

# Bioinorganic Markers of a Loss of the Crystalline Lens Capsule Barrier Properties and Consequent Age-Related Cataract Development

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**Abstract**—The age-related cataract development consequent upon a loss of the lens capsule barrier properties proved to be associated with accumulation of sodium, calcium, phosphorus and potassium. For the first time the use of spatial cluster and correlation analyses showed that the physical light scattering in the crystalline lens volume depends on changes in the lens matter elemental composition. The fields of elevated concentrations of sodium, calcium, phosphorus, potassium and chlorine conformed to the lens capsule geometry and their clustering was similar to that of opacity fields in the lens body. The accumulation geometry of the elements in the lens body that are commonly seen in the aqueous humor of the anterior chamber, can be considered evidence for excessive transitioning of their compounds through the lens capsule shell, while its spatial connection with transparency changes—proof of participation in cataractogenesis.

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Irreversible age-related changes in the crystalline lens transparency are widespread in higher animals and humans. Because of the high cataract prevalence it is considered a socially important disease. The problem of a loss of lens transparency is well-known also in the domestic and ornamental animal husbandry. This explains frequent use of the domesticated solidungulates, cats and dogs, as models for studying of the cataract genesis [1–5].

Crystalline lens is a biological biconvex lens consisting of the regular protein molecule aggregates. In most mammals, the crystalline lens matter has mechanical elasticity and it is transparent in visible range. The protein aggregates have a relatively high refractive index, and their regular packing provides light transmission without a significant light scattering. In addition, the electrolyte-containing distances between the organized protein units, at the boundaries of which a diffuse light scattering could be possible, are shorter than a half of a wavelength in the short-wave part of transmitted light. Therefore, a limited migration of chemical substances within the lens volume does not affect significantly the lens transparency. The organized protein aggregates consist of a large variety of specific proteins (33–45 wt %) the ratio of which varies slightly in different higher ani-

mals. The interstitial electrolyte including inorganic substances, carbohydrates and their derivatives, the reducing agents of glutathione and cysteine, cysteine itself and ascorbic acid account for 55–67% of the lens mass. Outside, the lens is covered by a thin unstructured shell, with basal membrane and a monolayer of the epithelium-like cells.

Despite numerous theories and hypotheses of cataract pathogenesis, there is still no consensus about the mechanisms underlying this disease. Protein disorganization and modification, in particular, due to photochemical reactions, is believed to be the most important factor leading to changes in the lens matter and arrival in its volume of the newly formed corpuscles that disturb light transmission [6–11].

Insufficient understanding of the mechanism leading to the disease prevents development of the pharmaceutical methods for its treatment. Radical surgery remains nowadays the main method of the age-related cataract treatment both in humans and animals.

In this study, analysis of the sodium, calcium, phosphorus and potassium accumulation showed that a loss of the lens capsule barrier properties underlies also the mechanism of the age-related cataract development.

## MATERIALS AND METHODS

The subjects of this study were 30 isolated human crystalline lenses. Bioinorganic changes during cataract genesis were studied on the isolated lenses (20 samples) that differed in the degree and localization of

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opacity (only in nucleus or in nucleus and the cortical layers). The lenses with opacifications were gathered during the extra or intracapsular cataract extraction. Bioinorganic characteristics of the transparent lenses (conditionally from healthy donors) was made using 10 cadaver lenses obtained from cadaver eyes enucleated no later than 12 h after the donor death. The age and ethnic comparability of the groups was taken into account.

From the pre-frozen samples, two flat-parallel blocks ( $0.80 \pm 0.05$  mm thick) were cut out under visual control along their optical axis. One of the blocks was used to estimate the light scattering ability, while another one, "mirror," was used for analysis of the chemical element distribution over the section plane. Chemical microanalysis was performed using a Zeiss EVO LS-10 microscope (Zeiss, Germany) with an OXFORD X-Max<sup>50</sup> energy dispersive spectrometer (OXFORD, United Kingdom).

We have earlier developed an original small-angle light scattering photometer (the patent RU 169521) that makes possible evaluating of the light scattering ability in the lens matter with the spatial resolution up to 3  $\mu$ m in vitro in a 1 mm-thick flat-parallel block cut out the length matter. The photometer provides an opportunity of estimation of the deflected light relative power simultaneously for each of the directions or for the entire set of directions to which a laser beam passing through the matter deviated (the wavelength 630 nm). For 422–895 points of each of the lens block, the data were obtained on the total power of light deflected by the angles 5° to 15° during the beam pathing through 0.80 mm of the matter. The qualitative (semi-quantitative) chemical composition was determined in all geometrically matching points of the "mirror" plane of an adjacent lens block. Evaluation of the chemical element distribution with a scanning electron microscope was performed without sputtering in a low vacuum mode (70 pA) at a voltage accelerating from 20 to 25 kV and 400–520 pA current per a sample (cathode LaB6). To minimize contraction in the rarefied atmosphere of the microscope, the samples were pre-frozen at –70°C.

Measurements were made in a single coordinate space, which allowed a correlation analysis and comparison of spatial clustering of various parameters including spatial modeling (Splat) in the GeoDa software (Luc Anselin, GPL, United States).

## RESULTS AND DISCUSSION

The small angle optical light scattering within the lens matter volume has been characterized quantitatively for the first time. The light power deflected by  $10^\circ \pm 5^\circ$  when passing 1 mm of the lens matter ranges from 1 to 86% in the conditionally transparent lenses and from 16 to 100% in lenses with opacifications. Thus, there are pronounced optical defects in the

transparent lenses too, but their insignificant proportion in the lens volume fails to interfere with formation of a proper light projection.

Section analysis at the qualitative (semi-quantitative) level showed the chemical element distribution in the conditionally transparent lens matter and in that of the lenses with opacifications.

A strong statistically significant correlation was found between changes in the lens light scattering ability and the total content of calcium ( $R = 0.759$ ;  $p < 0.001$ ), a strong negative correlation was between changes in the lens light scattering ability and the total content of potassium ( $R = -0.815$ ;  $p < 0.001$ ), moderate positive correlation was between changes in the lens light scattering ability and the total content of sodium ( $R = 0.603$ ;  $p < 0.001$ ), and the average negative correlation was between changes in the lens light scattering ability and the total content of phosphorus ( $R = -0.607$ ;  $p < 0.001$ ). The patterns of chemical element distribution in the lenses with opacifications were characterized by significant paired correlations between sodium and calcium ( $R = 0.634$ ;  $p < 0.001$ ), phosphorus and potassium ( $R = 0.596$ ;  $p < 0.001$ ) (Fig. 1).

In the conditionally transparent lenses, none of the chemical elements measured was substantially clustered in space. As calculated for the maximum likelihood model that links transparency with Na (paired with Ca) as well as with P (paired with K), the maximum Moran index value in the transparent lenses was lower than  $I = 0.74$  to be within the range  $I = 0.54$ – $0.58$  as a rule. With opacification development, a significant spatial clustering was observed and the Moran index increased to  $I = 0.98$  (at the minimum value recorded  $I = 0.72$ ).

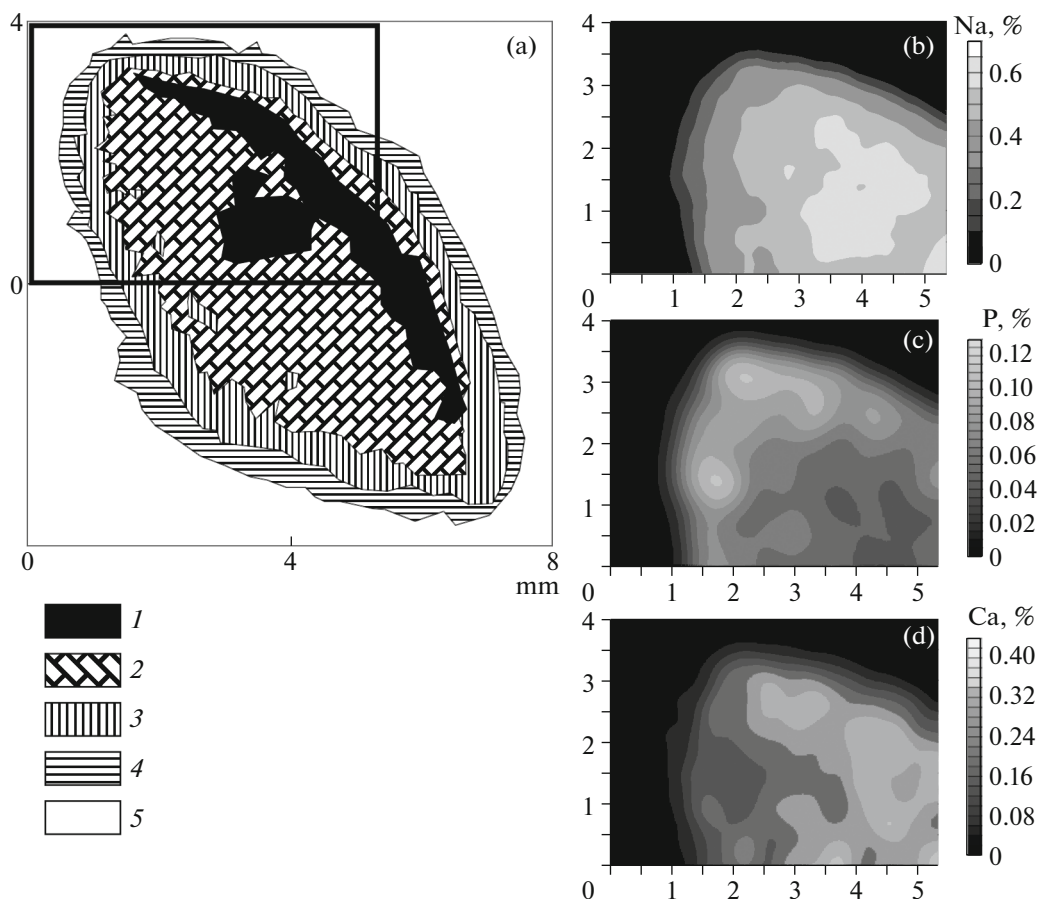
The data obtained on the lens elemental chemistry can serve as indicators of a process that have a certain spatial geometry in the lens volume and lead to development of opacifications. A pattern of chemical element pathological accumulation enables us to verify different competing biochemical or physical-chemical models of cataract genesis proposed by various authors.

The fact that the fields of elevated concentration of the main inorganic components (Na, Ca, P, K, and Cl) in aqueous humor of the lens anterior chamber correlate with the light scattering ability and conform to geometry of the lens capsule suggests an important role of a loss of the capsule barrier properties in development of the age-related cataract.

## COMPLIANCE WITH ETHICAL STANDARDS

*Conflict of interests.* The authors declare that they have no conflict of interest.

*Statement of compliance with standards of research involving humans as subjects.* The ethic norms and the laws of Russian Federation were observed. All proce-



**Fig. 1.** Sagittal section of the crystalline lens with opacifications. Typical distribution in the lens volume of (a) the light scattering ability of the lens matter, (b) the content of sodium, (c) the content of calcium. In the solid frame in (a), the field corresponding to the fields in other figures (b–d) is shown, for which purpose the correlation and cluster models were constructed (see description in the text). The map of the light scattering ability is given in the percentage of power of the light deflected by the angle  $10^\circ \pm 5^\circ$ ; in legend: (1) 80–100%; (2) 60–80%; (3) 40–60%; (4) 20–40%; (5) 0–20%.

dures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants involved in the study.

## REFERENCES

- Duncan, G. and Bushell, A., *Exp. Eye Res.*, 1976, vol. 23, no. 3, pp. 341–353.  
[https://doi.org/10.1016/0014-4835\(76\)90133-0](https://doi.org/10.1016/0014-4835(76)90133-0).
- Hightower, K.R. and Dering, M., *Invest. Ophthalmol. Vis. Sci.*, 1984, vol. 25, no. 9, pp. 1108–1111.
- Goralska, M., Nagar, S., Colitz, C., Fleisher, L., and McGahan, M., *Invest. Ophthalmol. Vis. Sci.*, 2009, vol. 50, no. 1, p. 305.  
<https://doi.org/10.1167/iavs.08-2230>.
- Goralska, M., Fleisher, L., and McGahan, M., *Invest. Ophthalmol. Vis. Sci.*, 2007, vol. 48, no. 9, p. 3968.  
<https://doi.org/10.1167/iavs.07-0130>.
- Goralska, M., Nagar, S., Fleisher, L.N., and McGahan, M.C., *Mol. Vis.*, 2009, vol. 15, p. 2404.
- Ostrovskii, M.A., Fedorovich, I.B., El'chaninov, V.V., and Krivandin, A.V., *Sens. Syst.*, 1994, vol. 8, nos. 3–4, pp. 135–146.
- McCarty, C.A. and Taylor, H.R., *Am. J. Ophthalmol.*, 1996, no. 37, pp. 1720–1723.
- West, S.K., Duncan, D.D., Munoz, B., et al., *J. Am. Med. Ass.*, 1998, vol. 280, pp. 714–718.
- Delcourt, C., Carrier, I., PontonSan'chez, A., et al., *Arch. Ophthalmol.*, 2000, vol. 118, no. 3, pp. 385–392.
- Tyuzikov, I.A., *Vopr. Dietol.*, 2017, vol. 7, no. 1, pp. 47–54.
- Weinert, B.T. and Timiras, P.S., *J. Appl. Physiol.*, 2003, vol. 95, pp. 1706–1716.

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