PERSPECTIVE

Collagens in ocular tissues

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Collagens – general comments

There has, within the past decade, been an explosive growth in the literature concerning collagens, their function, and their influence on cell behaviour. The purpose of this review is to outline briefly the fundamental characteristics of collagens and to relate their distribution and function to the requirements of various ocular tissues.

Collagens are a family of highly characteristic fibrous proteins, constituting a quarter of the total protein in mammals, and are produced by many cell types. At least 12 different types of collagen have been described in mammalian tissue (types I–XII) and these differ from one another in their structure, function, and tissue distribution.¹ The first collagen types to be identified were those present in striated fibrils (I–III). Subsequently it was appreciated that collagens also existed in other forms such as basement membranes (IV and V) and as filaments (VI, VII, IX, and XI). At the present time only collagen types I–IX have been located within ocular tissues.

STRIATED COLLAGENS

Striated collagens are easily recognised by transmission electron microscopy since they possess a striking 65 nm banding pattern (Fig 1). Cells secrete collagen as tropo-collagen molecules which are then assembled into striated collagen fibrils outside the cells. The cellular synthesis and extracellular assembly of tropocollagen molecules into collagen fibrils are diagrammatically summarised in Figure 2.

Figure 1 High magnification of human sclera illustrating the classic banding pattern of the striated collagen fibrils (CF). These fibrils have been labelled with goat primary antibodies against type V collagen. The primary antibodies have been made visible in the transmission electron microscope by incubation with rabbit anti-goat antibodies that have been tagged with 10 nm diameter gold particles. Note absence of immunogold particles from nucleus (Nu) of fibrocyte. These particles are seen as dark spots. (LR white, $\times 62\,000$, bar = 0.5 μm .)

While all striated collagen fibrils exhibit a similar 65 nm banding pattern, each fibril can contain one of a variety of collagen types (types I–III and V). Although a striated fibril can be composed of a single collagen type, co-assembly of two collagen types within the same fibril (heterotypic fibrils) has been described.² Accurate identification of individual collagen types requires electron microscopical immunogold labelling of the fibrils with antibodies against the specific collagen type (Fig 1).

All connective tissues in which morphologists have noted the presence of collagen fibres contain type I collagen. The function of type I collagen is to give tissue tensile strength. Type II collagen is principally present in cartilage and type III collagen is associated with tissues and organs that require a motile structural scaffolding such as uterus, arteries, skin, intestines, and lung.³ Type V collagen occurs in many situations and one of its many functions may be the anchoring of basement membranes to their underlying stromal matrix.⁴

BASEMENT MEMBRANE COLLAGENS

Basement membranes are continuous, thin mats of

Figure 2 Diagram of collagen type I synthesis. Collagen α chains are initially synthesised in the form of pro- α -chains (top) that contain extension peptides that will later be removed. Three pro- α -chains combine to form a triple stranded helix (tropocollagen) within the cell before secretion into the extracellular matrix. Note that the carboxyl extension peptides of the triple stranded helix are covalently linked together. Extracellular tropocollagen molecules are arranged in a quarter staggered array in the striated fibril. Striated fibril shows detail of five bands (a–e) that comprise the 65 nm repeat D period.

Figure 3 Basement membranes of retinal vessel. The lamina lucida (ll) and lamina densa (ld) are quite distinct. The basement membrane of the endothelial cell (end) is fused with that of a vascular supporting cell (V) so that the lamina fibroreticularis is absent. The basement membranes in this illustration have been labelled for type IV collagen which is present only in the lamina densa. (Cryoultramicrotomy, $\times 38\,000$, bar = $0.5\ \mu\text{m}$.)

specialised extracellular matrix that underlie all epithelial cell sheets and endothelial tubes; basement membranes also surround individual muscle cells, fat cells, and all neural connective tissue cells. The basement membrane separates cells from adjacent connective tissue and in certain sites serves as a highly selective filter (for example, kidney glomerulus).⁵ Basement membranes have many functions such as the determination of cell polarity, the induction of cell differentiation, and the supply of 'highways' for cell migration.⁵ Conventional transmission electron microscopy of a typical basement membrane (Fig 3) reveals a three layered structure consisting of a lamina lucida, a lamina densa, and a lamina fibroreticularis (reviewed by Abrahamson⁶ and Inoué⁷). This widely accepted nomenclature of the basement membrane⁸ has recently been challenged by Kadoya and Yamashina⁹ who considered the electron lucent lamina lucida to be an artefact of glutaraldehyde osmium tetroxide fixation.

The principal components of basement membranes are type IV collagen, laminin, and heparan sulphate proteoglycan.⁶ Type IV collagen is a non-fibrillar collagen which is only found within basement membranes.⁶ Type V collagen has also been detected, biochemically, in basement membranes of human placenta, skin, muscle, lungs, bones, and cartilage.¹⁰ In addition, type V collagen has been shown immunohistologically, to be co-distributed with type IV collagen in basement membranes of the kidney.¹¹

Basement membranes form a three dimensional scaffold to which, for example, vascular cells are attached. The structural elements of the basement membrane scaffold are most probably collagen types IV and V. Their insolubility and stability make collagen types IV and V (in addition to collagens I–III) ideal scaffolding proteins.¹²

FILAMENTOUS COLLAGENS

Filamentous collagens can be subdivided into pericellular and matrix collagens. They sometimes form loosely aggregated fibrils with little or no periodicity but are frequently much finer than striated collagen fibrils. Types VI, VII, IX, and X are classified as filamentous collagens.

Although type VI collagen occurs in the vicinity of striated collagen fibrils, it is absent from the fibrils themselves, and is located to a fine filamentous network associated with the striated fibril bundles.^{13,14} In skin, type VI filaments are highly concentrated around endothelial basement membranes and form a loose sheath around the blood vessels, as well as nerves and fat cells. Burgeson¹⁵ has suggested that the loose sheath of type VI collagen filaments serves to separate blood vessels, nerves, and fat cells from bundles of striated fibrils which make up tissue compartments: this also provides free movement between striated fibril bundles and cellular elements.

Anchoring fibrils are specialised fibrous structures thought to secure epithelial basement membranes to the underlying connective tissue matrix (Fig 4).¹⁵ Type VII collagen has a distribution which correlates with that of anchoring fibrils, being present at the epithelial stromal interface of cornea, amnion, oral and vaginal mucosae, and adjacent to the basement membrane of the dermal epidermal junction of skin.¹⁵

Type VIII collagen is synthesised by cell cultures of bovine corneal endothelial cells and aortic endothelial cells.^{16–19} It is predicted that this collagen type is a minor component of most mesenchymal tissues including the vascular tree and, as it forms part of the basement membrane, type VIII collagen may be closely associated with the cell surface. In vitro studies indicate that type VIII collagen is essential for the maintenance of the endothelial cell phenotype.²⁰

Type IX collagen is unique among the collagens in being covalently attached to a glycosaminoglycan, chondroitin sulphate.^{21,22} The function of type IX collagen is unknown but it appears to be associated with the surfaces of banded cartilage fibrils with a portion of the triple helix projecting out at an angle from the fibril surface which may serve to terminate increases in fibril diameter.¹⁵

Ocular collagens

The distribution of collagen types within ocular tissues is indicated in Table 1.

Cornea

BIOCHEMICAL DATA

Collagen forms 71% dry weight of the cornea²³ and biochemical analysis reveals that the mammalian cornea contains substantial amounts of type I collagen. Collagen types III (more than 10% dry weight),^{24,25} V (about 5%),^{24,25} and VI (not quantified)²⁶ have been biochemically identified in the human cornea. Type IV collagen has not been detected and there is general agreement that type II collagen is absent from the human cornea.^{24,27–31} Biochemical data concerning collagen types VIII–XII are not available.

COLLAGENS IN CORNEAL EMBRYOGENESIS

The chick cornea has been the principal tissue used to study

Figure 4 Diagrammatic representation of basement membrane complex. Hemidesmosomes rivet epithelium to underlying lamina densa (basement membrane) by means of anchoring filaments which cross the lamina lucida (lamina rara externa) at right angles. Anchoring fibrils project into Bowman's layer from the lamina densa, attaching the lamina densa to anchoring plaques and form a large scaffold throughout the sub-basal lamina (lamina rara interna) which entraps large numbers of collagen fibrils.^{42,43} It is the entrapped collagen fibrils that prevent the anchoring fibril network from being pulled out of Bowman's layer. The anchoring fibril network prevents the lamina densa from separating from Bowman's layer⁴⁴ and hemidesmosomes prevent the epithelium from separating from the lamina densa. Thus is the epithelium anchored to Bowman's layer.

Table 1 Summary of distribution of various collagen types in ocular tissues

CORNEA	
Biochemistry	III, V, ^{24 25} VI ²⁶
Embryogenesis	I, ^{2 30 33-37} II, ^{30 33} V, ^{2 34-37} IV, ³ VI, ^{IX} ^{32 40 41}
Basement membrane complex	IV, ^{28 29 52} +/− ^{31 53-57} VI, ⁴⁹ VII ⁴⁵⁻⁴⁸
Bowman's layer	I +/− ^{27 31 39} − ^{28 29} III + ^{31 39} − ²⁷ V, ^{VI} ⁴⁹
Stroma	I, ^{27-31 34-7 59} III, ²⁷⁻³¹ V, ^{28 29 34-37 49 60} VI ^{38 40 41 49}
Descemet's membrane	IV, ^{28 31 59} VI (−ve), ^{14 26 49 64} VIII ⁶⁵
SCLERA	
	I, ⁷⁶⁻⁷⁹ III, V, VI, ⁷⁹ VIII ⁶⁶
TRABECULAR MESHWORK	
Corneoscleral meshwork	I, III, ^{77 78 82 83 85} IV, ^{77 78 82 93} V, ⁸⁵ VIII ⁶⁶
Core of trabecular beams	I, III, ^{83 85} V, ⁸⁵ VI ^{84 85}
Basement membrane of trabecular beams	I, III, ⁸³ IV, ⁸⁵ V, ⁸³ VI ⁸⁴
Cribriform layer	I, ⁸⁵ III, ⁸³ IV, ⁸⁵ VIII ⁶⁶
Long-spacing collagen	VI + ⁶⁴ − ⁸⁵
IRIS	
Stroma	I, III ⁷⁸
Vascular basement membranes	I, IV, ^{96 99} VI ¹⁰⁰
Dilator muscle basement membrane	I, IV, ⁹⁶ VI ¹⁰⁰
CILIARY BODY	
	I, III ¹⁰³
Basement membrane of pigmented epithelium	I, III, IV ¹⁰⁴
Basement membrane of non-pigmented epithelium	I, III, IV ¹⁰⁴
Vascular basement membranes	I, IV, ¹⁰⁴ VI ¹⁰⁰
Stroma	I, III ¹⁰⁴
Ciliary muscle	I, III, IV, ¹⁰⁴ VI ¹⁰⁰
LENS CAPSULE	
	I, III, ¹¹⁴ IV ^{103 108-111 113 114}
VITREOUS	
	II, ^{30 117-119} IX ^{21 22}
RETINA	
Vascular basement membranes	I, ^{106 120-123} IV, ^V ^{120 121}
of large vessels	I, ¹⁰⁶ III, IV, ^V ^{106 120 121}
of small vessels	I, ^{106 120 122 123} IV, ^{106 120} VI ¹⁰⁶
CHOROID	
Bruch's membrane	I, III, IV, V, ¹²⁶⁻¹²⁹ VIII ⁶⁶
OPTIC NERVE HEAD	
Central retinal artery	I, III, IV, V, ¹³² VIII ⁶⁶
Prelaminar	III, IV, ^V ¹³⁰
Cribriform	I, III, IV, ¹³¹⁻¹³² VIII ⁶⁶

the involvement of collagens in ocular development (reviewed by Linsenmayer *et al.*³²) and is of particular interest because various collagen types participate, sometimes transiently, in this complex process. The primary stroma is laid down by the corneal epithelium and consists of types I and II collagen which are co-assembled into each striated fibril.^{30 33} Invading pericorneal mesenchymal cells differentiate into stromal fibroblasts and synthesise heterotypic fibrils of types I and V collagen.^{2 34-37} Type VI collagen appears very rapidly at this stage in embryogenesis³⁸ and is thought to direct the migration of invading fibroblasts.³⁹ In a transient stage in embryogenesis, filaments of types IV, VI, and IX collagen^{40 41} extend from the epithelial side to the endothelial side of the developing cornea. Type IV collagen is present during this phase in a non-basement membrane form, consisting of long strings radiating from the epithelial basement membrane to deep within the corneal stroma.³² The mature stroma is mainly synthesised by the stromal fibroblasts, the heterotypic fibrils consisting of types I and V collagen. Type VI collagen is thought to stabilise the whole structure.³² Type IX collagen appears in the embryonic chick cornea at the same time as endothelial cells begin migrating along the posterior surface of the stroma. It is not known if endothelial migration is induced by, or triggers the deposition of type IX collagen.³² Type IX collagen is only present for a short period and disappears rapidly before primary stromal swelling. This suggests that type IX collagen maintains the compactness of the primary stroma, probably by forming bridges between striated fibrils and its removal may be the trigger for the invasion of pericorneal mesenchymal cells into the primary stroma.³²

THE EPITHELIAL BASEMENT MEMBRANE COMPLEX

Corneal epithelial adhesion is maintained by a specialised structure, the basement membrane complex which anchors the epithelium to Bowman's layer. Although a basement membrane complex is present in the skin, that of the cornea is a more highly developed form.^{12 42-44} This highly developed

adhesion complex may compensate for the lack of a rete peg system which is present in the epidermis (see Fig 4 for a diagrammatic representation of the epithelial basement membrane complex). Several workers have demonstrated that anchoring fibrils contain type VII collagen as a primary structural element.⁴⁵⁻⁴⁸ Type VI collagen is present in the anchoring plaques.⁴⁹ The constituents of anchoring filaments are not yet identified.

The well established concept that anchoring plaques are trapped in the meshwork of collagen fibrils in Bowman's layer^{42 43 50} has been questioned by Wasano and Yamamoto⁵¹ who noted very little entrapment of corneal connective tissue elements by the anchoring fibril network.⁵¹ We found that type VI collagen, in addition to being present in anchoring plaques forms a filamentous network around striated collagen fibrils and therefore may serve to attach the anchoring plaques to this filamentous network in Bowman's layer.⁴⁹ Thus anchoring plaques may be secured within Bowman's layer by virtue of their incorporation within this filamentous network of type VI collagen.

The nature of the basement membrane of the corneal epithelium remains controversial. Although some immunofluorescent studies report the presence of type IV collagen beneath the human corneal epithelium,^{28 29 52} other investigations failed to detect it in this region.^{31 53-57} The most exhaustive study⁵³ found type IV collagen to be abundantly present in the conjunctival and limbal basement membranes but immunoreactivity disappeared within a short distance of the start of Bowman's layer, despite the use of a large number of unmasking treatments to expose the antigenic epitope. Loose adhesion between Bowman's layer and the epithelial basement membrane is a feature of diabetes in which basement membrane remains attached to epithelium removed from Bowman's layer.⁵⁸ This phenomenon is attributed to decreased penetration of anchoring fibrils in the diabetic cornea.⁵⁸

STROMAL COLLAGENS

The distribution of collagen types I–VIII is diagrammatically summarised in Figure 5. Various immunolabelling methods have been employed to demonstrate the presence of collagen types I,^{27-31 59} III,²⁷⁻³¹ V,^{28 29 34 34 37 49 60} and VI⁴⁹ in the human corneal stroma. In immunogold studies, labelling for all three collagen types is present on every fibril^{31 49} which suggests co-assembly (co-distribution) of these three collagen types within the same striated fibril. Co-assembly of type I and type V collagen has been demonstrated clearly in chick cornea using a double labelling technique^{2 35} and further evidence for their co-assembly has been derived from enzyme digestion studies of Fitch *et al.*⁶¹

Attempts to document the distribution of collagen types I–IV in the human cornea using immunofluorescence have not yielded consistent results. Type I collagen has been reported as both present in^{27 59} and absent from^{28 29} Bowman's layer of the human cornea. There is also some disagreement concerning the presence and distribution of type III collagen in humans. Ben-Zvi *et al.*²⁷ reported its absence in adults and presence only within the first 28 weeks of gestation. In contrast, Newsome *et al.*⁵⁹ found type III to be present in Bowman's layer and on the edges of stromal lamellae. Three other studies reported the presence of type III throughout the corneal stroma.^{28 29 31} It should be noted that the occurrence of type III collagen in the corneal stroma appears to be unique to the human eye.

Type VI collagen has been localised to fine filamentous structures in the human cornea.⁴⁹ These filamentous structures are quite distinct from striated collagen fibrils and are probably located between them.³⁸ Consequently it has been concluded that type VI collagen is not incorporated into the

Figure 5 Summary diagram of the distribution of collagens I–VIII in aged human cornea. Collagens I (blue circle), III (green square), V (brown square), and VI (red circle) are evenly distributed throughout Bowman's layer and the corneal stroma. Types V (brown square) and VI (red circle) are also present in the interfibrillar matrix. Type IV collagen (yellow circle) is present in Descemet's membrane. Both types VI (red circle) and VII collagen (green circle) are present in the basement membrane complex (BMC) immediately underneath the epithelium. Type VIII collagen (yellow square) is present in the anterior banded region (ABR) of Descemet's membrane. PNB=posterior non-banded region.

striated collagen fibrils but remains in the interfibrillar matrix.^{15 38 49 62}

DESCMET'S MEMBRANE

Although type IV collagen has been located in Descemet's membrane both by immunofluorescence^{28 59} and by immunoelectron microscopy,³¹ agreement is not forthcoming on its precise distribution. In two studies^{28 29} labelling was restricted to the stromal interface, but subsequently Newsome *et al*⁵⁹ observed two discrete layers, one at the stromal face and one at the endothelial face. This led to the suggestion that the unstained middle portion corresponded to the anterior banded region (Fig 5) observed in conventional electron microscopy. We found, by immunoelectron microscopy, that type IV collagen was concentrated at the endothelial face of Descemet's membrane.³¹ We also located types V and VI collagen between the most posterior lamella and the posterior non-banded layer (interfacial matrix; Fig 5).⁴⁹ As these collagens also encroached into a narrow margin of the anterior portion of Descemet's membrane, we suggest that they are involved in the tenuous welding of Descemet's membrane to the corneal stroma.

The elegant hexagonal pattern of the matrix present in the anterior region of Descemet's membrane has been ascribed to the presence of type VI collagen. This is because the node to node distance in its hexagonal matrix is similar to the distance between the globular domains of type VI collagen filaments.⁶³

However, immunofluorescence studies have not located type VI collagen in Descemet's membrane,^{14 26 64} either with or without treatment to increase the availability of type VI collagen to antibody binding (hyaluronidase; acetic acid).⁶⁴ In addition, the hexagonal matrix in our immunoelectron microscope study exhibited minimal labelling for this collagen type in the human cornea and we concluded that type VI collagen is not responsible for the hexagonal matrix morphology of the anterior banded region (Fig 5).⁴⁹ A recent study has demonstrated the presence of type VIII collagen in the hexagonal matrix of bovine Descemet's membrane by immunoelectron microscopy.⁶⁵ Type VIII collagen is secreted by most endothelial cells in culture, and was originally termed endothelial collagen.²⁰ In addition to Descemet's membrane, type VIII collagen has also been localised by light microscopic immunohistochemistry to the trabecular meshwork, the wall of Schlemm's canal, Bruch's membrane, the choroidal stroma, sclera, central retinal artery, and cribriform plates of the optic nerve head.⁶⁶

CELLULAR SYNTHESIS OF EXTRACELLULAR MATRIX

The unique compartments of the cornea make it ideal for the correlation of the immunomorphologic location of collagen types to the synthetic capability of the associated cell types. Several cell culture studies have been conducted on the biosynthesis of extracellular matrix components by corneal keratocytes, epithelial, and endothelial cells. Rabbit corneal endothelial cell cultures synthesise mainly type IV collagen plus small amounts of type III and VIII collagens.^{19 67} However, considerable caution must be exercised in assessing cell culture studies of extracellular matrix synthesis as the biosynthesis may be a tissue culture specific phenomenon. It is likely that organ cultures give more reliable indications of the biosynthetic activities in vivo. The following is a case in point. Radiolabelled proline was incorporated by cultured bovine corneal endothelial cells mainly into type III collagen⁶⁸ plus small amounts of collagens I, IV, and V, but only types I and IV collagen were radiolabelled in organ cultures of bovine Descemet's endothelial complex.⁶⁸ It was concluded that type III synthesis was a tissue culture induced phenomenon specific to bovine corneal endothelial cells. Perhaps the most satisfactory method of determining the synthetic capability of extracellular matrix associated cells is by the determination of mRNA expression of specific extracellular matrix components by individual cells (in situ hybridisation).

TRANSPARENCY AND FIBRIL DIAMETER CONTROL

The almost perfect transparency of the cornea is thought to be due to the uniform diameter and regular interfibrillar distance of the striated fibrils within the stroma.⁶⁹ The presence of type V collagen is one of many factors influencing fibril diameter.³⁶ Type V collagen has a greater concentration in the cornea than in sclera, tendon, bone, and dermis^{70–72} and is thought to influence fibril diameter in the corneal stroma through its longer helix^{70 73} and the presence of a unique terminal globular domain.⁷⁰ Uncontrolled accretion of type I collagen onto the surface of a fibril would be prevented by incompatibility with the 'non-fitting' type V molecule already present on the fibril surface. In vitro fibrillogenesis studies employing mixtures of collagen types I and V, show that the average diameter of the heterotypic fibrils formed decreases as the ratio of type V collagen in the mixture is increased.⁷⁴ The regular interfibrillar distance is probably achieved by the proteoglycans chondroitin (dermatan) and keratan sulphate forming bridges between the fibrils.⁷⁵ However, fundamentally important though they be, consideration of proteoglycans is outside the scope of this review.

ADVANTAGES OF COLLAGEN CO-ASSEMBLY

Co-assembly of two different collagen types may have the advantages of imparting regulatory information (such as influencing fibril diameter) and the need for more than one collagenase in turnover and degradation. An interesting hypothesis has been put forward by Linsenmayer *et al*³² on the importance of enzymes in corneal re-modelling. The release of collagenase I and collagenase V into the chick cornea would produce a loose filamentous matrix of random orientation, largely composed of type VI collagen, non-collagenous microfibrils, and proteoglycans. The activity of only collagenase I would also result in a loose filamentous matrix. However, the corneal lamellae would still have the same original spatial organisation as the skeleton would be preserved by the type V collagen fibrils. The surviving skeleton might then serve as a template for directing the assembly of a new connective tissue matrix similar in structure to the original stroma.

Scleral collagens

Type I collagen has been identified, biochemically, in human sclera with no apparent variation in its percentage of tissue mass between the equatorial and posterior regions.⁷⁶ Although four collagen types (I–IV) were studied by immunohistochemistry, only type I collagen was positively localised.^{77,78} Our fine structural immunogold studies of aged human sclera indicate that collagen types I, III, and V are present and may well be co-assembled within individual collagen fibrils, since every fibril exhibited labelling for all three collagen types at some point along its length.⁷⁹ Transverse sections of collagen fibrils revealed a preferential location of type V to the fibril surface. Both types V and VI collagen were localised to filamentous strands in the interfibrillar matrix. This filamentous material formed short bridges between adjacent fibrils. Such filamentous strands have been observed in both cornea and sclera that had been quick frozen and deep etched for scanning electron microscopy.⁸⁰ Both types II and IV collagen were absent from the scleral stroma.

COMPARISON OF SCLERA WITH CORNEA

The putative role of type V collagen in maintaining uniform fibril diameter in the corneal stroma has already been discussed. The collagen constituents of the cornea and the sclera are essentially the same, but scleral collagen fibrils exhibit a wide range of diameters and it therefore must be assumed that type V collagen present in the sclera does not play the same role as it plays in the cornea. This discrepancy requires further consideration. It may be advantageous for the scleral fibrils to have such a large variation in diameter: both large and small fibrils probably have a separate function that contributes to the biomechanical properties of the sclera. Large diameter fibrils, in having a greater density of cross linking between collagen molecules, impart greater tensile strength.⁸¹ Small diameter fibrils have a greater surface area of contact with the surrounding matrix and the increased amount of interaction between the fibrils and proteoglycans may endow greater creep resistant properties (the sliding of fibrils past each other ultimately resulting in stretching of the tissue).⁸¹

Trabecular meshwork

LOCATION OF MESHWORK COLLAGENS

The distribution of collagens in the meshwork is diagrammatically summarised in Figure 6. Three early light microscopical immunohistochemical investigations identified types

Figure 6 Distribution of collagens I–VI in aged human trabecular meshwork. Type I collagen (blue circle) is present in striated collagen fibrils in trabecular beams and the outer wall of Schlemm's canal as well as in basement membranes. Type III collagen (green square) is restricted to striated collagen fibrils. Type IV collagen (yellow circle) is localised over endothelial cell basement membranes and basal lamina-like material. Types V (brown square) and VI collagen (red circle) share a similar distribution to type III collagen (green square) except that they are not directly associated with the collagen fibrils.

I, III, and IV collagen in the trabecular meshwork.^{77,78,82} A subsequent immunofluorescence study described the distribution of collagens I, III, IV, and V.⁸³ The basement membranes of the trabecular beams contained types III, IV, and V collagen, the central core contained collagens I and III, while only type III collagen was detected in the outermost layer (juxtacanalicular meshwork; cribriform layer).⁸³ In a separate study, weak knot-like staining for type VI collagen was described within the basement membranes underneath the trabecular beam endothelium.⁸⁴ With the resolution of electron microscopy, types I and III collagen have been located to striated fibrils in the cores of the trabecular beams.⁸⁵ Montes *et al*⁸ have suggested that these two collagen types would impart tensile strength (type I) and resilience (type III) to the trabecular 'skeleton' – physical characteristics which are obviously an essential requirement for the biomechanical demands made on the trabecular beams.⁸⁵ One of our own surprising preliminary observations was the presence of type I collagen in the basement membranes of endothelial cells lining the trabecular beams.⁸⁵ This was unexpected because like types II and III, type I has been classified as an integral component of fibrous collagen forming the characteristic 65 nm periodic striated collagen fibrils.⁸⁶ No examples of such fibrils were observed in the endothelial basement membranes. This raises the interesting possibility that type I collagen, like type V, does not always form striated fibrils and merits further investigation.

Types V and VI collagen appear to be located to a fine filamentous network throughout the collagen fibril core of the trabecular beams.⁸⁵ We suggested that collagens V and VI

have a dual function in maintaining the structural integrity of the striated fibril core and adhesion between the striated core and the basement membranes of the trabecular endothelial cells.⁸⁵ This anchoring function has been attributed to very fine non-banded fibrils shown to contain type V collagen and which are enmeshed among the striated fibrils.⁴ The functional demands on the trabecular meshwork could explain the difference in distribution of type V collagen in comparison with that of the cornea. We see no reason to suspect that control of fibril diameter is as necessary in the trabecular meshwork. It is suggested that the network of types V and VI collagen permit the sliding of the collagen fibril cores relative to their adjacent endothelial cell monolayers. This arrangement would impart a greater degree of flexibility to a system that is subject to constant changes in configuration.

COLLAGENS AND AQUEOUS OUTFLOW RESISTANCE (CRIBRIFORM LAYER)

The suggestion that major resistance to outflow occurs in the cribriform layer⁸⁷ has stimulated numerous investigations into the nature and role of the various extracellular matrix components at that site.⁸⁸⁻⁹⁴ It would be very appropriate at this point to reiterate that the cells of the cribriform layer are surrounded by extracellular matrix constituents classified by their ultrastructural appearance as striated collagen fibrils, basal lamina-like material, granular-like material, fine fibrillar-like material, fibrous long-spacing fibres (long-spacing collagen), and dense plaques. As in the trabecular beams, collagens I and III are present within striated fibrils.⁸⁵ Basal lamina-like and fine granular-like material contain type IV collagen and the basement membrane of the lining endothelium of the inner wall of Schlemm's canal contains both types I and IV collagen.^{85,95} Immunocytochemical localisation of collagen types does not address the question of the origin of extracellular matrix in the cribriform layer which could have been washed through with the aqueous. Determining the contribution of indigenous cells would require the application of mRNA probes (in situ hybridisation) for each of the extracellular matrix components present.

LONG-SPACING COLLAGEN

Long-spacing collagen (fibrous long-spacing fibres), so described because of its 'zebra-like' alternating thick electron dense and lucent bands, is a hallmark of aging ocular extracellular matrix. An immunoelectron microscope study of tangential sections through the cribriform layer in the region of the scleral spur, localised type VI collagen to fibrous long-spacing fibres (long-spacing collagen).⁸⁴ However, this has not been confirmed by subsequent immunogold electron microscope studies on aged meshwork,⁸⁵ Bruch's membrane (unpublished observations), or corneal stroma⁴⁹ in which fibrous long-spacing fibres (long-spacing collagen) were also present. From our own studies it appears that collagen types I-VI are absent from fibrous long-spacing fibres and in addition it is unlikely that these structures will be found to contain collagen types VII to XII as collagenases do not eliminate the wide electron dense bands⁹⁶ (collagen types VII-XII are particularly sensitive to collagenases).⁹⁷ Thus there appears as yet no justification for considering long-spacing structures as collagen derivatives and the term, long-spacing collagen, may be a misnomer.

Iris

Striated collagen fibrils are the major component of the iris stroma, providing mechanical support for a tissue which undergoes continual changes in configuration. In iris vessels, basement membrane coats the abluminal surface of the

endothelial cells and completely surrounds the vascular supporting cells (myocytes and pericytes). Basement membrane is also present on the basal surfaces of the iris pigment epithelium – that is, the muscular extensions of the anterior pigmented epithelium and the posterior surface of the iris.

IRIS COLLAGEN TYPES

There is very little documentation of iris collagens either by immunomorphology or by biochemical analysis. Type I collagen appears to be the most prevalent collagen in iris tissue, being present both in striated fibrils and basement membranes.⁹⁸ Type III collagen is present within striated fibrils.⁹⁸ The presence of type I collagen in iris basement membranes^{98,99} is somewhat surprising as it is not traditionally viewed as a basement membrane component.⁸⁶ Within the iris vasculature it was found to be principally restricted to the extracellular matrix enclosing the vascular supporting cells.^{98,99} The reverse was true for type IV collagen which was mainly present in the subendothelial basement membrane. This pattern of distribution led us to suggest that within the iris vasculature the synthesis of type I collagen is a responsibility of the myocytes and pericytes, rather than the endothelial cells.⁹⁸ Conversely, type IV collagen appears to be synthesised by endothelial cells rather than the vascular supporting cells. Both collagens I and III are present in the striated fibrils of the double-layered sheath of the iris vessels.⁹⁸ Light microscopic immunohistochemistry has located type VI collagen to this region, and thus it may connect the vascular basement membranes to the surrounding vascular sheath.¹⁰⁰

Collagen types I, IV, and VI are present in the matrix in which the dilator muscle is embedded.^{98,100} It has been suggested that type VI collagen resembles type VII, in that it is involved in adhesion between basement membranes and the adjacent connective tissue.¹⁰⁰ Thus, the functional role of type VI collagen in this location is seen as adhesion of the dilator muscle basement membrane to the iris stroma.¹⁰⁰

Ciliary body

The physical barriers provided by the basement membranes of the ciliary epithelium may influence aqueous humour formation and transportation. In addition, the stromal components of the ciliary processes which surround the fenestrated capillaries may impede fluid exchange between the vascular lumen and ciliary epithelium. Marked morphologic changes arising from collagen deposition occur in the aging ciliary body.¹⁰¹ There is as yet no evidence that fibrosis in the ciliary processes has an effect on inflow but fibrosis in the circular and oblique components of the ciliary muscle is accompanied by a decrease in the amplitude of accommodation.¹⁰²

COLLAGENS OF CILIARY BODY

Biochemical analysis of the collagen content of the ciliary body is limited to one study of the bovine eye in which the presence of types I and III collagen was reported.¹⁰³ Striated collagen fibrils in the stroma exhibited strong labelling for types I and III collagen,¹⁰⁴ which is a labelling pattern characteristic of extensible tissues.¹⁰⁵ Both collagen types were present in the ciliary epithelial basement membranes and type I collagen was also present in vascular basement membranes.¹⁰⁴ The presence of type III collagen in the epithelial basement membranes was of great surprise, as this collagen type had not been localised in other studies of basement membranes of the aged human iris,⁹⁸ retina,¹⁰⁶ cornea,³¹ and trabecular meshwork.⁸⁵

In a light and electron microscopic study by Rittig *et al*,¹⁰⁰

Figure 7 Distribution of collagens I–VI in human ciliary body. Collagens I (blue circle) and III (green square) are present in the basement membranes (BM) of the pigmented and non-pigmented ciliary epithelium and ciliary muscle and over striated collagen fibrils in the ciliary body stroma. Type I collagen (blue circle) is also present in vascular basement membranes. Type IV collagen (yellow circle) is present in all the basement membranes. Type VI collagen (red circle) exhibits an asymmetric distribution over non-fenestrated regions of capillaries in the ciliary processes and subjacent connective tissue.

types IV and VI collagen were located in the ciliary muscle basement membranes and in vascular basement membranes. Type VI collagen was largely located over the non-fenestrated regions of the capillaries in the ciliary processes. The conclusion was that type VI collagen would mechanically anchor the capillaries without impeding water and solute movement through the fenestrations.¹⁰⁰ The distribution of collagen types I–VI in the ciliary body is summarised in Figure 7.

Lens capsule

According to biochemical analysis, lens capsule has a high collagen content (69% (SD8%))¹⁰³ comparable with that of cornea (69.2%) and sclera (76%).¹⁰⁷ The biochemical detection of type IV collagen in mammalian lens capsule has been widely reported,^{103 108–111} and is estimated to be about 30–40% of the dry weight of the lens capsule. Barnard *et al*¹¹² suggested that type IV collagen supplies the strength and flexibility of this basement membrane. Its presence in the lens capsule has been confirmed, both by immunofluorescence microscopy¹¹³ and by immunogold electron microscopy.¹¹⁴ If type IV collagen were the only collagen type present x ray diffraction studies should reveal a chicken wire meshwork arrangement which is peculiar to type IV collagen.¹¹⁵ However, the x ray diffraction studies of Barnard *et al*¹¹² failed to show this arrangement, but demonstrated 10 nm banded microfibrils: the latter may have been the result of the association of collagen types I, III, and IV.

The lens capsule increases in thickness with age and this accretion process appears to be due to completely uniform assembly of three types of collagen (I, III, and IV).¹¹⁴ The possibility cannot be excluded that this phenomenon is intrinsic to aging and that these collagens may not be present in the human capsule in the early decades (these data are not yet available). The occurrence of type III collagen in ocular basement membranes is peculiarly unique to the lens capsule and ciliary epithelial basement membranes. Type III collagen is associated with tissues that have elastic properties,³ an essential feature of the lens capsule which makes the lens more spherical in accommodation; a tendency which is opposed by tension on the zonular fibres. The role of type I collagen in the lens capsule is uncertain, but it has to be assumed that it imparts tensile strength.³

Vitreous

Vitreous collagen fibrils are oriented randomly to form an irregular mesh in which hyaluronic acid molecules are trapped and are inserted into the basement membrane of the Mueller and glial cells of the retina. The resulting architecture provides both a deformability and rigidity that supports the vitreous body against the surrounding structures. Vitreous collagen fibrils have a diameter of about 15 nm and an ill defined axial periodicity of 22 nm which is characteristic of embryonic or immature collagen fibrils.¹¹⁶ Type II collagen has been identified by various techniques in chick and bovine vitreous^{30 117–119} and is the major fibril-forming collagen. To our knowledge, type II collagen has not yet been identified in human vitreous. Type IX collagen is present on the fibril surface and its precise form appears to be tissue and species specific.²¹ That of chick vitreous is attached to an extremely long chondroitin sulphate chain,²² while that in bovine vitreous possessed a single chondroitin/dermatan chain.²¹ Bishop *et al*²¹ concluded that such differences are likely to be of importance in determining the biochemical and morphological characteristics of the tissue.

Retinal vessels

Collagens I, IV, and V have been localised by immunofluorescence to vessels of the human retina.^{120 121} Jerdan and Glaser¹²⁰ found that a broad spectrum of retinal vessels stained for types I and IV collagen, while types III and V collagen were restricted to the walls of larger vessels.

In our immunoelectron microscope study of aged human retinal vessels,¹⁰⁶ collagen types I, III, IV, V, and VI were found in larger vessels. Types I, IV, and V with small amounts of III and VI were present in small vessels, and types I, IV, and V in capillary basement membranes. With aging, the thickened walls of retinal vessels consisted mainly of types I, IV, and VI collagen.¹⁰⁶

Microvessel preparations from rabbit cerebrum as well as bovine retinal microvessels have been shown to contain type I collagen.^{122 123} On finding this collagen type in retinal vessels, including capillaries, Jerdan and Glaser¹²⁰ suggested that it is the presence of type I collagen that makes the human retinal vasculature so uniquely resistant to trypsin digestion. The structure of type I collagen has a greater stability to digestion by this protease.¹²⁴

Type IV collagen was more abundant in the outer basal lamina of the intramural pericytes than in that of the endothelial cells.¹⁰⁶ This difference may be very significant if, from it, one assumes that pericytes produce more type IV collagen than endothelial cells. There is considerable evidence that changes in ECM components, including collagens, can have profound effects on endothelial cell behaviour. For example, type IV collagen was shown by Roberts and Forrester¹²⁵ to inhibit retinal vessel endothelial

cell migration and proliferation in vitro, whereas type I collagen stimulated cell migration in vitro. Pericyte loss is an early abnormality in diabetes, presumably leading to a decrease in the amount of type IV collagen and thus removing the inhibiting influence that this collagen type has on endothelial cell migration and proliferation.

Bruch's membrane

Various attempts have been made to determine the collagenous constituents of Bruch's membrane using immunohistochemistry, and collagen types I and III–V have been demonstrated by light microscopy.^{126–129}

The ultrastructural location of collagen types I–VI in the five layers of Bruch's membrane is summarised in Figure 8. The 65 nm striated collagen fibrils contain types I, III, and V and the choriocapillaris basement membrane, collagen type IV.¹²⁸ Labelling for collagen type IV was dense over the basement membrane of the choriocapillaris but weak over the basement membrane of the retinal pigment epithelium.^{128–129} This unexpected discrepancy between epithelial basement membranes and endothelial basement membranes is not confined to Bruch's membrane as it has been noted between epithelial and endothelial basement membranes in cornea^{31–53} and ciliary body.¹⁰⁴ The discrepancy may

Figure 8 Distribution of collagen types I–VI in aged human macular choroid. Collagens I (blue circle), III (green square), V (brown square) were localised to striated collagen fibrils in the inner (ICL) and outer collagenous layer (OCL) of Bruch's membrane and in the choroidal stroma. Type I collagen (blue circle) is more prominent in the choroidal stroma than in Bruch's membrane. Collagen type IV (yellow circle) is localised principally to the basement membrane of the choriocapillaris (BM) although a little labelling is present over the retinal pigment epithelial basement membrane (RPE BM). Both types V (brown square) and VI collagen (red circle) are also present in the choriocapillaris basement membrane (BM), the latter being asymmetrically restricted to the choroidal side.

be due to differences in the availability of antigenic epitopes of the type IV collagen molecule (Essner, personal communication). We concluded from our own unpublished studies on the distribution of collagen types I–VI in macular Bruch's, that only the deposition of type IV collagen in focal thickenings of the choriocapillary basement membranes contributes to the age-related thickening of Bruch's membrane.

In contrast to type IV collagen, which is present throughout the entire circumference of the choriocapillaris, the distribution of type VI collagen was considerably more prominent on the choroidal side (unpublished observations). This suggests that type VI collagen is a product of pericytes which are only present in this location in normal tissue. This co-assembly is also apparent in aged human retinal vessels, where type VI collagen was associated with pericytes and not with endothelial cells.¹⁰⁶

The asymmetric distribution of type VI collagen in the choriocapillaris is also a feature of the fenestrated capillaries in the ciliary processes (see ciliary body). The asymmetric distribution in the ciliary processes suggests a mechanical anchorage of the fenestrated capillaries without impedance of aqueous flow production.¹⁰⁰ We suggest that type VI collagen has a similar function in Bruch's membrane. Considering the great extent to which the choroid can alter its volume in response to oxygen and carbon dioxide levels, anchorage of the choriocapillaris to the underlying choroid is obviously an essential feature of this system.¹⁰⁰ Impedance of nutrient flow from the fenestrated capillaries to the outer retina would presumably be undesirable.

The abundance of type IV collagen in the choriocapillaris basement membrane may act as an efficient inhibitor of endothelial cell migration¹²⁵ into Bruch's membrane in the normal situation. If, however, endothelial cells breach this barrier the presence of type I collagen in the inner and outer collagenous layers could augment the invasive behaviour of endothelial cells. The absence of type IV collagen beneath the retinal pigment epithelium may therefore be disadvantageous in limiting neovascularisation.

Optic nerve head

The nerve fibres of the optic nerve are enclosed and segmented by extracellular matrix that provides support and anchorage for the neural elements: the information on human material is based on light microscopic investigations. This extracellular matrix contains collagen types I, III, IV, V, and VI.¹³⁰ Types III, IV, and V collagen present in the prelaminar glial septa are thought to provide structural strength¹³⁰ thereby protecting the delicate axons which pass through the tissue. Type IV collagen present on the surface of the glial septa may serve to anchor the nerve fibres.¹³⁰ An age-related increase in the cores within the cribriform plates is associated with an increased density of collagen types I and III.¹³¹ An increase in deposition of type IV collagen in basement membranes surrounding blood vessels in the prelaminar and postlaminar regions could be associated with age-related axonal loss.¹³¹ Morrison *et al*¹³² mapped out the fine structural distribution of collagen types I–VI in the monkey optic nerve head by immunogold immunoelectron microscopy. Collagen types I, III, and V were located at striated fibrils in arterial walls, the core of striated fibrils within the lamina cribrosa beams and within the optic nerve septa. Both types IV and V collagen were present in the basement membranes of blood vessels and astrocytes. In an experimental model of glaucomatous optic neuropathy (cynomolgous monkey) extensive glaucomatous optic nerve damage was accompanied by abnormal deposition of collagen types I, III and IV within the laminar pores.¹³³ It is interesting to note that intraocular elevation can alter cellular metabolism in the lamina cribrosa:

there is greater gene expression for type I collagen in lamina cribrosa explant cells grown under high pressure.¹³⁴

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patients with Sorsby's fundus dystrophy and age-related macular degeneration was thought to be caused by thickening of Bruch's membrane interfering with metabolic exchange across Bruch's membrane between the choriocapillaris and the retinal pigment epithelium. The results may indicate that Bruch's membrane changes associated with peau d'orange in patients with PXE have little effect on function and seem not to interfere significantly with delivery of the metabolic substrates necessary for normal function to the photoreceptor cells.

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NOTICES

Medical Screening: The Way Forward

Medical screening provides many opportunities for the prevention of disease and handicap. What can it offer and what are its limitations? Based on several case studies, a one day conference entitled Medical Screening: The Way Forward, organised jointly by *BMJ* and *Journal of Medical Screening*, will be held on 26 January 1994 at the QE2 Conference Centre, London to examine the medical, scientific, ethical, social, psychological, and economic aspects of screening. For details: Pru Walters, BMA Conference Unit, BMA House, Tavistock Square, London WC1H 9JR. (Tel: 071-383 6605; Fax: 071-383 6400.)

Optics '94

Optics '94, an international exhibition on eye wear, technology, and equipment for optometry and ophthalmology will be held on 18-20 February 1994 at the World Trade Center, Singapore. A conference on better eye care will be held in conjunction with the exhibition. Further details: Lines Exposition & Management Services Pte Ltd, 318-B King George's Avenue, Singapore 0820. (Tel: (65) 2998611; Fax: (65) 2998633.)

International Society of Ocular Trauma

The 3rd International Symposium on Ocular Trauma will be held in Cancun, Mexico in March 1994. Further details: Secretariat, PO Box 50006, Tel Aviv, 61500, Israel. (Tel: (972) 5174571; Fax: (972) 5175674.)

Third Annual Scientific Meeting of the Australian Squint Club

The Third Annual Scientific Meeting of the Australian Squint Club will be held in Melbourne, Australia on 4-6 March 1994. Further details: Dr W E Gillies, 82 Collins Street, Melbourne 3000, Australia (tel: 61 3 654 5860; fax: 61 3 650 4404).

International Ophthalmic Excimer Laser Congress

The first annual United Kingdom International Ophthalmic Excimer Laser Congress will be held on 15 and 16 April 1994 at Redworth Hall Hotel and Country Club, County Durham. Details: Ms Judith Ritchey, Sunderland Eye Infirmary, Queen Alexandra Road, Sunderland, UK SR2 9HP. (Fax: 091-569 9275.)

Fourth Breton Workshop on Autoimmunity

The Fourth Breton Workshop on Autoimmunity will be held on 15-16 April 1994 in Brest, France. Further details: Secretariat, Laboratory of Immunology, Brest University Medical School Hospital, BP 824-29 609 Brest cedex, France. (Tel: (33) 98 22 33 84; Fax: (33) 98 80 10 76.)

European Society of Traditional Ophthalmology and Traditional Chinese Medicine

The 3rd international symposium of traditional medicine will be held on 12-22 May 1994 in Japan. Further details: Dr J Poletti, Société Européenne d'Ophthalmologie Traditionnelle, CHIC Tarbes, BP 1330, 65013 Tarbes Cedex, France. (Tel: 62 51 54 55; Fax: 62 51 51 62.)

American Academy of Optometry

A meeting of the American Academy of Optometry will be held on 28-30 May 1994, at the Amsterdam Marriott Hotel, Amsterdam, The Netherlands. Further details: Academy Office, 4330 East-West Highway, Suite 1117, Bethesda, MD 20814, USA. (Tel: (301) 718-6500; Fax: (301) 656-0989.)

International Conference on Biomedical Periodicals

The International Conference on Biomedical Periodicals will be held on 16-18 June 1994 in Beijing, China. Further details: Dr Yongmao Jiang, International Conference on Biomedical Periodicals, c/o Publishing House of Medical Journals, Chinese Medical Association, 42 Dongsi Xidajie, Beijing 100710, China. (Tel: 86-1-5133311 ext 362; Fax: 86-1-5123754.)

XXVIIth International Congress of Ophthalmology

The International Council of Ophthalmology will hold its XXVIIth Congress in Toronto, Canada on 26-30 June 1994. Further details: Secretariat, 275 Bay Street, Ottawa, Ontario, Canada K1R 5Z5. (Tel: (613) 563-1994; Fax: (613) 236-2727.)

Allied Health Personnel - International Congress of Ophthalmology '94

The first Allied Health Personnel Conference will be held in conjunction with the XXVIIth International Congress of Ophthalmology on 26-30 June 1994 in Toronto, Canada. Further details: Congress Canada, 191 Niagara Street, Toronto, Canada M5V 1C9. (Tel: (416) 860-1772; Fax: (416) 860-0380.)

Welsh Cataract Congress 1994

The Welsh Cataract Congress 1994 will be held on 8-10 September 1994. Details from: Eula Mae Childs, coordinator, Cullen Eye Institute, Baylor College of Medicine, 6501 Fannin, NC200, Houston, TX 77030, USA. (Tel: (713) 798-5941; Fax: (713) 798-4364.)

Third International Symposium on Ocular Inflammation

The 3rd international symposium on ocular inflammation will be held on 22-25 October 1994 in Fukuoka, Japan. Further details: Registration Secretary, c/o JTB Communications Inc, New Kyoto Center Building, 5F, Shiokoji, Shinmachi, Shimogyo-ku Kyoto 600, Japan.

Correction

We regret that, in the perspective in the August issue (1993; 77: 515-24), reference 134 was incorrect. The correct reference is:

- 134 Yang JL, Neufeld AH, Zorn MB, Hernandez MR. Collagen type I mRNA levels in cultured human lamina cribrosa cells; effects of elevated hydrostatic pressure. *Exp Eye Res* 1993; 56: 567-74.