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The Anatomy of the Limbus

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Summary
The limbus forms the border between the transparent cornea and opaque sclera, contains the pathways of aqueous humour outflow, and is the site of surgical incisions for cataract and glaucoma. Externally the epithelial cell border between conjunctiva and cornea possesses multipotential cells important for differentiation of the respective cell types. By the same token, the internal limbal border zone between corneal endothelium and anterior trabeculum appears to contain specialised cells some of which are activated to migrate and repopulate the trabecular meshwork after trabecular injury.

The oblique interface between corneal and scleral stroma determines the appearance of the surgical limbus whose landmarks vary around the circumference of the globe but predictably correlate with structures of the anterior chamber angle. The vasculature of the limbus derives in primates primarily from the anterior ciliary arteries.

Their superficial branches form arcades to supply the limbal conjunctiva and peripheral cornea. Perforating branches contribute to the vascular supplies of the deep limbal structures and the anterior uvea.

The term, limbus, denotes 'a border' between two different types of tissues. Most commonly it refers to the border zone between the cornea and sclera. Despite its small size, the limbus excites our interest because it demarcates the optically clear cornea from the conjunctiva and opaque sclera; it maintains nourishment of the peripheral cornea; it contains the pathways of aqueous humour outflow; and it is the site of surgical incisions into the anterior chamber for cataract and glaucoma. The limbus does not constitute a separate tissue but, rather, the border zone or the ocular globe separating cornea, conjunctiva, sclera, and uvea. Like geographic border zones around the earthly globe, there is no uniform agreement about its precise boundary lines; it is a very busy place; and groups representing quite disparite interests lay claim to it!

Gross Anatomy

Externally the limbus is formed by the junction of the corneal and conjunctival epithelia. Mechanisms for the differentiation within this epithelial border now appear to be related to multipotential corneal limbal cells.[1]

The radius of curvature abruptly changes at the junction of cornea and sclera creating a shallow furrow or sulcus externally, the external scleral sulcus. (Fig. 1) The internal scleral sulcus, formed by the scleral spur contains the canal of Schlemm and the aqueous humour outflow apparatus (Fig. 1).

Clinically, the limbus appears elliptical in shape with the long axis oriented horizontally.

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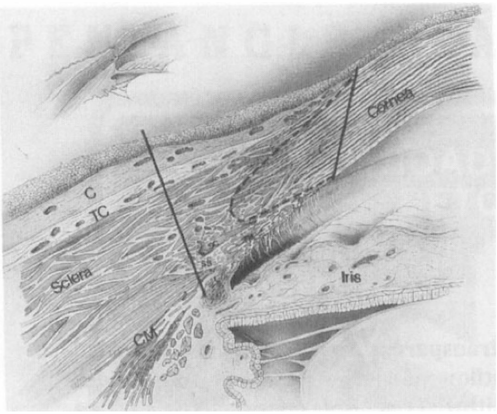

Fig. 1. *The Limbus. Solid lines demarcate the pathologist's limbus; the dotted line, the histologist's limbus. Conj = conjunctiva; TC, Tenon's Capsule; CM, ciliary muscle; TM, Trabecular Meshwork; SC, Schlemm's Canal; SS, Scleral Spur showing circumferential collagen fibres.*

The corneo-scleral interface is longer in the vertical meridia than horizontally as the sclera and conjunctiva extend further over the cornea (Fig. 2). Thus the external corneal diameter averages 10.6 mm vertically, but 11.7 mm horizontally (Fig. 2). This difference becomes important in planning surgery in the limbal region as discussed in the section on the surgical limbus. Just beneath the conjunctiva, the anterior extent of the capsule of Tenon, a dense, collagenous, connective tissue covering the globe, fuses with the episclera and the conjunctiva over a 1–2 mm band approximately two millimetre posterior to the junction of conjunctiva and cornea (Fig. 1). A zone of approximately one to two millimetre lies between the most anterior extremes of Tenon's capsule and the conjunctiva. The absence of Tenon's capsule in this narrow limbal zone may account for the tendency of some eyes to develop thin-walled cystic blebs after full thickness glaucoma filtration surgery.

Internally, the limbal zone begins at the junction of the peripheral extent of Descemet's membrane and corneal endothelium and the most anterior extent of the trabecular meshwork identifiable gonioscopically as Schwalbe's line (Fig. 3). However, in many eyes both Descement's membrane and corneal endothelium overlap the most anterior aspect of the meshwork, creating a border zone that recently has generated renewed interest (Fig. 4). The mechanism by which corneal endothelium respects this border is not understood, but occasionally it crosses over, overgrowing trabecular meshwork and iris and leading to secondary glaucoma. During embryologic development, the trabecular meshwork is covered by an endothelial layer confluent with corneal endothelium (Fig. 5). This membrane 'retracts' to the cornea only in the later stages of the third trimester (Fig. 3). In some primates, Descemet's membrane can overlap the trabecular meshwork with a significant operculum of tissue (Fig. 4). Proliferation of this tissue similarly has been described in the ICE syndromes and after trabecular trauma, especially laser trabeculoplasty.[4–7]

Histologic Anatomy

The limbus is defined differently by pathologists, by histologists and by ophthalmic surgeons. Pathologists define a limbal block of tissue bordered anteriorly by a line between the peripheral extremes of Bowman's and Descement's membrane and posteriorly by a line from scleral spur perpendicular to the tangent of the external surface of the globe (Fig. 1).

The histologic limbus relates to the identifiable junction of cornea and sclera observed in histologic cross section (Fig. 1). Since both

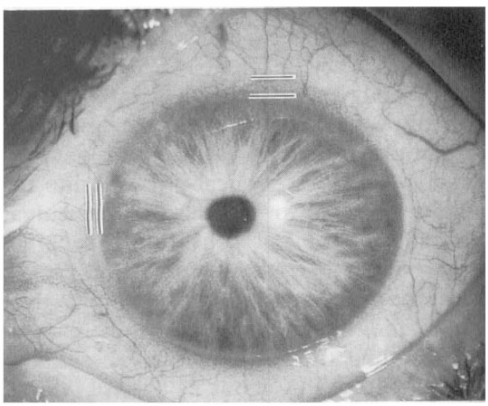

Fig. 2. *Anterior view of the external human eye showing the eliptical appearance of the external limbus, the broader limbal zone in the vertical than in the horizontal meridia.*

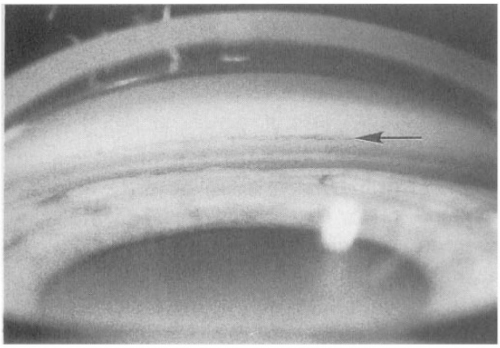

Fig. 3. *Gonioscopic view of the internal limbus in the angle of the anterior chamber, with some pigmentation of the line of Schwalbe (arrow) between the peripheral cornea above and the trabecular meshwork below.*

tissues consist primarily of collagen, this junction is difficult to define precisely with conventional histology although corneal collagen is somewhat less eosinophic than scleral collagen. Corneal collagen fibres typically are less than 600 Å in diameter. They are arranged in a highly regular lamellar pattern that lends a crystalline quality to the tissue and contributes to its transparency. Scleral fibres, on the other hand, are larger, 700–1,000 Å, more branched, are not parallel and follow a less regular pattern lending an opaque quality (Fig. 6). The scleral fibres extend further anteriorly on the external than the internal surface of the corneo-scleral junction. Thus in cross-section the histologic junction from external to internal follows a curvilinear diagonal from anterior to posterior almost to the inner wall of the globe before abruptly curving anteriorly to form the outer wall of the internal scleral sulcus and the Canal of Schlemm (Figs. 1, 6). This diagonally curvilinear corneo-scleral junction accounts for the appearance of the surgical limbus.

Although the limbus is a site of many disparite but important activities, it is not a very cellular place, being predominated by collagenous connective tissue. The most cellular areas of the limbus are the conjunctival-corneal epithelial border and the trabecular meshwork. Evidence is now accumulating for multipotential cells at the conjunctival limbus, and we recently have observed activity in the trabecular meshwork suggestive of multipotential cells near Schwalbe's line.[1,8,9] It has long been recognised that the trabecular cells have vast phagocytic capability and in fact detach themselves from the trabecular beams in a very macrophage-like manner when exposed to foreign material.[10,11] This denuding of the trabecular beams has been suggested as a contributor to outflow obstruction in some forms of glaucoma.[12] Whether or not trabecular cells have any regenerative capacity has remained controversial despite the affirmative observations of Rohen, Lutien-Drecall and colleagues after trabecular surgical injury.[13]

Preliminary studies in cats and organ-cultured human trabecular meshworks have demonstrated a low but regular rate of mitosis in trabecular cells that increases four to six fold after laser trabeculoplasty.[9] Of more interest has been that the majority (60%) of the newly dividing cells are concentrated in the most anterior region of the trabecular meshwork. During the weeks following laser trabeculoplasty the newly divided cells are seen to migrate into the region of the trabecular burns. These specific anterior meshwork cells were stimulated to divide, and to regenerate new trabecular cells that then migrated from the anterior meshwork to repopulate the laser damaged areas of the trabeculum. Special Schwalbe's line cells have also been observed in this area by Raviola but their function is not well defined.[10]

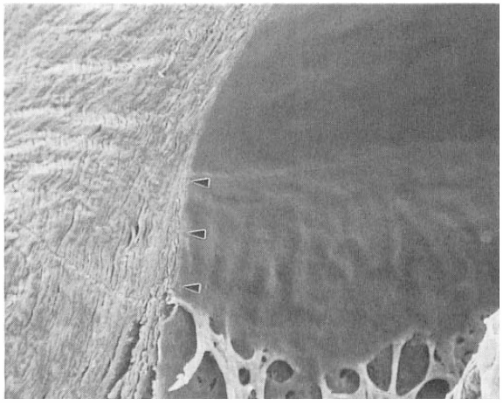

Fig. 4. *Anterior border zone between cornea and trabecular meshwork in an infant monkey, macacca fascicularis. The corneal endothelium overlaps the anterior trabeculum with a small operculum.*

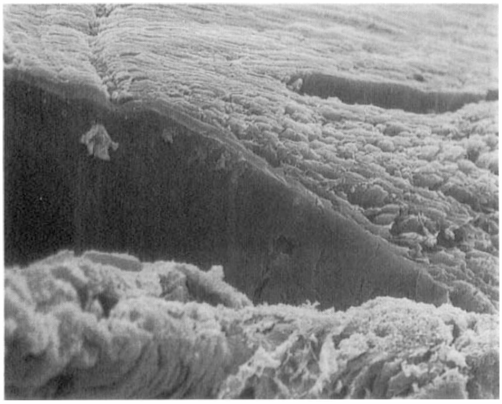

Fig. 5. *The Anterior Chamber Angle in a three-fourths term monkey (macacca fascicularis) with trabecular meshwork covered by primordial corneal endothelium.*

Surgical Limbus

The limbus is of more than purely anatomic interest to the ophthalmic surgeon because the majority of operations for cataract and glaucoma involve incision through this area. Familiarity with the anatomic landmarks in this area underpins correct performance of these operations. The surgical limbus is known among clinicians as the grey or blue zone because of the blue-grey appearance of this transition zone when viewed externally after the conjunctiva has been reflected away from the limbus (Fig. 7). The blue-grey ring spans a width of about 1.2 mm between the white of the sclera posteriorly and the transparency of the cornea anteriorly. The blue-grey appearance derives from a scattering of light through the oblique interface between cornea and sclera (Fig. 7). Since, in cross section, the demarcation line between cornea and sclera extends diagonally relative to the tanget of the globe, the relative contribution of opaque sclera gradually diminishes from the scleral to the corneal side (Fig. 6). The anterior segment surgeon can correlate the location of the intraocular structures with the external appearance of this blue grey surgical limbus in order to assure placement of his incision in the proper location (Fig. 7). Because the obliquity of the diagonal corneoscleral interface is less in the horizontal than in the vertical meridia, the blue/grey zone appears narrower in the horizontal than in the vertical meridia (Fig. 6). The most consistent external limbal landmark of internal structures is the posterior border of the blue/grey zone. It most closely corresponds to the internal junction of corneal and sclera which usually overlies the anterior trabecular meshwork in all meridia.[2] Thus, despite variations in the anterior (external) border of the surgical limbus, incisions well anterior to the posterior border should enter the eye anterior to the trabecular meshwork in the majority of cases regardless of the meridian of the incision.[2]

Many operations for glaucoma and cataract are now made under a split-thickness scleral flap where these limbal correlates change markedly again because of the obliquity of the corneo-scleral interface. Under a scleral flap of about 50% of the scleral thickness, the blue-grey transition zone is relatively displaced posteriorly compared to the appearance through the full limbal thickness (Fig. 8). Advancing from sclera to cornea, a sharply delineated white line is encountered that roughly corresponds to the level of scleral spur. Next the tissue appears greyish over the trabeculum giving way to clear cornea at about the level of Schwalbe's line (Fig. 8). Under a split thickness scleral flap, an *ab*

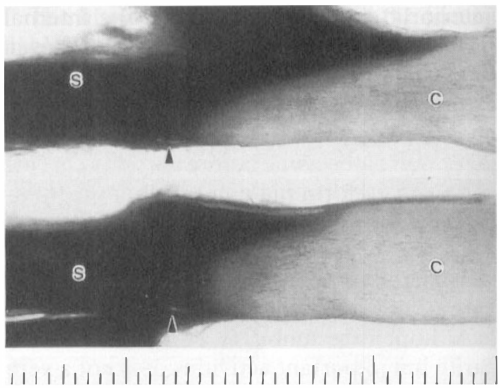

Fig. 6. *Retroilluminated 1 mm cross sections of limbus in the superior vertical (s) and the temporal, horizontal (t) meridia. Reference to Schlemm's Canal (arrows) and to scale below demonstrates the more oblique and elongated interface (histologic limbus) between cornea (C) and sclera (S) in the vertical meridia. The larger, more randomly arranged scleral fibres block transmission of the light (black) while the smaller, regularly arranged corneal fibres permit light transmission.*

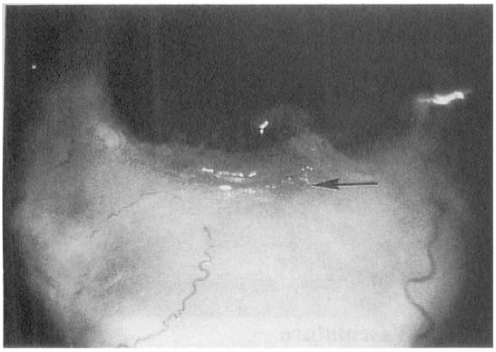

Fig. 7. *Limbus in human eye bank eye with cornea reflected showing the surgical limbus, blue-grey zone.*

externo incision at the posterior portion of the transition zone will enter the eye at about the level of scleral spur, while more anterior incisions will assure incision into the anterior chamber.

Aqueous Outflow Pathways

The limbus contains the principal conventional pathways of aqueous humour outflow: the trabecular meshwork, Schlemm's canal, and the aqueous collector channels (Fig. 9). The trabecular meshwork is a wedge shaped lamellar tissue stretched between the periphery of Descemet's membrane of the cornea anteriorly and the scleral spur posteriorly.

The meshwork exhibits three principal components. The uveal meshwork, the most internal component, consists of strands of uveal tissue extending from peripheral iris to the surface of the meshwork. The uveal meshwork varies in life from a few diaphenous fibrils, to densely pigmented iris processes (Fig. 9). Occasionally these processes are nearly confluent, but under normal circumstances the uveal meshwork appears to contribute little, if any, to total outflow resistance.

The middle trabecular componant, the corneo-scleral meshwork, comprises the principal bulk of the trabecular meshwork lying just distal or external to the uveal meshwork. It consists of perforated collagenous lamellae or beams suspended between Schwalbe's line and scleral spur or the ciliary muscle (Fig. 9). Each layer is normally covered by a monolayer of trabecular cells. These cells fabricate the extracellular matrix of the intertrabecular interstices, have extensive phagocytic capability under appropriate stimulation and normally undergo a slow rate of mitosis.[8,11,14,15] The corneo-scleral trabecular lamellae are interlaced with the tendenous fibrils from the longitudinal fibres of the ciliary muscle.[16] Some tendonous fibres insert directly into the scleral spur while others pass internal to the spur directly fusing with the trabecular lamellae.[16]

The third componant of the trabecular meshwork, the juxtacanalicular tissue or cribriform meshwork, lies immediately adjacent to the canal of Sclemm comprising its internal wall. It consists of a more loosely organised moderately cellular connective tissue through which aqueous must percolate to reach the lumen of Schlemm's canal.

The canal of Schlemm is a uniquely primate feature of the aqueous outflow pathways consisting of an identifiable circular endothelial lined channel encircling the trabecular meshwork collecting the effluent aqueous humour (Figs. 9, 10). The canal lies in a groove of sulcus within the sclera known as the internal scleral sulcus, bounded posteriorly by the scleral spur and externally and superiorly by the scleral collagenous fibres of the limbus (Figs. 1, 9). The mechanical arrangement of the scleral spur, the canal and the trabecular meshwork contributes to the maintenance of aqueous outflow resistance. The outer and posterior walls of the canal are rigid and col-

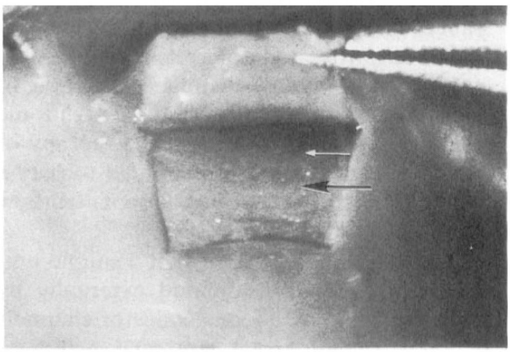

Fig. 8. *Surgical limbus under a split-thickness scleral flap showing relative posterior displacement of landmarks. White border (arrow) approximates internal location of scleral spur. Edge of clear cornea approximates internal location of Schwalbe's line (arrowhead).*

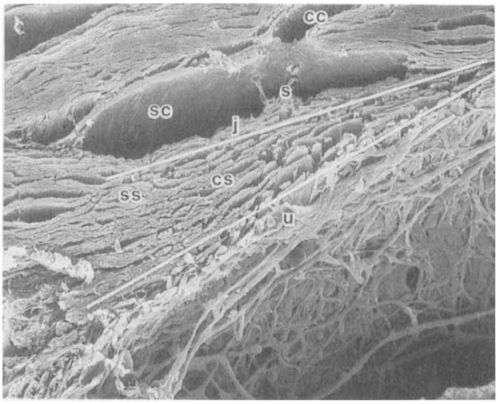

Fig. 9. *Scanning Electron Micrograph of cross section of aqueous outflow pathways in a human eye. U, Uveal Meshwork; CS, Corneo-Scleral Meshwork; JCT, Juxtacanalicular Meshwork; SS, Scleral Spur; SC, Schlemm's Canal; S, Septum; CC, Collector Channel.*

lagenous while the inner, trabecular wall is more elastic. The spur consists of circumferentially oriented collagen fibres (Fig. 1) that can be retrodisplaced by contraction of the ciliary muscle increasing the mechanical tension upon the trabecular meshwork, reducing its flow resistance, even in the postmortem eye.[17,18] The elastic meshwork can, under some circumstances, such as high intraocular pressure, collapse within the canal, obliterating its lumen or be stretched away from the canal by contraction of the ciliary muscle.[19] What role these mechanical alterations in the shape of the meshwork play in modulating outflow under normal circumstances is unknown but collapse of the canal has been postulated to contribute to some cases of open angle glaucoma.[20] Stretching of the meshwork is exploited with cyclotonic agents such as pilocarpine in the therapy of glaucoma and has been postulated to play a role in the enhancement of aqueous outflow after laser trabeculoplasty.[17,21]

The canal of Schlemm is not a simple unilumenal tube.[22] It is drained externally by multiple external aqueous collector channels that merge with intrascleral and episcleral veins[23] (Figs. 9, 10). Internal collector channels between the intratrabecular spaces and Schlemm's canal now appear to be blind-ended diverticulae.[24,25] The lumen of Schlemm's canal is intermittently divided into more than one lumen by multiple septae over at least 50% of segments of its lumen (Figs. 9, 10). Although these septae are located throughout the canal, the openings of the collector channels nearly always occur near septae, suggesting the septae may serve to keep the inner wall of the canal from collapsing against and occluding the collector channel ostea[19] (Fig. 9).

Limbal Vasculature

The vasculature of the limbus supplies the peripheral cornea, conjunctiva, episclera, limbal sclera, and peripheral uvea. The arterial supply derives from the anterior ciliary arteries extending from the rectus muscles.[23,26] The external limbal zone is served primarily by direct arteriolar branches from these arteries. Multiple terminal arterioles supply the peripheral cornea with straight branches that pass within the palisades of Vogt at the junction of conjunctiva and corneal epithelium to enter the peripheral cornea. These in turn are drained by venules reversing over the same direction. Other, recurrent, arterioles supply the peripheral conjunctiva from peripheral corneal arcades by looping posteriorly (Fig. 11). The venules from peripheral cornea and conjunctiva blend

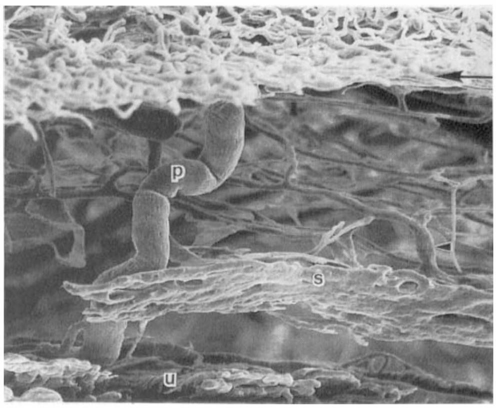

Fig. 10. *Methacrylate casting of the limbal vessels viewed by Scanning Electron Microscopy showing the peripheral conjunctival limbal vascular arcade (arrow) a perforating anterior ciliary artery (P) passing posterior to Schlemm's Canal (S) to enter the internal anterior uveal arterial system (U) of the iris and ciliary body. The Canal of Schlemm is drained by multiple collector channels (CC).*

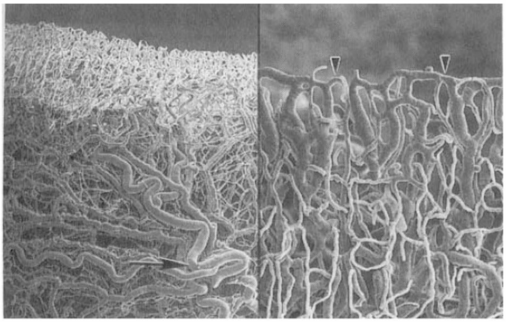

Fig. 11. *Methacrylate casting of the peripheral external limbal vasculature of the cynomologous monkey in Low (left) and High (right) magnification showing the arterial supply from the anterior ciliary artery (arrow) and the limbal archades supplying peripheral conjunctiva and cornea (arrowheads).*

with those of the episclera and Tenon's capsule and drain posteriorly into the orbital venous system. The sclera and internal limbal structures also receive the majority of their vascular supply from the anterior ciliary arteries. After the anterior ciliary arteries leave the rectus muscles, they pass anteriorly to form an episcleral anastomotic circle more complete in some non-human primates than in man.[26] Perforating arterioles from the episcleral circle and from the arterioles themselves penetrate the sclera to supply the ciliary muscle and the anterior uvea (Figs. 10, 11). Some fine intrascleral branches probably supply the limbal sclera down to the level of the outer wall of Schlemm's canal. A deep scleral plexus and intrascleral plexus of veins, in turn, drain this area, into the episclera veins. The aqueous collector channels draining Schlemm's canal sometimes merge with the deep scleral veins while others pass directly through the sclera visible in the episclera as aqueous veins, (of Ascher).[27]

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