The electron microscopy of the normal human lens

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A general review is presented of our current understanding of the fine structure of the lens, its capsule, and zonular fibers, and illustrated with original material from the human lens. The principal points emphasized include the nature of the capsule as a laminated basement membrane; the relative paucity of extracellular space; the presence of interdigitating cell processes in the epithelium and fibers as well as "tight" cell membrane junctions; the progressive increase in cell fibrosity in older and more deeply situated lens areas. A bibliography relating to lens ultrastructure is provided.

The principal physiological problems confronting the student of the crystalline lens may be briefly listed as: (1) How are metabolites exchanged throughout the depth of a relatively thick and avascular tissue? (2) What factors contribute to transparency and how are these influenced by various abnormal states? (3) What structural factors are involved in maintaining the structural integrity of the lens despite normal physiological deformations? To the solution of some of these problems electron microscopy has made significant contributions.

By any means, the lens is a difficult object to fix. This is particularly true of the poorly penetrating fixatives employed in electron microscopy. Solutions of osmium tetroxide even as high as 40 per cent (in carbon tetrachloride) fail to penetrate the full thickness of a mature rabbit lens during several days' exposure. Similar difficulties are encountered with glutaraldehyde and potassium permanganate. Yet many of the problems that interest us most concern the depths of the lens. Therefore with the exception of a single report on the tiny lens of the neonatal mouse no studies have been reported on other than the lens capsule, epithelium, and cortex. Studies of deeper regions have to date involved cleaving the lens before fixation and resulted in numerous difficulties of interpretation.

Electron microscopy reveals that the lens capsule consists of laminated faintly fibrillar sheets which lie parallel to the lens surface and which by all-current evidence represent replicated basement membrane. The laminated structure, however, is only apparent in sections perpendicular to the lamellar planes (Figs. 1, 2, 10). In studies of lens induction and formation in the chick and mouse all investigators have noted the presence of a basement membrane underlying the embryonic ecto-
Fig. 1. An electron micrograph of zonule fibers (z) and capsule fibers (c). Note the periodicity of the zonule filaments as best seen between the arrows and other fibers (f) at the capsule surface. These may also be zonule fibers. (×72,500.)
derm and then the lens plate prior to formation of the lens vesicle. A similar basement membrane overlies the surface of the optic vesicle. The basement membrane of the lens vesicle becomes the first extracellular coating of the embryonic lens. With growth of the lens this coating thickens by lamination and expands.

One may also note in passing that the inductive influence of the optic vesicle must pass through its basement membrane and that of the nearby ectoderm. In addition, no protoplasmic connectives of optic vesicle and lens plate have been observed, thus placing in doubt the concept of a "primary vitreous" originating by this means.10

Details of the separation of the lens from the ectoderm remain to be worked out, but a line of cytolyzing cells has been observed across the stalk of the lens vesicle,7 thus confirming numerous observations by light microscopy (summarized by Glücksmann13).

The lens capsule's identity as a basement membrane is based on its origin, similar response to P.A.S. staining, and similar dissolution by collagenase. The zonule fibers, while also P.A.S. positive, stain metachromatically and in this respect differ from the capsule.34 Fluorescent antibodies prepared against the capsule also stain many basement membranes elsewhere in the body.10 Similarly, fluorescent antibodies to basement membranes of kidney glomeruli stain the lens capsule.25 Unsolved problems relative to the capsule include the mechanism of its replication and expansion about the growing lens. It is possible that replication involves the secretion by the lens of molecules which interact with collagen precursors in the extracellular space. The participation of the lens in capsule formation follows from the observation that the lens capsule thickens during lens growth while nearby basement membranes underlying the skin ectoderm or overlying the optic vesicle do not thicken to the same extent if at all.7

Other fibrous materials are seen by elec-
Fig. 3. An electron micrograph of the lens epithelium illustrating small mitochondria (m), lipid droplets (l), as well as a terminal bar (t) on the interdigitating border of two cells. (x17,300.)

Fig. 4. An electron micrograph of a section tangential to the lens epithelium to illustrate the large intercellular spaces about the cells. A portion of a nucleus (n) is seen as well as numerous small cross-sections of interdigitating processes. (x8,350.)
Fig. 5. An electron micrograph of the borders of several epithelial cells just below the lens capsule (c). The nucleus (n) of one cell is also seen. Note the somewhat periodic character of the expansions of the intercellular space (arrow). (x27,500.)

Fig. 6. An electron micrograph of interdigitations of adjoining cells of the lens epithelium to illustrate how fine (arrow) the interdigitating processes may be. (x24,000.)
Fig. 7. An electron micrograph of the border between lens epithelium and underlying cortical fibers to show an occluded zone (arrow) as well as the Golgi region (g) of an epithelial cell. (×25,000.)

Fig. 8. An electron micrograph of a junction of two epithelial cells to show a terminal bar region (t) in some detail. The capsule is at (c). (×36,000.)
Fig. 9. An electron micrograph of a section almost tangential to the lens epithelium and at a level just above the lens cortex to show the numerous terminal bars (arrows) about the border of individual cells. The variation in the levels of the terminal bars appears to preclude their demonstration in a single section as a complete circumferential structure. (x7,700.)

Electron microscopy to be associated with the capsule. The zonule fibers which exist as an external coating of the capsule in the equatorial region have a suggestion of structural periodicity contrasting with the faintly fibrillar material of the capsule proper (Fig. 1). In addition, buried in the capsule are rare patches of other fibers (Fig. 1, f), some of which (Fig. 2) show a 500 to 600 A periodicity in preliminary measurements. The zonule striations have occasionally appeared as two main bands about 160 A apart, repeating at about 675 A intervals. It should be recognized however that periodicity dimensions are markedly influenced by processing procedures. Other fibers buried in the superficial capsule seem to resemble zonule material, possibly overgrown during capsule and lens growth. Jakus has also noted the presence of granular material. Students of the chemistry or immunochemistry of the capsule must take note of these contaminating materials which are probably chemically related although distinct.

Turning to the epithelium of the lens, one must note that as a structure formed by the invagination of ectoderm, the inner aspect of the lens ectoderm is the homologue of the outer surface of the cells of the embryonic ectoderm. The latter cells are separated by a network of terminal bars at their outer aspect and the cells of the lens epithelium are separated by these bars at their inner aspect (Figs. 3, 8, and 9). However, the terminal bars do not occur at a precise plane as in other epithelia and, short of serial sectioning, it is difficult to establish that they form a complete ring about each cell although this is probably the case. In the equatorial region where cells are transforming to fibers, terminal bars may sometimes be seen on the outer aspects of the ingrowing cells. In other epithelia a distinctive region of the terminal bar complex known as the
Fig. 10. An electron micrograph on a postequatorial zone of the lens epithelium to illustrate the lack of interdigitations in the region of growth of cortical fibers. (×35,000.)
Fig. 11. An electron micrograph illustrating an occluded zone (zo) involving the cell membranes of an interdigitating process and an adjoining cell. A cell nucleus (n) is evident as well as a nonoccluded intercellular border (b). (×55,600.)

Fig. 12. An electron micrograph to illustrate in detail an occluded zone, 105 Å in width, along the border of two epithelial cells. Note the dark intercellular line which characterizes such zones. (×165,000.)
Fig. 13. An electron micrograph illustrating the interdigitations of lens cortical fibers in longitudinal (left) and cross-section (right). Note the increased density of the process. (x72,000.)

Fig. 14. An electron micrograph illustrating a more complex interdigitation of two lens processes. (x65,600.)
Fig. 15. An electron micrograph of a border of two cells of the lens cortex showing an occluded zone (zo) as well as a mitochondrion (m). (×71,500.)

Fig. 16. An electron micrograph of a border of two lens fibers to show an occluded zone. (×60,000.)
occluded zone (zonula occludens) or tight junction has been described\textsuperscript{10} and has been the subject of considerable speculation as to its physiological import. In this zone the usual intercellular gap of 200 Å between the dark lines representing a part of the cell membrane in osmic tetroxide-fixed materials is narrowed to about 100 Å. These zones are not present in association with the terminal bars of the lens epithelia but do occur as patches at other levels between membranes of the epithelial cells (Figs. 11, 12) as well as between the membranes of lens fibers (Figs. 15, 16) or fibers and epithelial cells (Fig. 7). These probably correspond to Jakus\textsuperscript{15} “dense segments.” Before considering their possible significance, one should also note the presence of interdigitations of cell processes of adjoining epithelial cells (Figs. 3, 6, 8) (and lens fibers) as first reported by Wanko and Gavin\textsuperscript{30} and Cohen.\textsuperscript{6} It seems reasonable to assume that these make for a “stabilizing” of the lens structure which possibly serves to resist the effects of lens deformation during accommodation changes. In any event, these interdigitations are minimal in the zone of fiber growth (Fig. 10). The occluded zones occur not only as random patches between cells but can also occur between a cell face and the membrane of an indenting interlocking process (Fig. 11). In certain nervous tissues or in smooth or cardiac muscle, such zones have been shown to permit the passage of electrical perturbations from cell to cell without neurohumoral intervention.\textsuperscript{35} It is conceivable that differential metabolism of lens regions tends to generate potential differences and the tight junctions might permit electrical equilibration of the lens. Such a view is highly speculative, however. In the epithelium of the intestine the existence of the tight junction as a complete circumferential band between adjoining epithelial cells has been considered as an arrangement which might prevent intercellular passage of materials from the lumen of the intestine to the submucosa and compel intracellular passage.\textsuperscript{10} However, preliminary examination of tight junctions in the lens and its epithelium suggests an occurrence in patches rather than as a complete seal about the cells. It may well be that the tight junction’s primary role is adhesive. The facility with which electrical disturbances pass across tight junctions also suggests that they may permit intercellular movement of certain small molecules, with bypassing of the extracellular space. Another observation of possible significance, although based only on experience with a single fixative, is the existence of somewhat periodic dilatations of the space between adjacent epithelial cells (Figs. 5, 8) suggesting the possibility of fluid moving between cells by peristaltic passage. While spaces between epithelial cells are common (Fig. 4), it is possible that they are an artifact representing shrinkage of the cells.

The cytoplasm of the epithelial cells (Figs. 3, 5, 6, 7, 8, 10, 11) possesses the usual organelles including numerous small mitochondria, ribosomes, and oil droplets, and a Golgi zone is likewise present (Fig. 7) but is not prominent. The cytoplasm of the epithelial cells also contains fibrillar material similar in appearance to that seen in cortical cells (Figs. 5, 6, 7, 8, 11, 12). Indeed, there is a progressive increase in the concentration of cytoplasmic fibrillar material as one proceeds from the surface to the deeper-lying regions of the lens or to the older regions. In addition, the interdigitating processes of adjoining lens fibers also show an increase in fiber concentration (Figs. 13, 14).

As one proceeds deeper into the lens the osmiophilia of the cytoplasm, apparently due to an increasing concentration of fibrillar material, rises so sharply as to totally obscure other cytoplasmic components. At these levels the intercellular zones are far less dense than the cytoplasm and give sections of these layers the appearance of a negative image. One can still see interlocking processes and rare nuclei, the latter being less dense than the surrounding cytoplasm.
As previously noted, the cortex shows occluded zones of adjoining cell membrane both in patches along the length of fibers and on their interlocking processes. In addition, Wanko and Gavin, in the only study of suture areas, noted the presence of the type of desmosome now termed maculae densae, attachment plaques with an increase in the intermembranal space and complex bandings in the intercellular gap. These cell junctions are characteristic of the prickle cell level of the epidermis or conjunctiva.

Although the epithelium is relatively rich in mitochondria, the concentration of this organelle falls off sharply in the cortex except where cortical fibers reach the surface posteriorly. Insofar as the deeper cortex can be explored in the face of increasing background osmiophilia, the mitochondria appear to be quite rare, and it is probable that they are absent in the deeper regions of the lens which accordingly must then depend on an anaerobic metabolism.

A general observation on the lens is the apparent paucity of extracellular space in electron micrographs, a situation resembling that found in electron micrographs of central nervous system where measurements show a range of 5 to 15 per cent depending on the average size of cell processes in a given volume. This poses a number of problems relating to the transport of metabolites to more deeply lying regions of the lens.

References referring to lens development are 5, 7, 14, 33; the zonule 17, 21, 22, 23; the lens capsule 1, 2, 6, 15; the lens epithelium 6, 18, 30, 31; the lens fibers 4, 6, 11, 12, 20, 24, 26-29. An electron micrograph of isolated alpha crystallin may be seen in the paper of Bloemendahl and associates.

REFERENCES

American Institute of Biological Sciences, p. 161.


