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The Circulation of Fluid at the Limbus (Flow and Diffusion at the Limbus)

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Summary

Diffusion of molecules, e.g. ions, proteins, etc. and flow of water takes place across the physiological limbus. This 'structure' is estimated to be a zone approximately 1 mm in width and to bridge the anatomical limbus. The source of most molecules that diffuse into the corneal stroma across the limbus under normal circumstances is the perilimbal vascular system with its rich capillary bed. Small ions are lost within 2–3 mm of movement into the stroma, whereas larger molecules may diffuse to the centre of the cornea. Diffusion or flow may be bidirectional; however, it is anticipated that the majority of the flow, albeit small, is inward toward the cornea. The sclera, compared to the corneal stroma, has been found to be less resistant to fluid flow, but more resistant to diffusion of ions and larger molecules. Under normal circumstances, there have been few substances identified of importance that diffuse or flow across the limbus in either direction. There are a number of substances that traverse the limbus that are of importance in disease states, e.g. Wilson's disease, with the corneal Kaiser-Fleischer ring and angiogenic factors stimulating corneal neovascularisation. Under normal circumstances, the limbus would, therefore, seem to be more important as a zone that restricts flow and diffusion rather than an area of active molecular movement.

The physiological limbus of the cornea is estimated to be a band or zone approximately 1 mm in width, that bridges the anatomical limbus. The flow of fluid and diffusion of molecules in the structures on either side of the limbus, as well as across it, are of importance in considering the normal physiology and pathophysiology of the eye. This paper will concentrate on the diffusion and flow in the normal physiological state. In addition to the physiological limbus itself, the movement of substances in the perilimbal vasculature, with its rich capillary bed, the sclera, and the corneal stroma will be considered as each has a significant influence on the movement of substances across the limbal area.

The anatomical and biochemical factors that affect flow and diffuse within the individual structures, including the physiological limbus, will be discussed. Also, the important fluid, i.e water and the important solutes, e.g. ions, nutrients, amino acids, proteins, and experimental markers will be addressed relative to their movement in the individual structures as well as across the limbus. The factors that affect the movement of individual par-

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ticles, such as collagen content and alignment, as well as ground substance concentration, will be addressed.

Important Anatomical and Biochemical Considerations

The corneal stroma is approximately 78% water by weight, compared to 68% for the sclera. The corneal stroma is approximately 15% collagen, 4.5% mucopolysaccharide (0.7% keratan, 0.3% chondroitin sulfates) and 1% salts. The difference in relative hydration of the stroma and sclera have a differential effect on fluid flow and molecule diffusion.

Collagen in the corneal stroma is laid down in lamellae, running parallel to one another. In the human cornea, there are approximately 300 lamellae centrally and 500 at the limbus.[1] Bowman's layer is approximately 12 microns thick, made of fibrils similar in appearance, but smaller in diameter, to those in the deeper stroma. There also is irregular interweaving of the fibrils anteriorly that is not seen in the deeper stroma. Theoretically, this arrangement would create greater resistance to flow and diffusion; however, this most likely is of a negligible degree. The collagen fibrils in the cornea have been measured between 22.5 nm and 36 nm.[2-4] The interfibrillar space has been measured at >30 nm. The regular array of the collagen fibrils with a relatively constant interfibrillar space will limit the movement of particles in the cornea. The maximum diameter of a molecule that is able to move within the stroma is 12 nm which is considerably less than the interfibrillar space. The space between the fibrils is filled with a ground substance made up principally of glycosaminoglycans (GAGs) that most likely contributes to resistance to diffusion.

The sclera is made up of fibrous bands that tend to divide and recombine frequently. They do, however, appear to be continuations of the collagen fibrils from the lamellae in the cornea that run uninterrupted across the limbus. In contrast to the cornea, the sclera has been found to have numerous elastic fibres.[5] The individual collagen fibrils in the sclera are of unequal diameter and range from 30 to 300 nm.[6,7] The fibrils in the outer layer of the sclera have been shown to have an overall average diameter of approximately 250 nm, in comparison to the fibrils in the inner layer, with an average diameter 150 nm. Fibrils near fibroblast are of a finer diameter.[8]

Bowman's zone stops abruptly at the anatomical limbus. The deeper collagen fibrils, however, as stated above, course uninterrupted from the corneal stroma into the sclera. The diameter of the fibrils becomes larger within the limbal region, compared to the diameter of the fibrils in the corneal stroma. Furthermore, the fibrils at the limbus take on the appearance of twisted bundles of fine fibrils.[9]

The concentration of ground substance, made up mostly of GAGs, is higher in the corneal stroma than in the sclera. The region of the limbus has an intermediate density of this ground substance. The specific GAGs in the various structures differs. The stroma is predominantly keratan sulfate; whereas, the sclera is dominantly dermatan sulfate. Chondroitin in the central cornea yields to chondroitin sulfate toward the periphery of the cornea and limbus. This has been borne out by observations indicating that the GAGs are different at the limbus.[10,11] There also have been differences shown in the anterior stroma relative to the posterior stroma in that the keratan to chondroitin sulfate ratio is greater anteriorly.[12] The variation in GAGs within the stroma may or may not have physiological significance relative to flow or diffusion; however, the higher concentration of GAGs in the stroma compared to the sclera would exert significantly different effects on diffusion and flow.

There are three other structures that appear at the limbus that bear comment. There is a rich vascular plexus surrounding the cornea within the episcleral tissue. The capillary plexus penetrates a short distance into the anterior portion of the corneal stroma, and may serve as a source of various blood products that might gain entry into the stroma directly at the limbus, as opposed to entering first into the sclera, where they will diffuse very poorly before reaching the limbus and stroma. There also are endothelial lined lymphatics at the limbus.[13,14] However, there is no evidence that these enter into the corneal stroma. There are, however, a large number of nerves that course from the sclera, cross the

limbus into the stroma. There are perineural channels through which substances might move;[15] however, the overall volume of space available for movement is so small that these spaces cannot be considered to be significant channels for substance movement.

Diffusion in Corneal Stroma, Sclera and Limbus

The movement of substances in the corneal stroma in general can be expected to follow the laws of simple diffusion. There are, however, a number of other factors that come to bear, including the compactness of the tissue, i.e. the mechanical limitations to movement, the affects of ground substance, the loss of substances across the anterior and posterior surfaces, and the hydration of the tissue. Since we are dealing principally with the normal physiological state, little will be said about the oedematous cornea; however, it must be noted that resistance to flow and diffusion decrease significantly as tissue hydration, i.e. swelling, oedema, increase.

The stroma can be viewed as a somewhat open structure with interfibrillar spaces greater than 30 nm; however, the limiting molecular size for diffusion within the stroma has been found to be approximately 12 nm. The ground substance must, therefore, be restricting solute movement. Molecules of a size less than 12 nm do diffuse relatively well within the stroma, even though there is some effect other than space availability contributed by the GAGs.

There has been contradictory evidence in the past; however, there seems to be consensus at present that diffusion and flow within the stroma is equal in all directions. In other words, the movement of fluid and ions seems to be equally free or restricted both from anterior to posterior (moving across the cornea) and going from limbus to limbus (moving along the cornea). This has been well demonstrated experimentally using fluorescein and mammalian haemoglobin.[16]

Table I lists the measured obstruction to diffusion along the cornea and Table II, the obstruction to diffusion across the cornea.[17] The term obstruction in this context relates to how many times more slowly the substance moves in corneal stroma than in normal saline at the same temperature. The apparent increase in resistance or obstruction across the cornea for several molecules compared to along the cornea is explainable by the fact that the cellular layers were left intact for the substances that appeared to move more slowly across the cornea. It would be expected that the cellular layers would significantly impede movement, and therefore explain the apparent high obstruction value. In comparing the obstruction values for different substances, it can be seen that there is not a linear relationship between molecule size and resistance to diffusion.

The obstruction to diffusion of solutes in the sclera has been tested for a few substances.[18] The obstruction to diffusion of small ions is approximately six times that of the substance in stroma. The obstruction for larger molecules approaches forty times in sclera compared to cornea. The findings for the diffusion of dyes was somewhat unexpected, as only small molecular weight negatively charges dyes, such as fluorescein, were found to diffuse at all in the sclera. In other words, the resistance to diffusion of molecules in the sclera is much greater than the resistance to diffusion of molecules in the stroma.

There have been no specific measurements of the obstruction to diffusion across the limbus; however, it can be expected that the one mm wide physiologic limbus would be a gradual transition zone bearing the characteristics of the stroma toward the corneal side and sclera toward the scleral side. It is expected that the transition across this zone would be gradual and not abrupt. There most likely is a minimal amount of diffusion of both small ions and larger molecules across the physi-

Table I *Obstruction to diffusion along stroma*

Substance	*Diameter*	*Obstruction*
O_2	0.5	1.9
Na	0.66	1.9
Br	0.5	2.7
Cs	0.5	2.2
Fluorescein	1.1	5
Albumin	7.4	8
Mammalian Hb	6.4	10
IgG	12 × 3.5	27
Planorbis Hb	18.5	∞

Table II *Obstruction to diffusion across stroma*

Substance	*Obstruction*
THO	1.6
O_2	2.1
Na^*	6
Fluorescein*	18

*Cell layers intact

ological limbus in normal circumstances; however, there probably is quantitatively little movement in either direction.

By measuring non-metabolised substances, it has been possible to demonstrate the diffusion of substances across the limbus and through the stroma. As stated above, there probably is a little movement inward of small ions, as well as movement of larger molecules. The major factor affecting diffusion most likely is molecular size. Small ions that diffuse into the stroma are most likely lost across the surfaces within 2–3 mm of the limbus. Larger molecules that are less readily lost across the surfaces will continue to diffuse and may reach the centre of the cornea, as has been shown for albumin and IgG. The degree of loss across the surfaces will affect the concentration distribution across the stroma.

Albumin is a molecule that has been shown to diffuse across the limbus and through the stroma. Its concentration at the limbus is approximately one-fifth of its concentration in the blood. The concentration at the centre of the cornea is approximately one-third of that at the limbus. This concentration difference is consistent with albumin having its source in the blood, entering the peripheral cornea, diffusing centrally, with loss across the corneal surfaces.[19] Fluorescein has been similarly shown to enter in the periphery and diffuse centrally with loss across both surfaces. IgG has also been found in the stroma; however, the central concentration is only approximately 30 per cent less than that at the limbus, indicating that smaller amounts of IgG are lost across the surfaces.[20]

In the past, there has been considerable debate about the source of nutrients to the cornea. It has now been shown that the major source of nutrients is across the corneal surfaces and not from the limbus. The vascular supply at the limbus is limited; therefore, it should be expected that most of the nutrients would come from across the anterior and posterior surfaces and that breakdown products would be similarly lost across these surfaces. Deprivational experiments have established that, indeed, the major source of nutrients is from either the aqueous or tears, e.g. glucose from the aqueous.[21] It is, however, expected that a limited supply of nutrients could come into the cornea from across the limbus; however, the high rate of metabolism would suggest that the small supply of nutrients from this source would be used up very rapidly in the far peripheral cornea.[22,23] It would also be expected that the major breakdown products of carbon dioxide and lactic acid would be lost across the surfaces and not principally across the limbus.

The potential for amino acids being supplied to the cornea across the limbus exists; however, it would be expected that such a supply would be grossly inadequate for the central regions of the cornea. There probably is some diffusion of amino acids cross the limbus into the stroma; however, most likely the major supply of these substances to the cornea is from the aqueous humor.

There are a number of angiogenic factors that may diffuse from stroma across the limbus to sclera and blood vessels preceding and during times of neovascularisation. The egress of angiogenic substances would be expected; however, specific identification of the substances active in corneal neovascularisation has been less than complete. There have been angiogenic factors from damaged cells[24] and extracts of epithelial cells[25] that could diffuse across the limbus and initiate corneal neovascularisation. Multiple other factors have been shown to induce corneal neovascularisation; however, it has not been established that they have a role in the pathophysiology of disease. These factors include fibroblast growth factor, epidermal growth factor, prostaglandins, active amines, lactic acid, and products from tumours. It is known that stromal oedema up to the limbus is necessary, but not sufficient, for corneal vascularisation; however, it is not known whether such oedema is necessary for the

angiogenic factors to be able to diffuse out of the cornea.

Factors Affecting Fluid Flow

There are several factors that apparently affect fluid flow in the sclera, stroma and across the limbus. The fluid pressures are of importance and will be dealt with in detail below. The GAGs, separate from their effect on fluid pressure, also apparently exert a significant resistance to fluid flow. The passive loss of fluids or active transport would also affect the movement of fluids in the tissues.

The swelling pressure of the stroma and sclera is attributable to the GAGs. As the tissue swells, the interfibrillar spaces increase, while the diameter of the fibrils remains constant. The swelling pressure of the cornea is 75–85 gm/cm^2 for corneas of normal thickness. Consistent with the difference in distribution of GAGs, noted in a previous section, as well as the difference in lamellar interweaving, the anterior stroma absorbs water less than the posterior.[26] Under normal circumstances, it has also been noted that the posterior stroma is somewhat more hydrated than the anterior stroma.[27] It is expected, however, that the swelling pressure in the anterior and posterior stroma would be equal. As noted above, swelling occurs in the ground substance with increase in interfibrillar space. As the cornea swells, there is an associated decrease in resistance to fluid flow attributable to other than the increase in interfibrillar space.

The swelling pressure of the sclera is 20–30 gm/cm.^{2} Thus sclera swells in saline by only 15% of its weight. This lesser swelling is most likely attributable not only to the fact that there is a lesser concentration of GAGs in the sclera, but to the dense interweaving of the collagen fibrils that would mechanically restrict swelling. It also has been noted that the swelling pressure of the sclera is very close to the colloid osmotic pressure of blood. It, therefore, has been suggested that there may be a simple fluid equilibrium between the water in the scleral tissue and that in the neighbouring vessels.[28]

The resistance to the flow of water in the corneal stroma is great, and mostly attributable to the high concentration of ground substance.[28] The resistance to flow is probably the same in all directions, i.e. across the cornea from anterior to posterior and along the cornea from limbus to limbus. It should be noted, however, that the overall resistance to water flow in the corneal stroma in any direction is approximately ten times greater than that in the sclera.[30] This most likely is secondary to the different GAG concentrations. The resistance to flow in both tissues decreases rapidly as the tissue hydrates.

The resistance to diffusion of ions is, therefore, greater in the sclera than in the stroma; whereas the resistance to water flow is greater in the stroma than in the sclera.[31] The major roll of the limbus in the normal state may be to prevent unwanted flow, i.e. water from vessels and sclera into the stroma, and unwanted diffusion, i.e. ions etc from the stroma to the sclera.

It is expected that there would be some flow of water across the limbus into the cornea in the extreme periphery. This would be expected because of the difference in fluid pressures in the stroma compared to that in the sclera and the perilimbal capillaries. The estimated 50 mmHg of pressure acting across the 1 mm physiological limbal zone would be expected to produce a fluid flow rate of 0.05–0.1 mm/hr centrally,[32] which would abate rapidly. However, there has been no demonstration of increased hydration in the perilimbal corneal stroma.[33] The absence of increased tissue hydration in the perilimbal cornea noted above may be attributable largely to the endothelial pump activity or possibly to the difference in fibril interweaving in the limbal region mechanically restricting expansion, or to the difference in swelling pressure in the limbal region[11] attributable to the previously noted difference in GAGs in this region.[10] This degree of fluid flow along with the diffusion and rapid loss of small ions across the anterior and posterior surfaces of the cornea might explain the presence and distribution of the Kaiser-Fleischer ring in Wilson's disease, the deposition of gold in the peripheral cornea with systemic gold therapy, as well as contribute to more normal peripheral corneal deposition such as arcus senilis and limbal girdle of Vogt.

The major considerations presented here

have been directed to fluid flow and diffusion between sclera and cornea across the limbus. There also would be expected, on the stromal side of the limbus, significant exchange across the surfaces that would be similar to those across the more central cornea. These are not considered here, as they are more suitable to a paper on the maintenance of normal corneal hydration and nutrition.

A constellation of important factors, including anatomical and biochemical considerations, have been discussed that affect fluid flow and diffusion across the one mm wide physiological limbus. The summation of these factors results in what appears to be little fluid flow or substance diffusion across the limbus in the normal physiological state. There are, however, significant exchanges of molecules in both directions across the limbus in a number of pathophysiological states. The principles of fluid flow and diffusion that govern this tissue and lead to little movement in the normal state also govern the movement in the pathophysiological states. The principles, phenomena, and influencing factors described in detail above for the normal physiological state can be applied in endeavouring to understand the various disease states and the associated underlying pathophysiology.

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