Some fine structural features of the ora serrata region in primate eyes

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The morphological transition between the pars plana and the neural retina at the ora serrata has been studied in two species of monkey and in a wide range of ages of the human eye. Some difficulty is had in distinguishing the precise cell type at the transition. The most characteristic change appears to be in the type of junctional complexes binding the pigment epithelium to the nonpigmented cells of the pars plana and to the neural epithelium. In the pars plana both zonulae occludentes and desmosomes are found, but on the retinal side of the ora serrata only desmosomes are present. The transition actually occurs between the columnar epithelial cells of the pars plana and two or three cells of somewhat uncertain or undifferentiated type, presumably Müllerian cells, on the retinal side. On both sides of the ora serrata, small intercellular spaces, representing remnants of the optic cavity, are observed. These spaces become confluent posteriorly, into which incompletely differentiated photoreceptors protrude. These consist, at first, only of an inner segment, to which is added, more posteriorly, a cilium and then a primitive outer segment. This transitional zone is approximately 0.28 mm. in width, varying somewhat between the temporal and nasal side in adult human eyes. The series of transitional stages is identical in two species of monkeys and in human eyes of all ages studied and is very similar to embryonic stages of human photoreceptor development.

Our knowledge of the anatomy of the region of the pars plana and the retina adjacent to the ora serrata depends almost entirely on the observations of light microscopists made many years ago. These descriptions, found in standard texts, simply state that at the ora serrata a rather abrupt transition of the columnar nonpigmented epithelial cells of the pars plana to the neural cells of the retina occurs, and that the photoreceptors immediately behind the ora serrata appear to be malformed. A more complete knowledge of this area is important, because the pathologic changes which often affect this region, even in young individuals, may lead in many instances to retinal detachment. To morphologists a knowledge of the structural alterations necessary to make this abrupt transition from a multilayered neural epithelium to a single layer of columnar epithelial cells is important. If the transition is as abrupt as it appears in the light microscope, a rapid modification in

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Fig. 1. Photomicrograph of the ora serrata region of a 2½-year-old human eye. The ora serrata (indicated by the arrow) is easily distinguished by the difference in staining of the pars plana (P.P.) and the retina (R.). The nonpigmented epithelium of the pars plana shows the typical extracellular pockets present in these cells up to the ora serrata line; Bruch's membrane (B), pigment epithelium (P.E.), choroidal capillaries (c.c.), outer segment (O.s.), inner segment (I.s.), external limiting membrane (E.L.M.). Glutaraldehyde-OsO₄ fixation embedded in Epon and stained with toluidine blue. (The line in each micrograph represents 1μ.)

Fig. 2. Photomicrograph of the ora serrata region of a 7-year-old human eye. The transition between the pars plana and the retina is indicated by the arrow. This photomicrograph demonstrates that the retina and the pars plana epithelium are in close contact with the pigment epithelium on both sides of the ora serrata line. No photoreceptor outer or inner segments can be seen adjacent to the ora serrata. To the left of this area, outer (O.s.) and inner photoreceptor segments (I.s.) may be seen, and in this region, there is no close contact between the pigment epithelium and the retina. Note the small and medium sized cysts (c) in the retina; pigment epithelium (P.E.), external limiting membrane (E.L.M.), choroidal capillaries (c.c.). The extracellular pockets in the nonpigmented cells of the pars plana are clearly shown. Glutaraldehyde-OsO₄ fixation embedded in Epon and stained with toluidine blue.
the function of the cells in this region is indicated. It is most important to learn if this may be correlated with structural changes in the basement membranes which cover the outer surface of the two layers and the structure of vessels which maintain the metabolism of the overlying tissue. Perhaps, of greatest interest in this region is the relationship of the outer pigment epithelium to the inner neural retina, both of which are derived from the optic cup and, in embryonic life, are separated by the large optic vesicle cavity. This paper is concerned with a description of the morphology of the ora serrata.

Materials and methods

Five human eyes (2½, 7, 29, 65, and 71 years of age) and two species of monkey eyes (Macaca mulata and Saimiri sciurea) were used in this study. The human eyes were obtained at surgery for small tumors affecting the most posterior portion of the globe or the anterior segment. In all cases, the ora serrata regions studied were considered normal by clinical examination and light microscopy. The monkey eyes were examined under the slit lamp and with an ophthalmoscope, and the globes were removed under anesthesia. Immediately after enucleation the eyes, both human and monkey, were opened by an equatorial incision well posterior to the ora serrata. The two halves were immersed either in 2 per cent osmium tetroxide (buffered to pH 7.4 with Millonig's buffer) for 1½ hours or in 2 to 3 per cent glutaraldehyde, prepared in the same buffer for 20 minutes, after which they were postfixed in 2 per cent osmic acid for 1½ hours. After the tissue had been in either fixative for a few minutes, the portion containing the ora serrata was further dissected so that flat pieces including sclera, choroid, pars plana, and peripheral retina were isolated and continued to fix as described. The tissues were then rapidly dehydrated in cold ethanol, and after removal of the sclera they were embedded in Epon.

One to 2µ thick sections were routinely cut for light microscopy. Only those blocks in which no retinal detachment was found were used for thin sections cut with a glass knife on an LKB ultratome. They were stained with uranyl acetate and lead citrate and examined with a Siemens Elmskop I.

During fixation the neural retina often separated from the pigment epithelium in osmic acid fixed but not in the glutaraldehyde postosmicated tissue. Therefore, most of our studies were based on this type of fixed material and the first group used for reference. Occasionally, some swelling of mitochondria with broken cristae was noted in the glutaraldehyde postosmicated tissue, but this does not affect our conclusions.

Results

All observations reported apply equally to the human eyes of all ages studied and to the two species of monkeys unless specifically excepted. Figs. 1 and 2 are light micrographs of the ora serrata region. The transition at the ora serrata appears to be quite abrupt. The staining characteristics of the pars plana epithelium serve to emphasize the sharpness of the transition. Fig. 2, which is of an eye 7 years of age, shows some cysts within the neural retina, very close to the ora serrata. They are a regular component of this area and were not found more posteriorly. These figures also demonstrate that a well-developed photoreceptor layer does not start immediately at the ora serrata but assumes a reasonably normal appearance only about 280 µ from it. This varies somewhat from the temporal to the nasal side. Bruch's membrane is readily demonstrable in both illustrations.

No vessels were seen in the retina proper of this region near the ora serrata, and no abrupt change was noted either by light

Fig. 3. Electron micrograph of the ora serrata region of a human eye aged 2½ years. This micrograph demonstrates the cells located at the ora serrata. The first cell (center of the figure) on the retinal side appears to be undifferentiated. Its cytoplasm contains large numbers of dense, probably RNP, particles. It is followed by a Mullerian cell (M.c.). The densely staining cell to the left is a nonpigmented cell (N.P.E.) of the pars plana. Note the attachment bodies (desmosomes, D.) joining the pigment epithelium to the undifferentiated cell and to the nonpigmented epithelium. Small lacunae may be noted between the nonpigmented and pigmented epithelium (P.E.) in which processes may be seen; centriole (cent.).
Fig. 3. For legend see opposite page.
Figs. 4 and 5. For legends see opposite page.
or electron microscopy in the capillary layer underlying the retina and the pars plana at the ora serrata. The capillaries under the pars plana became more irregular in size and spacing anteriorly. Bruch's membrane was structurally very similar on both sides of the ora serrata, as observed by Nakaizumi.\(^5\) Myelinated nerves were sometimes found immediately outside the choriocapillaris on both sides of the ora serrata, but their distribution was quite variable. According to Hogan,\(^4\) the internal limiting membrane of the ora serrata region is composed of the plasma membrane of the glial cells, the basement membrane, and the vitreous humor cortex, with a space between the plasma and basement membranes on the pars plana side but none on the retinal side. We found such a space between both the pars plana and the glial (Müllerian) cells and their respective basement membranes.

The electron micrographs (Figs. 3 to 10) illustrate regions beginning in the pars plana area through the ora serrata and progressing posteriorly toward the equator of the globe. The nonpigmented (the vitread cell layer) cells of the pars plana are firmly attached to the pigmented epithelium (the sclera cell layer) up to the ora serrata. Posterior to this for a short distance, the neural retina also is attached firmly to the pigment epithelium by desmosomes, but gradually neural retina becomes separated from it more posteriorly and apparently forms, at first, a discontinuous space and, finally, a continuous space in which the inner and outer segments of the photoreceptors are first seen. The size of the zone of transition from the ora serrata to normal photoreceptors varies, but is about 250 \(\mu\)m.

The pigment epithelial cells on both sides of the ora serrata appear to be quite similar. They are more columnar in shape than those posteriorly, with pigment granules, mostly of the mature type, in the apical and mitochondria in the basal zone. Basal infoldings are present but are not as elaborate as those in the ciliary processes nor as those in the fundus. They appear even more simple in the younger specimens. Rough-surfaced endoplasmic reticulum, free RNP particles, and vesicles are found throughout the cytoplasm. There are junctional complexes, mostly desmosomes (some zonulae adherentes and zonulae occludentes) at their lateral surfaces, as noted by Bairati and Orzalesi.\(^7\)

The nonpigmented epithelial cells of the pars plana contain abundant vesicles, mitochondria, filaments, and large numbers of unevenly distributed RNP particles. The cytoplasm has a rather dense appearance. Rough-surfaced endoplasmic reticulum and Golgi complexes are located prominently in the apical portion, facing the pigment epithelium. The structure of the nonpigmented epithelial cells was quite uniform up to the ora serrata. Our observations are in agreement with those of earlier workers in that they possessed “extracellular pockets” and were attached to each other, as described by Fine and Zimmer-

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**Fig. 4.** Electron micrograph of the ora serrata region of a 7-year-old human eye. This micrograph shows the junction complexes between the pigment and nonpigment epithelium of the pars plana in this region. The arrow indicates the space between these two cell layers, which contains microvilli from nonpigment epithelium. Pigmented epithelium (P.E.), nonpigmented epithelium (N.P.E.), pigmented granule (P), desmosomes (D), zonulae occludentes (z.o.), zonulae adherentes (z.a.).

**Fig. 5.** Electron micrograph of a 7-year-old human eye in the ora serrata region. This micrograph shows the junction complexes on the retinal side of the ora serrata. The arrow indicates the large space between the pigmented epithelium and the retina which is much larger than those shown in Fig. 4 and contains numerous and longer cell processes. Note the two incompletely differentiated neurons (n) enclosed by Müllerian cells (M.c.). One of the neurons contains two centrioles (cent.), desmosomes (D).
Fig. 6. For legend see opposite page.
man and by Bairati and Orzalesi, who applied the modern terms (desmosomes, zonulae adherentes, and zonulae occludentes) to their junctional complexes.

The type and distribution of intercellular junctions were studied in the areas immediately anterior and posterior to the ora serrata. The nomenclature used and the identification of the various junctional complexes follow closely that proposed by Farquhar and Palade. Three types were seen, zonula occludens which are tight junctions consisting of the fused opposing cell membranes, adjacent to which the cytoplasm is variable in density. It is believed that these tight junctions (zonula occludens) form a continuous belt around the cell effectively separating the lumen apical to the cell from the intercellular space. Such continuity of the cell junction can rarely be proved and was not achieved in the present experiments. Zonula occludens are located more apically than are the other junctional complexes. The term “terminal bars” used in light microscopy doubtless refers to these structures. Examples of zonula adherens were found. It is an intermediate type of junction, not necessarily continuous, and there is a space evident between the opposing cells about 200 Å in width which is filled with an amorphous material. The cell membranes in this junction are strictly parallel and extend for 0.2 to 0.5 μ. The adjacent cytoplasm is dense. Many examples of the third type, macula adherens, were seen. This is commonly known as a desmosome, a term we employ because it is shorter and so often used. It is a disc-shaped discrete plaquelike attachment body on which bundles of cytoplasmic fibrils converge. The adjacent cytoplasm is dense and the space between the cell membranes is about 240 Å.

The identity of the cell type immediately posterior to the ora serrata is not as well defined as might be supposed from observation of light micrographs. This is because the first cell (Fig. 3) on the neural retinal side of the ora serrata does not appear to be as well differentiated as those more posteriorly. However, the cytoplasmic appearance of the “undifferentiated cell” is closer to that of a Müllerian cell than to that of a neuron or a pars plana cell. It contains electron dense particles which are mainly free RNP mixed with glycogen particles, vesicles, Golgi complexes, and mitochondria, but there is very little rough-surfaced endoplasmic reticulum. Centrioles are often seen in these cells.

Posterior to the “first” cell are typical Müllerian cells containing glycogen particles, short rod-shaped profiles of rough-surfaced endoplasmic reticulum and small dense mitochondria, usually in the outer portion near the external limiting membrane. Smooth-surfaced endoplasmic reticulum and fine filaments are located throughout the cell processes which traverse the whole thickness of the retina. Müllerian cells in this region possess microvilli which project into the lacunae, mentioned below, toward the pigmented epithelium. Their nuclei are located in the midportion of the retina.

Fig. 4 illustrates this transition zone in a 7-year-old human eye. There are marked differences in the junctional complexes attaching the cells to each other on the pars plana and the retinal side of the ora serrata.
Fig. 7. Electron micrograph of the retina near the ora serrata of a 29-year-old human eye. This micrograph shows the "mushroom" appearance of the inner segments (I.S.) of the photoreceptors typical of this area in both monkey and human eyes. Note that the retina is completely separated from the pigment epithelium. External limiting membrane (E.L.M.).
Zonulae occludentes, desmosomes, and probably some zonulae adherentes are found between the pigment and nonpigment epithelium on the pars plana side of the ora serrata. Occasionally, small intercellular spaces, or lacunae, occur between the two epithelia which represent the old optic vesicle cavity and contain microvilli, derived both from the pigmented and nonpigmented epithelium.

On the neural retinal side of the ora serrata, i.e., posterior to it, the Müllarian cells are not only attached to each other, but also occasionally to the pigment epithelium by desmosomes. However, no zonulae occludentes are found in this region (Fig. 5). This attachment of Müllarian cells to the pigment epithelium was not found posterior to a clearly recognizable photoreceptor, i.e., one which had an inner or outer segment and protruded sclerad past the external limiting membrane. The first identifiable neurons, which are only a few cells removed from the ora serrata, appear to be undifferentiated and are always completely enclosed by Müllarian cells, i.e., lie entirely internal to the external limiting membrane. These cells have the cytoplasmic characteristic of neurons, the mitochondria are concentrated in the portion of the cytoplasm toward the pigment epithelium as in photoreceptors, but they are not arranged in layers as they are more posteriorly. They possess no cell processes, and no normal synaptic connections exist in this region. Small spaces (lacunae) between the retina and the pigment epithelium are also found in this region, as in the pars plana area, but are larger as one progresses posteriorly and contain longer processes derived from both the pigment epithelial and Müllarian cells. These processes are very similar, excepting in length, to those found anterior to the ora serrata. The apparently isolated spaces finally became confluent posteriorly. The terminal bars linking Müllarian cells to one another were seen immediately behind the ora serrata and were continuous with those which formed the external limiting membrane more posteriorly.

In this area, slightly more posteriorly (Figs. 6 and 7), some of the photoreceptor neurons are found to possess short, round, primitive inner segments, most of which have a rounded "mushroom" appearance and protrude past the external limiting membrane into the lacunae. The cytoplasmic organelles of these incomplete inner segments are qualitatively normal and characteristic of inner segments. The attachment bodies, which constitute the external limiting membrane and bind them to the adjacent Müllarian cells, have the same structural characteristics as in the retina more posteriorly.

A steady progression of stages in which the outer segments first appear and then gradually reach normal adult structure and size can be found posteriorly. Examples of these are shown in Figs. 8 and 9. Some of them may consist of as little as a primitive cilium protruding from a poorly formed inner segment, as shown in Fig. 10, A, or primitive outer segments containing disoriented, or fragments of lamellae which are always attached to an inner segment via a connecting cilium (Fig. 10, B and C). The poorly organized lamellae are characteristic of the peripheral photoreceptors and not thought to be due to poor fixation. Such disorganization is not found a very short distance posteriorly. These malformed photoreceptors consist mostly of cones, as determined by the structure of their synaptic connections. The poorly developed synaptic connections were similar to cone pedicles of embryonic cones and not like rod spherules. The primitive "pedicle-like" structures with synaptic ribbons were a part of the cell body and not a specialized protruding portion of it (Fig. 11). Beyond this point, posteriorly well-developed, although short, photoreceptors are found (Fig. 10, D). These have spherules or pedicles which make synaptic connections, presumably with bipolar dendrites, but the body of the bipolar cell is not seen in this location. The first definitely identifiable bipolar cell is located still more posteriorly. The appearance of the entire ora serrata region is shown in Fig. 12.
Fig. 8. For legend see opposite page.
Figs. 8 and 9. These micrographs are of a region somewhat more posterior to that shown in Figs. 6 and 7 and are of a 2.5-year-old human retina. In this region, the first signs of an outer segment appear. The apical portion of the photoreceptor inner segment (I.s.) contains ribosomes (rb, Fig. 9). The membranes forming the disks of the outer segments (O.s.) are frequently highly irregular in arrangement. Pigment epithelium (P.E.).
Fig. 10. For legend see opposite page.
Discussion

The pigment epithelium is much more firmly united with the nonpigmented epithelium in the pars plana up to the ora serrata than it is, a few microns posteriorly, to the retina. On the pars plana side, zonulæ occludentes and desmosomes are responsible for the strong attachment. At the ora serrata, one finds a few desmosomes still attaching the pars optica retinae to the pigment epithelium, but these occur in a very narrow band posteriorly. This may explain why, in most cases of retinal detachment, the two layers are found still attached at this point as they are also in the detachments often seen in histological preparations. From the present observations of the fine structure of these cells, no reason for the firm attachment in the pars plana region can be found other than the junctional complexes. The cells of the two layers do not have extensive interdigitations, and, in fact, occasional small spaces are found between them. It is easy to understand how the neural retina is so readily separated from the pigment epithelium posteriorly because contact is maintained only by the delicate processes of the pigment epithelium and the outer segment, with no indication of attachment bodies.

Ueno and Inomata\(^6\) reported that, at the transition from the ciliary body to the retina, the nonpigmented cells of the pars plana were in contact with Müllerian cells. The electron micrographs examined in the present study indicate that these are not typical Müllerian cells, but rather are “undifferentiated” in morphology. Their structure has some of the characteristics of both the Müllerian and nonpigmented cells of the pars plana. However, we agree that these cells are much more similar to Müllerian cells than to neurons.

The graded series of photoreceptors, ranging from an undifferentiated neuron near the ora serrata to complete sensory cells more posteriorly, duplicates the embryonic development of these cells. Electron microscopic studies of the morphogenesis of the human retina have been made by Lerche\(^9\) in embryos of 4.5 cm. (10 weeks) to 16 cm. (22 weeks), and by Yamada and Ishikawa\(^11\) in embryos from 10 to 36 weeks’ gestation. If the morphology of the peripheral photoreceptors described here is compared with that of the differentiating cells reported by these authors, we find that the gradation from the ora serrata almost precisely duplicates the embryonic stages. Area \(I\), shown in Fig. 12, contains photoreceptors equivalent to those found in the retina of a 10 to 22 week fetus. In this area, the receptors are not differentiated, having neither an outer nor inner segment nor central connection. The neural retina is in close apposition with the pigment epithelium, excepting where separated by lacunae, and the terminal bars uniting adjacent Müllerian cells form an external limiting membrane. These Müllerian cells are in contact with the pigment epithelium, as they are in this stage of embryonic development and completely enclose the neuronal cells. Further posteriorly (area \(2\)) the photoreceptor cells are similar to those in the developing retina of a 23 week to 36 week fetus. In this area, and in embryos of this age, the inner seg-

**Fig. 10.** Electron micrographs of the outer segments of the photoreceptors found near the ora serrata in several human eyes, \(a\), Cilium on the apical aspect of a photoreceptor. It is not associated with an outer segment. Müller cell processes (M.c.p.). \(b\), Photoreceptor showing the connecting cilium (c.c.) and a partially formed outer segment (O.s.). \(c\), A more fully developed outer segment of a photoreceptor. Note the close relationship of the pigment epithelial cell processes (P.c.p.) to this poorly developed outer segment (O.s.). pigmem epithelium (P.E.). \(d\), A micrograph of one of the most peripheral complete photoreceptors with an essentially normal, although very short, outer segment (O.s.), inner segment (I.s.), connecting cilium (c.c.).
Fig. 11. Electron micrograph of one of the most peripheral photoreceptors in this human retina aged 7 years. The mitochondria are concentrated in the scleral portion of the cell. Note particularly the rudimentary "pedicle" with synaptic membrane which suggests that this photoreceptor is of the cone type. Next to it is an intraretinal space commonly found in this area which is not believed to be artifact. Müllerian cells (M.c.), mitochondria (M), Golgi apparatus (G), synaptic ribbon (Sr.). Glutaraldehyde postosmication fixation.
Fig. 12. Schematic representation of the structural arrangement of the ora serrata region in primate eyes. Pigment epithelium (P.E.), nonpigmented epithelium (N.P.E.), Müllerian cell (M.c.), ora serrata (O.S.), undifferentiated neuron, probably a photoreceptor (R), undifferentiated cell (1st), zonulae occludentes = —— >; nucleus = N; lysosome = △. (1) Region adjacent to the ora serrata, containing undeveloped photoreceptors. (2) More posterior region containing partially developed photoreceptors.

ment consists of a mass of cytoplasm protruding past the external limiting membrane, and lying within the optic vesicle cavity. Most of the photoreceptors can be differentiated into either cones or rods by the morphology of their synaptic connections, if present, and some possess a primitive outer segment, lying in the space between them and the pigment epithelium. None of the sacs of the rudimentary outer segments were open to the exterior, i.e., obviously formed by infolding of the plasma membrane. Occasionally, an inner segment had a cilium but no outer segment. More posteriorly, the photoreceptors appear to be quite normal in structure, as in the fundus of the retina. Therefore, the retinal zone just posterior to the ora serrata contains all stages of embryonic differentiation of photoreceptors. Since this condition was found in young adult monkeys and in human eyes of wide age range, it seems that it represents a normal condition of arrested development rather than merely a long delayed, but still active, completion of differentiation, which presumably would be attained in older eyes. A similar sequence of morphological stages is also found in some retinal degenerations, which might suggest that we are dealing with a related or degenerative phenomenon in the present case. This does not seem to be likely because it was found in all individuals studied, in very young as well as old eyes, and in three species.

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