Electron microscopic study of galactose-induced cataract

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Cataractous lenses induced by galactose feeding were studied electron microscopically. The early changes were increased reactivity of the epithelium, edema of the lens cells, and intercellular cyst formation in the bow and posterior zones. These changes continued with further feeding of galactose until the lens cells became irreversibly damaged. The reactive epithelial cells formed marked cell proliferations.

Key words: galactose cataract, crystalline lens, lenticular cyst, subcapsular vacuoles of the lens, ultrastructure.

Histological studies have revealed that the changes in galactose cataract are very similar to those of diabetes, and the first morphological change is the appearance of hydrotic lens fibers. Biochemical findings suggest that the osmotic changes occurring early in these cataracts are caused by the accumulation of sugar alcohol formed by the enzymatic reduction of sugars. In the early stages of galactose cataract, alteration in potassium flux and amino acid concentrating process is also seriously affected.

The present paper documents the ultrastructural changes occurring in the lens at different stages of experimental galactose cataracts in the rat. Previous studies by Friedenwald and Rytel by light microscopy and by Brini and Porte by electron microscopy emphasized the accumulation of fluid within the lens fibers. The present study points to involvement of lens fiber cells and epithelium and demonstrates accumulation of fluid both within and outside of the cells.

Materials and methods

Albino rats weighing approximately 50 grams each were placed on a high galactose diet. Opacities occurred in the equatorial region on the third day and became progressively more severe until the formation of a nonreversible mature cataract in 2 weeks. The details of this experiment have been described previously. The animals were killed by intraperitoneal injection of an overdose of sodium pentobarbital on the third, fifth, and fifteenth days of the galactose feeding. After removing the cornea and the posterior portion of the globe, the whole tissue was fixed in 4 percent glutaraldehyde solution in pH 7.2 phosphate buffer for 20 minutes at room temperature. During this fixation, the lens was carefully dissected out by cutting the connecting zonules with a pair of fine scissors and then cut into halves with a sharp razor blade. At the end of the initial fixation in the glutaraldehyde, the shell-shaped lens
halves were cut into smaller pieces. To avoid the lens substance sticking to the blade, it was important that the tissue be immersed in the fixative all the time. Without washing, the tissue was transferred into 1 per cent osmium tetroxide in the same buffer solution for 90 minutes at a cold temperature. At the end of the postfixation, the tissue was trimmed into smaller pieces measuring 0.5 mm³, dehydrated in a series of ethanol, treated with propylene oxide, and embedded in epoxy resin. Thin sections were stained with both uranyl acetate and lead citrate and examined by electron microscopy.

Variation of the osmotic pressure of fixatives did not cause appreciable difference in preservation of the lenticular tissue as long as the tissue was trimmed into smaller pieces in the early stage of the fixation. Identical results were obtained by 1 and 8 per cent glutaraldehyde solutions.

Tissues were regularly obtained from the anterior center, bow area, and the posterior center for this study. Although the nuclear portion of the lens was not processed in the present experiment, the sectioning of this was not difficult. Sections, 0.5 μ thick, of the epoxy-embedded lenses were stained with toluidine blue and examined by light microscopy. Several normal rat lenses were processed similarly as controls. Portions of the lenses were also embedded in paraffin and prepared by conventional histological techniques.

Results

Lenses of young normal rats showed a relatively constant structure. Fine structural findings of the normal rat lens were not particularly different from those of

Fig. 1. Anterior center epithelium of the normal rat lens. Nuclei are lightly lobulated. The basal portion of the epithelial cells attach to the anterior capsule, and the apical portions attach to the apexes of the elongated and nonnucleated lens cells. The lens epithelium consists of relatively dense ground matrix in which moderate numbers of mitochondria (m) and rough endoplasmic reticulum (er) are seen. Tj = tight junction; d = desmosome. (×8,800.)
other animals described by earlier investigators. The outer surface of the lens was covered with the capsule, which is the basement membrane of the lens cells. The anterior capsule was markedly thicker than the posterior. The outer limit of the capsule consisted of fine fibrillar substance and was not sharply demarked by any membrane. The flat epithelial cells formed a single layer in the anterior portion by joining to each other with marked infoldings of the plasma membrane. Nuclei seemed to be slightly lobulated but uniform in size. They had a moderate concentration of chromatin and no prominent nucleoli. The cytoplasm generally consisted of a compact ground matrix, rich in fine fibrils and granules. There were no specific microorganelles to characterize this epithelium. The mitochondria were small and moderate in number. Rough endoplasmic reticulum, free ribosomes, and well-developed Golgi’s apparatus were relatively abundant. Small and sparse lysosomes and randomly arranged microtubules were other regular components (Fig. 1). There were numerous tight junctions and desmosomes at the apical portions of the cells, joining with the adjacent epithelial and anterior lens cells.

The epithelial cells elongated gradually toward the equatorial zone. The bow ar-
Arrangement of the nuclei was formed by heightening the cell bodies and keeping the nuclei in the center of the elongated cells. The cytoplasmic appearance of the nucleated lens cells at the bow area was similar to that of the anterior epithelium. However, toward the deeper zone the elongated cells lost their nuclei and became more compact (Fig. 2). The most common microorganelles began to disappear in this area. Free ribosomes were relatively well preserved up to the deeper cortical zone (Fig. 3). Microtubules were extremely well developed in the lens cells of this zone. Cell membranes formed numerous loops in which tight junctions were apparent (Fig. 2).

Anterior cortex was formed by apical portions of the elongated cells. Because of the difference of the length of the cells, the cross section of the anterior cortex showed irregular sizes of the cells. However, that of the posterior lens showed regularity in cell sizes.

Because of the extreme compactness of the tissue, some fixation artifacts were difficult to avoid. Fine vacuoles occurred commonly in the anterior epithelium at the off-center zone and in the posterior cortex immediately beneath the capsule. However, these artifacts were easily distinguishable from the pathological abnormalities in this experimental condition.

Fig. 3. Equatorial cortex of the normal rat lens. This high magnification photograph shows homogeneous fine granularity of the cytoplasm, free ribosomes (r), and microtubules (mt). Lens cells are held to each other by tight junctions (Tj). G = remnant of Golgi's apparatus. (x44,000.)
Initial vacuolar stage (3 day feeding). The equatorial cortex of the lens began to show small vacuoles at this stage. The rest of the lens remained clear clinically. These changes were reversible by removing the galactose from the diet.

The early ultrastructural abnormalities were found in the anterior lens epithelium and in the superficial cortex of the bow area. Many epithelial cells became dense by an increase of free and membrane-bound ribosomes. The intercellular spaces were commonly widened. Some cells were edematous and their apical portion often protruded into the subadjacent cortex (Fig. 4). One of the striking changes at this stage was marked intercellular cyst formation in the superficial cortex of the equatorial and posterior zone. Although small cystic separations of the lens cells at these areas were commonly seen as one of the artifacts, the cysts in the present condition were more conspicuous than simple artifacts. Lens cells became irregular in size and density and were extensively vacuolated. Numerous small cysts formed between the cell membranes which were separated in several places, sparing only

Fig. 4. Three day feeding. Epithelial cells are separated by large intercellular vacuoles. Some cells are extremely dense (D), partially due to increase of ribosomes. Some cells protrude into the anterior lens substance which seems to be swollen (←). (x6,700.)
the sites of the tight junctions (Figs. 5 and 6). Fine vesicles often accumulated at the portion of the lens cells which formed the wall of the cyst. Many cysts contained thin proteinaceous substance.

The lens cells themselves were not damaged severely at this stage. However, the cytoplasm was vacuolated and the granularity was coarser than normal, especially in the anterior portion. Although irregularity of the density in the anterior cortical cells was a normal finding, it appeared to be more conspicuous in the experimental lenses. Occasional anterior lens cells were already liquefied at this stage. The liquefied protein was extremely electron dense and amorphous. This material was often found in the intercellular spaces.

Intermediate vacuolar stage (7 day feeding). The lenses became markedly opaque on the seventh day of the high galactose diet. The vacuolated appearance of the bow and posterior portions was more pronounced, but the cataractous changes could still be reversed to certain degree. Light microscopic examination revealed frequent mitoses and several small foci of cell proliferation in the epithelium at this stage.

Electron microscopy showed marked irregularity of the epithelial cells in size and shape. Many anterior epithelial cells had

![Image](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933001/)
proliferated to a layer, 2 or 3 cells thick, in several places. The cytoplasm of these cells was rich in ribosomes and Golgi's apparatus (Figs. 7 and 8). Some dark-staining cells appeared to grow over the swollen cells. The intercellular cysts which were seen in the superficial equator and posterior cortex in the earlier stage became large and were coalesced into large vacuoles (Fig. 9). The cytoplasmic substance of the cortical cells had become coarse and irregularly granular. Cell membranes of these cells were broken, and liquefied proteinaceous substance was found in several places (Fig. 10). Also, localized foci of marked liquefaction of the lens cells were found in the anterior cortex (Fig. 11). The epithelial cells were severely vacuolated, and the normal appearance of the lens cells was totally absent in these areas. Liquefied lens protein formed droplets of varying sizes and density. Some droplets were surrounded by cell membrane. The dark-staining material was often found in the intercellular spaces of the epithelium (Figs. 11 and 12).

The swollen posterior lens cells showed a rounded configuration instead of the normal hexagonal shape in cross sections (Fig. 13). The rounded cells formed increased intercellular spaces in which microvilli-like processes were extended from the pathological lens cells. The lens cells escaping liquefaction often formed filamentous substance in the cytoplasm.

There was increased cellularity in the

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**Fig. 6.** Three day feeding. Equatorial cortex. Granularity of the cell is somewhat coarse and irregular. Cells are held only at the tight junctions (→) and form intercellular cysts. (×28,800.) Inset shows vesicle formation in the cytoplasm of the cyst wall. (×37,500.)
vitreous and in the posterior chamber at this stage. Some cells seemed to be of an inflammatory nature. The capsule remained normal.

**Advanced stage (15 day feeding).** After the feeding of 10 days or more, the lens became densely opaque. The whole cortical portion was liquefied and the lenticular nucleus also became opaque. The changes at this stage were totally irreversible.

The anterior epithelium became markedly irregular and formed numerous nodular foci of the proliferated cells (Fig. 14). Smaller cellular nodules were found in the posterior side also. The proliferated cells seemed to maintain the original appearance of the lens epithelium, but those which were elongated were similar in appearance to the normal cells of the bow area. These cells were joined tightly with several desmosomes and tight junctions. The cortical lens cells surrounding the proliferated cells were totally liquefied. The liquefied cells had lost their individual

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**Fig. 7.** Seven day feeding. Anterior epithelial cells have proliferated into 3 cell layer. The cells attached to the capsule are electron dense because of rich ribosome content. Proliferated lens cells have prominent Golgi's apparatus (G). Lens cells in this area show an intracellular vacuole (v), liquefaction (I), and coarse granules (c). (*×8,500.*)
Fig. 8. Seven day feeding. Higher magnification of the proliferated lens cells show rich Colgi's apparatus (G) and rough endoplasmic reticulum (er). The elongated cell (*) shows the appearance of the lens cell of the bow area. The anterior lens cells have been liquefied. (×17,900.)
cell membranes and were replaced by coarse electron-dense granules. The electron-dense proteinaceous substance was also often found in the intercellular spaces of the proliferated cells as well as intra-
cellularly (Fig. 14). The capsule of this area showed localized thickening corresponding to the proliferation of epithelial cells. The posterior lens was liquefied and was occupied by droplets of various sizes and densities (Fig. 15). Groups of these droplets were found in pools of relatively light-staining fluid accumulated in the cortex. Fragmented cell membranes were seen scattered in the protein droplets.

Some lens cells which had escaped the severe degeneration formed islands in a liquefied lake. The cytoplasm of these cells showed extremely coarse granularity and formed microvilli-like protrusions at presumably the apical ends of these cells (Fig. 16). Marked increase of the cellularity in the posterior chamber and adhesion between the iris epithelium and the anterior capsule were found frequently.

Discussion

Biochemical studies have revealed that in galactose cataract the sugar alcohol, dulcitol, is formed in the lens fibers and accumulates to a level sufficient to cause osmotic swelling. Other biochemical changes appear secondary to the retention of dulcitol and concomitant lens swelling.7

Electron microscopic studies of galactose cataract at various stages strongly support the concept that osmotic disturbances play an important role in the pathogenesis of this type of cataract. The present findings, however, differ somewhat from those observed by Friedenwald and Rytel1 in their light microscopic studies. These investigators found the accumulation of fluid was intracellular and not extracellular. The present electron micrographic studies revealed that, although there is evidence of cellular swelling and intracellular vacuoles, the striking feature at the early stage is the extracellular appearance of cysts which are especially numerous in the bow and posterior regions of the lens. These cysts

Fig. 9. Seven day feeding. Light micrograph of the thick section of the equatorial zone shows marked vacuoles in the superficial cortex. Deep cortical cells maintain the normal appearance in this area. (×480.)
Fig. 10. Seven day feeding. Lens cells in the deep cortex of the equatorial zone show swelling (S) and liquefaction (L). Cell membranes have broken and disappeared (†). Liquefied lens protein forms droplets. Some are surrounded by membrane (‡). Ribosomes are completely absent. (×8,700.)
appear as lakes between the lens cells and frequently are restricted by tight junctions. They may result from some active mechanism which is attempting to remove the excess water from the lens cells. This mechanism may move water out of the cells more rapidly than its passage through the extracellular space. The restriction imposed by the tight junctions results in the observed pools of fluid. However, in many of the cysts, irregularity of the cell membranes appears frequently. Although these may be the result of oblique sectioning, they may also represent breaks in the membranes of the hydropic lens cells. Since the cysts frequently contain proteinaceous material, they may have been turned by spillage of intracellular material

Fig. 11. Seven day feeding. Severely liquefied anterior portion of the lens shows vacuolated epithelium and proteinaceous droplets in the superficial cortex. The droplets show varying sizes and density. (x5,900.)
Fig. 12. Seven day feeding. Liquefied anterior lens substance flows freely between the cells. Arrows indicate small pools formed by the liquefied substance. Desmosomes (d) are well preserved. Ep = epithelial cells. (×51,800.)

Fig. 13. Seven day feeding. Cross section of the lens fibers at the posterior pole. These lens fibers have escaped the liquefaction but are markedly swollen. (×4,500.)
Fig. 14. Fifteen day feeding. Epithelial cells have proliferated into a thick layer and are elongated. The cells near the capsule are markedly separated by enlarged intercellular spaces. Rough endoplasmic reticulum and Golgi's apparatus are rich. Electron-dense liquefied lens protein is seen in the cytoplasm of the proliferated cell (→). (×4,500.)
Fig. 15. Fifteen day feeding. The posterior lens cells which are extensively liquefied contain droplets and fragments of cell membranes (>). (x11,500.)
Fig. 16. Fifteen day feeding. The deep cortical cells have escaped liquefaction although the cell cytoplasm shows an electron-dense, coarse granular substance. The cells are separated and form microvilli-like protrusions (→). (×17,800.)

from the swollen lens cells. The immersion of soluble lens in glutaraldehyde-phosphate solution and subsequently in osmium solution, media which are hypertonic, induces minute cellular separations. However, these artifacts are easily distinguishable from the pathological cysts.

The epithelial cells appear affected in the initial vacuolar stage. Cysts and vacuoles are present in the epithelial cells. There is a suggestion of increased metabolic activity of the cells as evidenced by the increase in the numbers of ribosomes. At this stage the cellular pump mechanism seems to be hyperactive as it attempts to correct the increase of permeability to cations. The marked swelling of the cortical lens cells immediately adjacent to the capsule both on the anterior and posterior surface offers a plausible explanation for the reported increase in permeability to cations and amino acids.

As the cataract progresses, the osmotic swelling becomes more prominent. The swelling of the lens cells continues to induce large intracellular vacuoles or to liquefy the cytoplasmic protein. Finally, cell membranes rupture to form large lakes of liquefied material. Although these changes were expected, the unusual aspect of the intermediate and the late stages is the proliferation of the epithelial cells. In the intermediate vacuolar stage, several areas of the epithelium appear as a double layer. When the nuclear opacity develops, the epithelium proliferates to form a multilayer. The reason for the epithelial proliferation may be an attempt to replace damaged cells. However, it may have been stimulated to correct the inability of the lens to maintain normal hydration. One of the most important mechanisms of survival for the lens or any cell is the cation pump process which excludes sodium ion. If sodium gains ready access into the lens, it will be accompanied by chloride ion resulting in a net increase in electrolytes which leads to osmotic swelling. As explained in previous publications, this kind of electrolyte change occurs in the later stages of galactose cataract. The existing cation pump may not be able to cope with the increase in cation permeability caused by lens swelling. Since the epithelium is the site of cation pump activity in the lens, the cell proliferation may be an attempt to reinforce the pump mechanism so that the lens is better able to remove sodium and reduce the osmotic swelling.
Marked electron density in the liquefied lens protein may be due to the heavy metal, which is used in the staining solution, bound to the liberated amino acids in the droplets.

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REFERENCES