δ-Crystallin synthesis and vacuole formation during induced opacification of cultured embryonic chick lenses

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Previous experiments have shown that embryonic chick lenses cultured either without their vitreous bodies or with their vitreous bodies in the presence of ouabain alter their intracellular concentrations of sodium and potassium, change their ratio of synthesis of δ-crystallin polypeptides, and develop opacities in cortical or epithelial regions, respectively. In the present investigation δ-crystallin synthesis was examined electrophoretically in the epithelial, cortical fiber, and nuclear fiber regions of the cultured lenses. The intercellular space was estimated by determining 3H-inulin uptake, and lens structure was visualized by light and electron microscopy. The results showed that the alteration in the ratio of δ-crystallin synthesis occurred in both the clear and cloudy regions of the cultured lenses, suggesting that this change in protein synthesis does not cause the opacities. Moreover, experiments with cycloheximide revealed that inhibition of protein synthesis neither induced nor prevented the development of opacities in lenses lacking the vitreous body or treated with ouabain. Intercellular and intracellular vacuoles were directly associated with the opaque regions of these lenses, suggesting a causal basis for the opacities. Ouabain-treated lenses with attached vitreous bodies had only intracellular vacuoles, rather than a combination of intercellular and intracellular vacuoles, which is consistent with the possibility that the intercellular vacuoles are caused by excretion of fluid from the hydropic cells.

Key words: δ-crystallin, intercellular vacuoles, intracellular vacuoles, lens, lens opacity, cataract, embryonic chick, ouabain, protein synthesis, sodium, potassium, electrophoresis, electron microscopy

Recently we reported that the embryonic chick lens developed a cortical opacity within 3 hr when cultured without its vitreous body, but remained clear for several days when cultured with its vitreous body still attached to the posterior capsule. The lens cultured without the vitreous body also showed an alteration in the ratio of synthesis of δ-crystallin polypeptides, which appears to be controlled by a change in the intracellular concentration of sodium and potassium. Experiments with ouabain indicated, that the opacity per se was not due to the alteration in δ-crystallin synthesis, since the fiber cells of ouabain-treated lenses remained clear while altering their pattern of δ-crystallin synthesis. As an extension of these studies, we have investigated whether the alteration of δ-crystallin synthesis is confined to the opaque regions of the chick lens cultured without the vitreous body, and examined the morphological basis for the opacification.

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Materials and methods

Fertilized eggs of White Leghorn chickens (Truslow Farms, Inc., Chestertown, Md.) were incubated for 15 days at 37°C. The lenses were excised with or without their vitreous bodies, and five to 10 lenses were cultured in approximately 10 ml of enriched Ham’s F-10 medium. In some cases 1 x 10⁻⁴M ouabain (Sigma Chemical Co., St. Louis, Mo.) was added to the medium.

For determining the amount of intercellular water, the lenses were incubated in 200 μCi of ³H-inulin (New England Nuclear Corp.; Boston, Mass.; 210 μCi/mg) in 10 ml of medium. The lenses were removed at the specified times, placed onto a glass filter, and quickly washed with water under a slight vacuum. After removal of the adhering radioactivity by this washing procedure, the lenses were transferred to scintillation vials with forceps, broken with a glass rod, dried overnight at room temperature, and assayed for radioactivity by scintillation counting in a toluene-based scintillation fluid containing 8.5% Bio-Solv (Beckman Instruments, Inc., Fullerton, Calif.). The radioactivity was normalized on the basis of the total water content of the lens at the time of explantation. This value (3.95 mg/lens) was established by obtaining the difference between the wet weight and the dry weight of 66 15-day embryonic chick lenses; the lenses were dried for 2 days at 70°C before the dry weight was obtained. Since the lenses were not dried under vacuum it is likely that they still contained some water.

Clear and opaque regions of the cultured lenses. Lenses cultured with their vitreous bodies remained transparent for 24 hr. By contrast, lenses cultured without their vitreous bodies developed peripheral opacities. Thus, when the anterior face of the lens was viewed, a transparent zone was evident in the middle, giving a doughnut appearance to the lens (see Fig. 1 of Piatigorsky and Shinohara¹). When lenses with attached vitreous bodies were cultured in the presence of ouabain, most of the cortical fiber cells and the nuclear fiber cells remained clear. Only the epithelial cells and perhaps a few cortical cells near the lens periphery became slightly opaque.
cloudy within approximately 15 hr. The ouabain-treated lenses did not have a discrete clear zone in the middle when viewing their anterior face (Fig. 1).

**Alteration in δ-crystallin synthesis in the cultured lenses.** Protein synthesis was examined in different compartments of the lens to determine if the alteration in the ratio of synthesis of the δ-crystallin polypeptides occurred differentially in the clear and opaque regions. Lenses were labeled for 3 hr with 35S-methionine under the conditions described below, and the proteins from the epithelial cells, cortical fiber cells, and nuclear fiber cells were separated electrophoretically and autoradiographed (Fig. 2).

Lenses cultured with their vitreous bodies for 3 hr synthesized the two sizes of δ-
crystallin polypeptides in a ratio of approximately 1:3 in favor of the lower-molecular-weight species in the epithelial cells, cortical fiber cells, and nuclear fiber cells. In the lenses cultured without their vitreous bodies, the ratio of synthesis of the δ-crystallin polypeptides changed to approximately 3:1 in favor of the higher-molecular-weight species in both the cortical and nuclear fiber cells. The epithelial cells of the vitreous-free lenses did not alter the ratio of synthesis of the higher- and lower-molecular-weight δ-crystallin polypeptides as did the fiber cells of these lenses. Finally, the epithelial cells, cortical fiber cells, and nuclear fiber cells of the lenses cultured with their vitreous bodies and in the presence of ouabain synthesized only the higher-molecular-weight δ-crystallin polypeptides after 24 hr of culture. Thus the alteration in the ratio of synthesis of the δ-crystallin polypeptides which occurs during opacification of the cultured lenses is not confined to the opaque regions.

Opacification in the absence of protein synthesis. Cultured lenses were treated with cycloheximide (10 to 50 μg/ml) in order to determine whether opacification was induced by or required protein synthesis. The cycloheximide inhibited incorporation of radioactively labeled amino acids into protein in the cultured lenses. Lenses cultured with cycloheximide with attached vitreous bodies for 24 hr developed cortical opacities within 3 hr when the vitreous bodies were removed. The lenses remained transparent in the presence of cycloheximide for at least 2 days if the vitreous bodies were not detached. Lenses cultured for 24 hr with their vitreous bodies and in the presence of ouabain and cycloheximide developed diffuse opacities as shown in Fig. 1.

Intercellular space in clear and opaque lenses. In order to determine whether the lens opacity was associated with an increase in intercellular space, cultured lenses were incubated with 3H-inulin. Inulin is known to penetrate the intercellular spaces but not the cells of lenses. Lenses cultured with their vitreous bodies in the presence or absence of ouabain accumulated extremely little 3H-inulin by 24 hr of culture. Thus the alteration in the ratio of synthesis of the δ-crystallin polypeptides which occurs during opacification of the cultured lenses is not confined to the opaque regions.

Fig. 3. 3H-inulin uptake by lenses cultured with their vitreous bodies in the presence or absence of 1 × 10^{-4}M ouabain or without their vitreous bodies in the absence of ouabain. Each point represents the average of at least three determinations. Standard errors are not drawn because they were too small to indicate on the graph.
Fig. 4. Meridional sections of cultured embryonic chick lenses showing their equatorial regions. (Methacrylate, 2 μm sections, PAS stain; ×133.) a, Lens incubated for 24 hr with its vitreous body attached. The structure remained normal except for the formation of small vacuoles just inside the capsule (arrow). b, Lens incubated for 24 hr without its vitreous body. Large vacuoles were formed in the elongating and recently elongated cells of the epithelium (arrow) and peripheral cortex (arrowhead). However, the fully elongated lens fiber cells located deeper in the cortex did not become vacuolated (asterisk). Note the changes in cell shapes, the altered elongation of epithelial cells, the cell debris and space between the epithelium and the cortex, the distortion of lens shape, and the partial detachment of the lens capsule, all of which were common to lenses receiving this incubation. c, Lens incubated for 24 hr with its vitreous body attached but with ouabain added to the medium. The subcapsular vacuoles which formed during all the incubations were larger following ouabain treatment (arrows) and vacuoles formed in a limited number of lens fiber cells of the cortex (arrowhead). Cell shape and lens shape remained close to normal.

Morphology of clear and opaque lenses. Lenses incubated for 24 hr with their vitreous bodies attached remained clear and showed only microscopic structural changes. Small vacuole-like spaces developed next to the capsule by a widening of the spaces between the basal interdigitations of both lens fiber and epithelial cells (Figs. 4, a, 5, and 6). Cell size, shape, and junctional complexes remained normal, as did the cytoplasmic content of microtubules, ribosomes, dense crystallin matrix, and limited endoplasmic reticulum.

In contrast, lenses incubated for 3 or 24 hr without their vitreous bodies exhibited changes in the equatorial regions (Fig. 4, b). Many intercellular and intracellular vacuoles were formed, and the cells displayed altered shapes and junctional relationships (Figs. 7 and 8). The formation of intercellular vacuoles is consistent with the increased uptake of 3H-inulin observed above. Some groups of cells had about the same density of vacuoles between and within the individual cells, whereas others had almost exclusively either intercellular or intracellular vacuoles. Epithelial cells and partially elongated fiber cells near the lens periphery of the equatorial region tended to have more intracellular vacuoles, whereas more elongated fiber cells a little deeper in the cortex had mainly intercellular vacuoles which were larger and more...
Fig. 5. Electron micrograph of fiber cells from the cortex in the equatorial region of an embryonic chick lens incubated for 24 hr with its vitreous body attached. Note that the cell junctions, cell shapes, and cell contents are normal for lens fibers of the cortex. (×7,000.)

Fig. 6. Electron micrograph of the basal portions of epithelial cells in the equatorial region of a lens incubated for 24 hr with the vitreous body attached. The subcapsular vacuoles seen by light microscopy (Fig. 4, a) were shown to be intercellular spaces (arrows) between the basal infoldings and interdigitations of these cells, and not spaces within the cells or between the lens capsule (asterisk) and the cells. Some cell debris was found in the enlarged intercellular spaces. (×11,500.)

numerous. In general, lenses incubated for 24 hr without vitreous bodies (Fig. 8) showed larger regions involved in vacuole formation, a higher proportion of intercellular vacuoles, and vacuoles which were larger in average size than lenses incubated for 3 hr without vitreous bodies (Fig. 7). Fully elongated fiber cells in deep regions of the cortex and cells of the anterior epithelium maintained their normal structure, as did cells of the lens nucleus.

Lenses incubated for 24 hr with their vitreous bodies and in the presence of ouabain showed minimal changes in cell shape. There were, however, appreciable numbers of vacuoles in the epithelial cells and in a limited number of elongated cortical fiber cells (Figs. 4, c, and 9). In the basal regions of the epithelium the widening of intercellular space and the separations between cell interdigitations were somewhat larger than those described in lenses cultured without ouabain (compare Figs. 6 and 10). All other vacuoles in the epithelium and cortical lens fiber cells were intracellular. Again, the deep cortex and lens nucleus remained free from vacuoles and shape or junctional changes.

Discussion

The present investigation suggests that the opacification of the cultured embryonic chick lens is not caused by the alteration of δ-crystallin synthesis which occurs concurrently, but is directly associated with the development of vacuoles. There was a good correlation between the region of the affected lens which develops vacuoles and the region which becomes opaque. There are numerous examples in the literature of vacuoles forming in lenses which develop cortical opacities.

The vacuoles which are associated with cortical opacities generally, although not always, represent intercellular spaces and are believed to result from lens hydration. Water influx into the lens cells initiated by the accumulation of sugar alcohol or sodium results in swelling of the fiber cells and formation of intercellular vacuoles. These vacuoles interrupt the well-aligned and tightly packed lens fiber cells found in transparent lenses. The mechanism of vacuole formation is not known. One possibility is that the hydropic cells excrete fluid which becomes trapped between junctions. This hypothesis is consistent with our observa-
Fig. 7. Electron micrograph of fiber cells from the cortex in the equatorial region of a lens incubated for 3 hr following detachment of its vitreous body. Vacuoles formed between (arrows) and within (arrowheads) the cells; cell junctions and cell shapes were altered. (×7,000.)

Fig. 8. Electron micrograph of fiber cells of a lens incubated for 24 hr without its vitreous body. The intercellular vacuoles (arrows) became much larger than those following 3 hr incubations (fig. 7), apparently their size being limited only by resistant cell junctions (circles). Intracellular vacuoles (arrowheads) were still present. (×7,000.)

Fig. 9. Electron micrograph of fiber cells of a lens cultured for 24 hr with its vitreous body attached and with ouabain added to the medium. Only intracellular vacuoles (arrowheads) were formed and the cell shapes, sizes, and junctional complexes were only slightly altered. (×7,000.)

Fig. 10. Electron micrograph of the capsule (asterisk) and the basal portions of epithelial cells in the equatorial region of a lens incubated for 24 hr with its vitreous body attached and in the presence of ouabain. The vacuoles in the intercellular spaces (arrow) were larger than those in lenses not treated with ouabain (Figs. 4, a, and 6), and they contained more cellular debris. (×11,500.)

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