Structural differences between regions of the ciliary body in primates

K. Ham, E. Lütjen-Drecoll, H. Prestele, and J. W. Rohen

The ciliary bodies from four rhesus monkey and six human eyes were subdivided into eight adjacent zones. The morphology of the ciliary epithelium and bordering stroma at the crests of the processes was examined electron microscopically in each region. In two of the monkey eyes (3 months and 18 years old) and in two of the human eyes (18 years and 55 years old) profiles of mitochondria, rough endoplasmic reticulum (RER) and Golgi complexes were counted at different locations, and the values for different zones within each individual eye compared by a multifactoral analysis of variance. Fenestrations in the endothelium of the capillaries in the stroma bordering the epithelium were also counted. The basal and lateral infoldings of the nonpigmented epithelium (NPE) and pigmented epithelium (PE) are most prominent in the anterior and middle areas of the pars plicata (zones I and II). Mitochondria and RER are significantly more frequent in this area. The stromal layer is very thin, and the capillary endothelia exhibit a large number of fenestrations. These findings suggest that the anterior portion of the pars plicata is involved in aqueous humor production. In the transition zones between the pars plicata and pars plana and in the pars plana (zones III to V) the number of mitochondria and RER profiles is decreased. In contrast, Golgi complexes are most frequent in zone IV, perhaps relating to the increased mucopolysaccharide production observed in this area. Special interconnections (wedgelike, mushroomlike, and fingerlike) were observed between the epithelial cells and between the PE and the stroma in zones III to V. These structures may serve as additional mechanical interconnections, which strengthen the tissue and thereby play an important role when it is put under tension during accommodation.

Key words: aqueous humor, ciliary body, ciliary epithelium, vitreous body, zonular apparatus.

The ultrastructure of the ciliary epithelium has usually been investigated in terms of aqueous humor secretion or the zonular epithelial attachment. Experimental studies in rabbits revealed at least two structurally and functionally different regions of the ciliary epithelium, but human data are lacking.

Recent studies in monkeys showed that the pars plicata and pars plana of the ciliary body reacted differently to paracentesis or prostaglandins. It therefore seemed likely that there were also regional differences in the ultrastructural organization of the primate ciliary body, especially the epithelium. We have now confirmed
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Fig. 1. Localization of 8 regions in the pars plicata and pars plana of the ciliary body. Ia, IIa, and IIIa, Tips of the ciliary crests (top regions); Ib, IIb, and IIIb, recesses of the ciliary crests (bottom regions); IV and V, pars plana.

Table I. Source of eyes studied

<table>
<thead>
<tr>
<th>Species and age</th>
<th>Pathological condition</th>
<th>Time of fixation</th>
<th>Studied quantitatively</th>
</tr>
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<tbody>
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<td>Rhesus monkey*</td>
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<td></td>
<td></td>
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<tr>
<td>3 mo.</td>
<td></td>
<td>Immediately after enucleation</td>
<td>Yes</td>
</tr>
<tr>
<td>7 mo.</td>
<td></td>
<td>Immediately after enucleation</td>
<td>Yes</td>
</tr>
<tr>
<td>4 yr.</td>
<td></td>
<td>Immediately after enucleation</td>
<td>Yes</td>
</tr>
<tr>
<td>18 yr.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Man:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 yr.</td>
<td>Malignant melanoma of choroid</td>
<td>Immediately after enucleation</td>
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</tr>
<tr>
<td>18 yr.</td>
<td>(Car accident)</td>
<td>4 hr. postmortem</td>
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<tr>
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<td>(Autopsy)</td>
<td>24 hr. postmortem</td>
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<tr>
<td>55 yr.</td>
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</tr>
<tr>
<td>67 yr.</td>
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</table>

*Macaca fascicularis.

this electron microscopically in rhesus monkeys and humans.

Material and methods

Four monkey (Macaca fascicularis) and six human eyes of differing ages were studied (Table I). The age of the monkeys was known exactly, since they were born in captivity. In the human cases of choroidal melanoma the tumors were so sharply defined and posteriorly located that the ciliary body could be considered normal.

Following enucleation the anterior portion of the globe was separated from the posterior portion behind the ora serrata. The ciliary body was then cut into 1 mm wide sagittal sections. The sections were fixed with 2.5 percent glutaraldehyde and embedded in Epon. Ultrathin sections were then made from the Epon blocks which had been trimmed into the following five zones numbered from anterior to posterior: zone I, transition area between iris and ciliary epithelia; zone II, middle portion of the pars plicata; zone III, transition area between pars plicata and pars plana; zone IV, middle portion of the pars plana; and zone V, posterior portion of the pars plana.
Fig. 2. Electron micrograph of the basal portion of the NPE in zone II (top region). Basal infoldings (arrows) and lateral interdigitations (asterisk) are shown. Note the concentrically arranged RER and the large amount of mitochondria (M). N, Nucleus. (Macaca fascicularis; \( \times 10,000 \).)

Only the crests of the ciliary processes proper were examined; the valleys between the processes were not studied. The tips of the crests are referred to as "top regions" and the recesses between tips are referred to as "bottom regions." The blocks containing zones I, II, and III were trimmed to isolate the top regions (Ia, IIa, IIIa) and the bottom regions (Ib, IIb, IIIb) (Fig. 1).

Quantitative evaluation. Overlapping photographs of two human and two monkey eyes (Table I) were taken at 2,300x and 5,000x of each of the five zones in different parts of the circumference of the eye. The sections were selected on the basis of their orientation being exactly sagittal (which could be determined from the position of the two epithelia) and of their exhibiting no artifacts. Several sections of one zone, usually taken from different parts of the circumference, were evaluated. The same cell was never used in more than one section. In each monkey eye 13 ciliary epithelial cells were evaluated in each of the eight zones/subzones. In human eyes 20 cells from each zone/subzone were studied. Cells in excess of these numbers were excluded, based on random numbers.

Countings of the cell organelles in the pigmented epithelium (PE) and the nonpigmented epithelium (NPE) of zones I to III were made on electron micrographs which were examined under a stereomicroscope at 50x magnification. The number of mitochondria, rough endoplasmic reticulum (RER), and Golgi complexes was determined in each zone. A slice of an organelle was counted as though a complete organelle had been seen.

The circumference of the blood vessels in the stroma was measured along the basal membrane, and the number of fenestrations in the endothelium of the vessels determined.

Statistics. The quantitative data were analyzed by two-factor analysis of variance (ANOVA) (program FO42 from the Statsys statistics program package at the Universitätsrechenzentrum Erlan-
Fig. 3. Electron micrograph of the ciliary epithelium and stromal layer of zone III. CC, Covering cells; CE, capillary endothelium; FP, fingerlike process; MP, Mushroomlike process; ST, stromal layer; WP, wedge-like process; Z, zonular fibers. (Macaca fascicularis, 3 months old; x7500.)

gen): factor 1, localization; factor 2, epithelium type. Replications numbered 13 in monkeys and 20 in humans. Given significant F values, localization comparisons were undertaken with the use of Tukey's method of multiple comparisons. The statistical tests each refer to an individual eye. Since our data represent frequencies, one requirement for ANOVA (normal distribution) is not fulfilled. Because the values determined for the Golgi complexes are small, those results must be looked at particularly critically. Furthermore, the number of applied tests is very large, so that chance "significance" can occur.

Results

Differences between the face top regions of the ciliary body

Epithelial layers. In the anterior part of the pars plicata (zones Ia and IIa) the cells of the NPE and PE are about the same size and nearly square. They are not in register. Cytoplasmic folds occur on the inner (basal) surface of the NPE. Intercalation between adjacent NPE cells is extensive (Fig. 2). The cell borders be-
Fig. 4. Electron micrograph of the apical portion of the NPE and PE in zone III. Note the mushroomlike cytoplasmic processes of the PE (arrows) and the wedgelike processes of the NPE (asterisk). G, Golgi complex. (Macaca fascicularis; x15,000.)

Between the NPE and PE area are relatively smooth; that is, mushroomlike processes do not occur as they do more posteriorly in the ciliary epithelium.

In the basal portion of the PE, fine-fingered, extensively branched cytoplasmic folds are seen, into which no stromal tissue penetrates.

In the NPE of zone Ia occasional pigment granules are seen; in the NPE of zone IIa, no pigment granules are seen. No other major structural difference between the cells are found. Between zones IIIa and V the form and structure of the ciliary epithelium changes. In monkeys the NPE becomes flatter, whereas the PE gradually becomes higher and higher; the overall effect is an increase in height of the epithelium. In humans the NPE is higher than the PE, and both epithelial layers take on a more extended cylindrical form. The basal foldings in the cytolemma in the NPE proceeding from zone IIIa to zone V become increasingly lower and more simple. The lateral infoldings become less numerous (Fig. 3). Long, mushroomlike cytoplasmic processes of the PE, exhibiting many desmosomes, appear in large numbers and extend into the apical cell membranes of the NPE. In addition, long, wedgelike cytoplasmic processes in the apical part of the NPE extend between the lateral junctions of the PE (Fig. 4). Within these processes as well as in the adjacent cytoplasm of the NPE, there are occasional small straight bundles of fine filaments which resemble the rootlets of cilia (Fig. 5).

In zones IV and V the basal infoldings of the PE are more simply formed and less numerous than in the more anterior cells. Stroma frequently penetrates into the basal portions of the PE, so that connective tissue interlockings between the stroma and epi-
Regional differences in ciliary body

Fig. 5. Electron micrograph of the striated filaments (F) in the apical cytoplasm of the NPE in zone IV. Arrows indicate apical cell junction between NPE and PE. (Macaca fascicularis, 3 months old; x30,000.)

...thelium occur; this is especially prominent in zone III (Fig. 3).

The cell organelles (mitochondria, RER, and Golgi complexes) are more numerous in the NPE than in the PE (Fig. 6). The mitochondria are found mainly in the basal portion of the cytoplasm of the NPE in all five zones. They are noticeably large and long and are of the crista type. In the PE the mitochondria are smaller than in the NPE and are found along the basal and apical borders of the cells. In the basal area they are usually seen between the infoldings of the cytolemma. In the middle part of the PE cells, where the nucleus is found, mitochondria are infrequent. RER is found mainly near the nucleus or in the apical portion of the NPE cells as single cisternae or parallel stacks. In addition there are concentric systems of closely spaced cisternae, which are found only in the basal part of the cytoplasm. In the apical area of the PE single cisternae and occasional stacks of ergastoplasmic cisternae are observed. The Golgi complexes in the NPE are found to the side of or apical to the nucleus (Fig. 7). Golgi complexes are infrequent in the PE, but when they occur, they appear in the apical part of the cell.

With the use of ANOVA, global differences between the NPE and PE in regard to the three types of cell organelles were found (p < 0.01 for all three factors). Combining the two epithelia, differences between the five zones in the number of mitochondria and amount of RER become apparent. Subsequent pair comparisons revealed that this finding is the result of differences within the NPE only. With one exception (more RER in the PE of the younger monkey in zone II than in zone V) there were no noticeable differences between different regions of the PE (Fig. 8).

Struma. The basal membranes of the ciliary epithelium in monkeys are thin and exhibit a uniform structure. In humans they are generally thicker in the pars plicata than in the pars plana and are irregularly shaped in the various zones. The fenestrations in the capillary endothelium are very...
numerous in zones Ia and IIa, especially the latter. Proceeding posteriorly, they become less common until in zones IV and V they are no longer observed (Fig. 9). Furthermore, the stromal layer between the ciliary epithelium and the vascular wall becomes thicker toward the posterior zones in humans. In zones IV and V more cells, collagenous fibers, and elastic fibers of middle diameter appear. The elastic fibers represent, in part, posterior tendons of the ciliary muscle. The membrana limitans interna is noticeably thin in zones I and II but slowly thickens posteriorly and combines more and more with the zonular fibers. Unmyelinated nerve fibers were frequently found in the stroma and between PE cells in all zones.

**Differences in the structure of the ciliary epithelium between top and bottom region of the ciliary crests**

*Ciliary epithelium.* The NPE is flatter and the superficial cytoplasmic infoldings are fewer in number and more simply shaped in the bottom region than in the top region. In the bottom region irregular interconnections are observed between the membrana limitans interna and the end offshoots of the zonular fibers and between the membrana and the ciliary epithelium. These interconnections are particularly prominent in zone IIb of the younger human. Cyto-
Fig. 7. Electron micrograph of the apical portion of the NPE in zone IV. G, Golgi complex; M, mitochondria; MP, mushroomlike processes of the PE; N, nucleus. (*Macaca fascicularis; 5×30,000.*)

<table>
<thead>
<tr>
<th>MONKEY</th>
<th>HUMAN</th>
<th>HUMAN</th>
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<tr>
<td>top young</td>
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<td>la</td>
<td>Ib</td>
<td>IIa</td>
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Fig. 8. Comparisons of the NPE of different regions. Region along abscissa compared to region along ordinate. Number of profiles in abscissa region significantly greater: *p <0.05; **p <0.001 (Tukey method). Mitos, Mitochondrial profiles; ER, RER profiles; Golgi, Golgi apparatus profiles. Roman numerals II and III on ordinate indicate IIa and IIIa when comparison is with Ia to IIIa, but indicate IIb and IIIb when comparison is with b regions.
Fenestration

- - 18-year-old male
- - 55-year-old female
- - 3-month-old monkey
- - 18-year-old monkey

Fig. 9. Number of fenestrations found in the capillary endothelium of the stromal vessels. Abscissa = region; ordinate = number of fenestration/100 μm circumference.

lemmic infoldings of the basal PE are less developed in the bottom regions, but finger-like stromal processes are observed. Differences in the form and distribution of pigment granules are not observed.

Regional differences in the frequency of cell organelles are less clear in the bottom regions than in the top regions. They are found in the NPE, but not the PE. In the younger human, differences are observed only in frequency of RER. The average frequency of mitochondria in the older human is greater in zone Ib than in zones IIb and IIIb.

Stroma. No differences between the top and bottom regions with regard to the basal membrane of the capillary wall and the ciliary epithelium are observed. Fenestrations in the capillary endothelia are found relatively seldom in the bottom region.

The stromal lamella between the vessels and the epithelium has approximately the same thickness in all zones. Bundles of unmyelinated nerve fibers are found in the bottom regions of the stroma and between the PE cells.

**Age differences in the ciliary epithelium.** In the NPE of the older monkey the mitochondria are on the average more numerous than in the younger monkey. In contrast, the frequency of the RER in the older monkey is less than in the younger one. The basal membranes in the anterior portion of the older eyes are clearly thicker than those in the younger eyes, and the membrana limitans interna in zone III is definitely thicker than in the other zones.

More fenestrations could be counted in the capillary endothelium of the stroma in the older monkey than in the younger one (Fig. 9). In the ciliary epithelium and particularly in the NPE, numerous lipid inclusions are found in older eyes. Similar differences were found in the corresponding regions of the younger and older human eyes. However, the time between death and fixation differed in the two human eyes.

**Discussion**

Electron microscopic and quantitative analysis of the ciliary epithelium of four monkey and six human eyes reveal clear regional differences in structure which probably correlate with differences in function (Fig. 10). In the strict sense, the statistical tests serve only to eliminate random variability within the individual eye. Although our experience has been that structural patterns of this kind found in one eye can be generalized, we realize the need for study of more eyes.

In the top regions of the NPE of the anterior portions of the ciliary body (zones I and II) the frequency of mitochondria is significantly higher than in the posterior portions (particularly zones IV and V). The profiles of RER are more frequent in zone II than in the other zones. Concentric
RER lamellae are found basally in zones II and III. The most extensive infoldings of the cell membrane (including both basal and lateral portions of the cells) are found in the NPE of the anterior ciliary body (zones I and II). There are also numerous infoldings in the basal portion of the PE. In contrast to the NPE, in the PE no significant differences exist between the five zones in the number of these cell organelle profiles. The pigmented epithelial cells of the anterior two zones lie on a thin straight basal membrane; the stromal layer is very slender, and the fenestrations in the capillary endothelium are clearly more numerous than in the pars plana.
Most authors agree that the fenestrations in the capillary endothelium and the infoldings of the cytolemma along the basal surface of the epithelial cells, which include mitochondria, are involved in the production of aqueous humor. These cellular structures are particularly numerous in the top region of the pars plicata (especially in zone II); on the basis of structure it must be assumed that the top regions are primarily involved in aqueous humor production. This assumption is also supported by the fact that the stromal layer between the fenestrated capillary walls and the ciliary epithelium is extraordinarily thin, thereby encouraging fluid exchange between the vessels and ciliary epithelium.

Mitochondria are more numerous in old age, whereas the other cell organelles examined are less numerous. In old age the stromal layer and the basal membrane become much thicker, a finding also reported by other authors. Mitochondria are more numerous in old age, whereas the other cell organelles examined are less numerous. In old age the stromal layer and the basal membrane become much thicker, a finding also reported by other authors.

The number of fenestrations in the capillary endothelium in zone II is greater in the older eye than in the younger eye. The thickening of the basal membrane and the widening of the stromal lamella in old age must make aqueous humor production more difficult. It could be that to compensate for this difficulty, the ciliary processes develop more capillary fenestrations and more mitochondria in the NPE. However, it is also possible that an increased number of fenestrations in the capillary endothelium leads to an increased escape of plasma, thereby causing a hyalinization of the stroma and a thickening of the basal membrane. At present, one cannot distinguish cause from effect.

We do not know why a relatively large amount of RER arranged in both stacked and circular forms is found particularly in zone II. Since RER is involved in protein synthesis, one wonders whether proteins or their precursors are produced in this portion of the ciliary body.

Golgi material is more common in the NPE of the pars plana, especially in zone IV, than in the pars plicata, whereas RER is much more rare in the posterior zones. Golgi material is thought to function in the synthesis of mucopolysaccharides. Our findings are thus consistent with those of Fine and Zimmermann, who found an increased amount of mucopolysaccharides in this area and related it to vitreous body metabolism. However, the total amount of Golgi material in these zones is so small that one must assume that the mucopolysaccharides necessary for the regeneration of vitreous are also produced by other cells.

The mitochondria and the basal and lateral infoldings of the cytolemma of the NPE are much more infrequent in posterior regions than in anterior regions. In addition, fenestrations in the capillary endothelium are very rare. These findings indicate that the pars plana has only a minimal role in aqueous humor production.

The functional significance of filaments resembling the rootlets of cilia is not known.

Mushroom-shaped interlockings between the epithelial are observed more commonly in zones III to V than in the anterior regions. Apical, wedgelike extensions of the NPE are also seen. The stromal layer is substantially wider than in the anterior portion and includes many elastic fibers. Fingerlike stromal structures appear along the border between the PE and the ground plate. Both the mushoomlike projections of the PE and the wedgelike projections of the NPE contribute to an increase in the interconnecting surface area between the PE and NPE. Stromal infoldings increase the anchoring surface between the PE and stroma. This latter finding correlates well with those of Zimmermann and Rohren, who were able to demonstrate penetration of the PE into the stroma, beginning in the transition area of pars plicata to pars plana and continuing up the ora serrata. The stromal-PE interconnections are clearly increased in old age. In addition, Rohren and Zimmermann showed that varying the degree of contraction of the ciliary muscle...
changed the form of the NPE in the same region. Rohen and Rentsch\textsuperscript{12} proposed a theory of accommodation to explain these changes. According to their theory, tension from the accommodative apparatus is centered in long "supporting fibers" during relaxation and is transferred to more anteriorly located "tension fibers" during accommodation. The increase in surface area between the NPE and PE and along the basal PE could be a prerequisite for the cell shift which takes place during accommodation. However, the increase could also be result of stress which occurs during the transfer of tension between the zonular fibers and ciliary epithelium and stroma.

A surprising finding was that in the ciliary processes of the pars plicata, the basal and lateral infoldings of the NPE of the bottom region are clearly less prominent than those in the top region. Additionally there are practically no fenestrations in the endothelium of the stromal capillaries in the bottom region. This is an indication that the bottom region probably plays only a subordinate role in aqueous secretion. In recent scanning electron microscopic studies,\textsuperscript{22} it was noted that the NPE of the bottom region serves as the insertion point for wide-based zonular fibers. This might be the explanation for the structural differences observed.

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\textbf{REFERENCES}

18. Fine, B. S., and Zimmermann, L. E.: Light...


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