sizes; they were arranged irregularly, mostly in the central part of the lens, gradually becoming less in number towards the periphery (Fig. 2). At 2 or 3 places toward the center of the lens, there were also tiny, globular, grayish white, amorphous masses. The rest of the lens, including the capsule, was clear. No such crystals were visible in the cornea or in the conjunctiva. The fundus could be seen through the peripheral part of the lens when the pupil was dilated, and an annular myopic crescent with chorioretinal degenerative changes characteristic of pathological myopia, was visible both at the central and peripheral part of the retina.

Right Eye: Vision under homatropine was finger counting at 1 foot. The lens showed a deposit of polyhedral prismoid crystals similar to that in the left eye, with more or less similar arrangement. The fundus also showed evidence of pathological myopia in the form of a myopic crescent and chorioretinal degenerative changes, both at the central and the peripheral region.

General Examination: The patient was a slight but otherwise healthy individual. A Wassermann reaction was negative. Alkaline serum phosphatase was 2.6 Bodansky units. Blood inorganic phosphate was 3 mg., and serum calcium was 9.8 mg. %. The albumin-globulin ratio was 2. The Van den Bergh reaction was direct negative and indirect positive. The urine on microscopic examination did not reveal the presence of any tyrosine crystal, and the free amino acid was only 3.5 mg. within 24 hours.

Fig. 1.—Photograph of the left eye.



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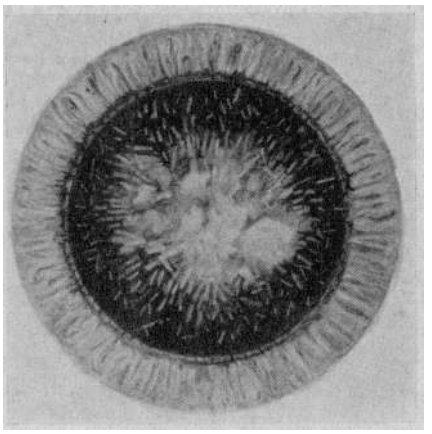

Fig. 2.—Sketch of slit-lamp appearance of the lens (left).

On July 3, 1961, the right lens was removed by intracapsular method. Immediately after removal, the lens was placed in a dry test tube, which was kept in a freezing mixture (common salt and ice) within a thermoflask and was taken to the Physiological Laboratory, University College of Science, Calcutta. There the lens was subjected to various tests to determine the nature of the crystals. There was an uneventful recovery following the operation, and the vision in the right eye came up to 6/18 partly with +4 O.D. with +1 O.D. cyl. 180°.

Laboratory Examination of the Lens.—1. Physical Studies: The lens, when examined under an ordinary microscope, was found to contain polygonal crystals which, when observed under a phase-contrast microscope, resembled solid crystals of the shape of polyhedrons and rhombohedrons (Figs. 3 and 4). They were very irregularly distributed, mainly in the nuclear portion of the lens. The lens was formol-fixed, and further examination of 10μ frozen sections under an ordinary and phase-contrast microscope revealed the presence of various cracks and folds on the body of the crystals (Fig. 5). Clusters of smaller crystals were seen at places to radiate from a spot.

Fig. 3.—Whole lens observed under ordinary microscope, irregular distribution of crystals. Reduced 11% from mag. × 96.

Attempts to isolate the crystals with the help of a micromanipulator were unsuccessful because of the viscous substance in which the crystals were embedded. However, it was observed that the crystals were denser and more compact than the surrounding lens substance. Histochemical methods of investigation were therefore adopted, and formol-fixed frozen sections were used.

2. Histochemical Studies: Results of these are shown in Tables 1-6.

Observations During Microincineration of the Sections: Crystals heated at 250 C for 2 hours separated out into several sheet-like layers, some of which showed definite charring. Some crystals seemed to withstand even 3,000 C of temperature for 4 hours, while the surrounding tissue was completely reduced to ash.

Comment

Clinically, there was no actual opacity of the lens in either eye, apart from aggregation of opaque polyhedral crystals, which obstructed vision. The arrangement of the crystals also did not resemble any known type of congenital cataract, such as the floriform or the coralliform type.

Fig. 4.—The whole lens observed under phase-contrast microscope. Prismatic appearance of the crystals; reduced 11% from mag. × 96.

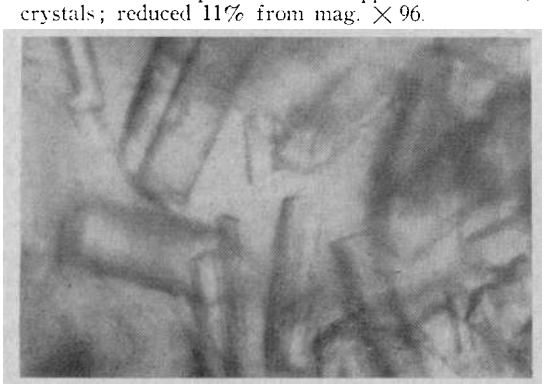

Fig. 5.—Crystals observed under phase-contrast microscope, showing polygonal prism-like shape with cracks. Mag. × 96.

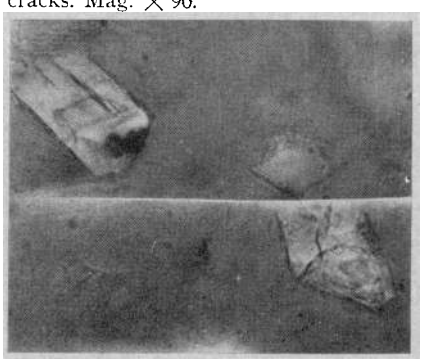

TABLE 1.—*General Staining*

Stains Used	Result
1. Eosin-hematoxylin	Crystals unstainable
2. Leishman-Giemsa	Same

Histochemically the crystals did not contain any detectable amount of lipid, calcium, phosphate, carbonate, or silicon in either free or bound form. More intense PAS-positive reaction might have been due to the presence of PAS-positive mucoprotein [18] in the crystals of compact form.

The clear blue bromophenol blue staining of the crystals indicated their protein nature. But the failure of eosin or Giemsa-Leishman to stain them in contrast to the surrounding lens substance, and the different staining of the crystals by Masson's trichrome method in contrast to the general lens protein, indicated that the crystalline protein was different from the surrounding protein. Some aggregates of fine amorphous particles in the lens also gave a staining reaction similar to that given by the crystals. Four different types of proteins, in the form of soluble

TABLE 2.—*Detection of Organic Substances*

Tests	Results	Inference
	Lipids	
1. Sections extracted in lipid solvent methanol:chloroform (2:1) at 60 C for 24 hr. followed by methanol: chloroform (1:2) for another 24 hrs. at 60 C [19]	Crystals insoluble in lipid solvent	Absence of almost all of the known lipids with the possible exception of some forms of ceroids and some lipoidal pigments
2. Sudan staining according to Kay [17]	No staining of crystals	Possible absence of neutral lipids
3. Schultz reaction for cholesterol [27]	No reaction	Absence of cholesterol
	Polysaccharides	
4. Periodic acid-Schiff reaction according to Hotchkiss [16]	Very faint but deeper staining of crystals in comparison to surrounding tissue.	Crystals probably contained higher concentration of either tissue polysaccharide [16] or unsaturated lipid [28] or both

TABLE 3.—*Detection of Inorganic Constituents*

Tests	Results	Inference
1. Detection of free carbonates according to Bunting [5]	No bubble formation with disintegration of crystals	Absence of free carbonates
2. Detection of calcium salts by Alizarine red S method [10]	No trace of calcium detected	Absence of calcium
3. Detection of inorganic phosphates by the method of Cheng [7]	No staining of crystals	Absence of phosphates
4. Reactions of the above mentioned inorganic substances in sections reduced to ash	No staining	No unusual concentration of bound forms of calcium, phosphate or carbonate [18]
5. Detection of silicon by chemical procedure [15] in section reduced to ash	No trace of silicon	Absence of silicon

TABLE 4.—*Detection of Proteins*

Tests	Results	Inference
1. Masson's trichrome staining [21]	Crystals stained red in contrast to light blue staining of the general lens substance	Probably indicated that crystals contained some component which differed in composition from the surrounding tissue
2. Mercury-bromophenol blue method of staining general protein [4]	Crystals stained clear blue and the surrounding tissue grayish	Indicated that the crystals were protein in nature somewhat different in composition from the adjacent tissues

TABLE 5.—*Detection of Amino Acids*

Tests	Results	Inference
1. Xanthoproteic reaction as described by Lillie[20]	Intense yellow staining of crystals	Indicated the presence of tyrosine, tryptophan and phenylalanine and like substances in higher concentration
2. Millon's reaction according to Bensley and Gersh[2]	Crystals were stained deep red	Indicated presence of higher amount of tyrosine
3. Sakaguchi's staining for arginine as modified by Serra[24]	Crystals stained deep red in contrast to pinkish staining of surrounding tissue	Indicated presence of arginine in higher amount in the crystals

TABLE 6.—*Detection of Thiol Group (SH Group)*

Tests	Results	Inference
Detection carried out according to Hammett[13]	SH group not detected in the crystals	Absence of SH group in the crystals

α-crystallin, β-crystallin, albumin, and insoluble albuminoid have been identified in the lens.[1] The histochemical tests for protein, however, did not give an idea as to which variety of the lens protein was crystallized.

Of the amino acids which crystallize, cystine, tyrosine, and leucine are notable. The cystine crystals reported by Cogan et al.[8] and the crystals of the "spear cataract" of Vogt which were made of cystine were morphologically different from the crystals in the reported case. Further, there was no evidence of cystinosis or of Lignac-Fanconi syndrome[9] in this case. On the other hand, the more intense color with Millon's, xanthoproteic, and Sakaguchi's reactions showed increased content of tyrosine and arginine in the crystals. Amino-acid analysis of the protein fractions of bovine lens has revealed that the β-crystallin portion is relatively richer in tyrosine and arginine contents.[3] It was consequently apparent that the crystals were made of β-crystallin. But the SH group known to be associated with β-crystallin[14] was completely absent in the crystals. A decline in the SH group is a common observation with senile cataract and normal aging of the lens. Moreover pure β-crystallin is not stable in solution, even at 0 C, unless some α-crystallin is present.[23] It is reasonable to suggest that the precipitation of the β-crystallin was probably due to the decline of the α-crystallin content, owing to some unknown cause, and the absence of the SH group was due to aging process.

The observation on microincineration was most confusing. The crystals which withstood a temperature of even 3,000 C could only have been constituted by some inorganic component apart from the protein, but the nature of this inorganic component could not be determined.

Summary and Conclusion

1. A case with polyhedral prismoid crystals in the central part of the lens, with rather similar distribution in each eye, is reported.

2. One of the lenses was removed by intracapsular method, and histochemical analysis of the crystals was done.

3. There was no detectable amount of lipid, calcium, phosphate, carbonate, or silicon in the crystals.

4. The crystals showed marked reactions for protein containing chiefly tyrosine and arginine which are known to be the main constituents of the β-crystalline portion of the lens protein. However, the SH group known to be associated with β-crystallin was completely absent, perhaps due to aging process.

5. On microincineration, the crystals left behind a refractile residue, which could only be some inorganic substance. The nature of this substance, however, could not be de-

tected, since the material available was insufficient.

6. The underlying mechanism for crystal formation can only be guessed. The crystal formation was possibly due to the decline (for reasons unknown) of the α-crystallin content, which is thought to keep β-crystallin in a stable condition.

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