

Chapter 38
Vitreous Biochemistry, Structure, and Clinical
Evaluation

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BIOCHEMISTRY

COLLAGENS

As is true for connective tissues everywhere, collagen is an important structural protein in vitreous. Gross¹ initially claimed that vitreous collagen fibrils are morphologically distinct from collagen in other connective tissues. However, Swann and coworkers² demonstrated that the amino acid composition of the insoluble residue of vitreous is similar to that of cartilage collagen and later identified that it is most similar to cartilage collagen composed of alpha 1, type II chains.³ Comparisons of the arthrogenic and immunologic properties of collagens from bovine articular cartilage (type II) and vitreous showed that the two were indistinguishable by these assays.⁴ Subsequent studies⁵ demonstrated that, although vitreous collagen contains an alpha 1, type II chain similar to cartilage collagen, there is a lower alanine content. Furthermore, these studies found that vitreous collagen has additional peptides present as uncleaved extension chains containing an amino acid composition different from the alpha chain component. The investigators concluded, however, that the overall similarities in amino acid composition and in the types of cyanogen bromide cleavage peptides indicate that the fibers of the central and posterior peripheral regions of the vitreous are composed of a collagen that should be classified as type II. Linsenmayer and collaborators⁶ measured *in vivo* synthesis of types I and II collagen in chick embryo vitreous by radioimmunoprecipitation after tritiated proline labeling and found that more than 90% of the labeled material in the vitreous was type II collagen. Snowden⁷ provided further physicochemical evidence in support of the similarities between vitreous and cartilage collagens. This may explain why certain clinical phenomena, such as inborn errors of type II collagen metabolism in arthro-ophthalmopathies, manifest phenotypic expression in each of these two tissues.

There are, however, distinct differences in the chemical composition of vitreous and cartilage collagens that are only partly due to the presence of terminal peptide constituents in vitreous collagen.⁵ Swann and Sotman⁸ have demonstrated that the carbohydrate content of pepsin-solubilized vitreous alpha chains is significantly greater than cartilage alpha chains, indicating that the carbohydrate side chains of vitreous collagen are largely composed of disaccharide units similar to those found in basement membrane collagen. They proposed that these distinct chemical features are related to the special structure of the mature vitreous fibrils *in vivo*. Liang and Chakrabarti⁹ have shown that there are differences between bovine cartilage and vitreous with respect to collagen fibril growth, melting temperature, and fluorescence with a hydrophobic fluorescent probe. These investigators and others¹⁰ proposed that vitreous collagen should be considered a "special" type II collagen. Schmut and associates¹¹ employed differential salt precipitation of pepsin-solubilized collagen from bovine vitreous and found that

type II collagen is the major component of native vitreous fibers.

Gloor¹² pointed out that the collagen content is highest where the vitreous is a gel. Ayad and Weiss¹³ studied bovine vitreous collagen to determine whether the gel-like structure of vitreous could be explained on the basis of chemical composition. Their findings demonstrated that type II is the major vitreous collagen, but collagens composed of $\alpha 1$, $\alpha 2$, and $\alpha 3$ chains as well as C-PS disulfide-bonded collagen were present in concentrations similar to those in cartilage. In contrast to cartilage, however, vitreous type II collagen was significantly more hydroxylated in the lysine and proline residues. The $\alpha 1$, $\alpha 2$, and $\alpha 3$ collagen chains were interpreted by Van der Rest¹⁴ to represent type IX collagen, although Eyre and colleagues¹⁵ felt that there was evidence to indicate the presence of type V collagen in vitreous. Furthermore, with respect to the disulfide-bonded collagen, vitreous had three times more C-PS1 and C-PS2 collagens than cartilage, although the molar ratio of C-PS 1 to C-PS2 in both was 1:1, suggesting that in both tissues these collagens are components of a larger molecule. Other studies¹⁴ demonstrated that these disulfide-linked triple-helix fragments were actually derivatives of type IX collagen. In this regard vitreous is again similar to cartilage, insofar as both contain type IX collagen,¹⁵ although the two tissues differ in the sizes of type IX collagen chains.¹⁶ Hong and Davison¹⁷ have identified a procollagen in the soluble fraction of rabbit vitreous that was identified as type II by segment-long-spacing banding patterns. Detection of a propeptide extension only at the N-terminus prompted these investigators to conclude that this was a novel type II procollagen. Such distinctive characteristics are possibly related to the unique physiologic roles of vitreous, in particular, its mechanical function.¹⁸

Individual vitreous collagen fibrils are organized as a triple helix of three alpha chains. The major collagen fibrils of the corpus vitreus are heterotypic, consisting of more than one collagen type. Recent studies of pepsinized forms of collagen confirm that the corpus vitreus contains collagen type II, a hybrid of types V/XI, and type IX (Fig. 1).

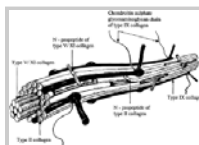


Fig. 1. Vitreous collagen fibril structure. This schematic diagram of the structure of the major heterotypic collagen fibrils of vitreous is largely speculative but based on the current knowledge of the structure and biophysical attributes of the constituent molecules, particularly in how they would assemble to form fibrils. (From Bishop P: The biochemical structure of mammalian vitreous. Eye 10:64, 1996.)

Type II Collagen

Type II collagen, a homotrimer composed of three identical alpha

chains designated as $[\alpha 1 (II)]_3$, comprises 75% of the total collagen content in vitreous. When first synthesized as a procollagen and secreted into the extracellular space, type II collagen is highly soluble. The activity of N-proteinase and C-proteinase enzymes reduces the solubility and enables type II collagen molecules to cross-link covalently in a quarter-staggered array. Within this array are likely to be N-propeptides, which probably extend outward from the surface of the forming fibril.¹¹ This may influence the interaction of the collagen fibril with other components of the extracellular matrix. Recent studies¹⁹ combined immunolocalization with Western blot analysis of macromolecules extracted from bovine vitreous collagen fibrils and found that the pN-type IIA procollagen is located on the surface of the vitreous collagen fibril. The finding²⁰ that type IIA procollagen propeptides specifically bind transforming growth factor-beta 1 ($TGF_{\beta 1}$) and BMP-2 supports the concept that, in certain circumstances, these growth factors and cytokines interact with vitreous fibrils to promote the cell migration and proliferation that result in proliferative diabetic retinopathy and proliferative vitreoretinopathy.

Type IX Collagen

Type IX collagen is a heterotrimer that is disulfide-bonded with an $[\alpha 1 (IX) \alpha 2 (IX) \alpha 3 (IX)]$ configuration. This heterotrimer is orientated regularly along the surfaces of the major collagen fibrils in a "D periodic" distribution, in which it is cross-linked onto the fibril surface. Type IX is not a typical collagen but is a member of the fibrillar-associated collagens with interrupted triple helixes (FACIT) group of collagens. It contains collagenous regions described as COL1, COL2, and COL3 interspersed between noncollagenous regions called NC1, NC2, NC3, and NC4.^{21,22} In vitreous (as opposed to cartilage) the NC4 domain is small and, therefore, not highly charged and not likely to exhibit extensive interaction with other extracellular matrix components.²³ In vitreous, type IX collagen always contains a chondroitin sulfate glycosaminoglycan chain,^{21,22} which is linked covalently to the $\alpha 2$ (IX) chain at the NC3 domain; this enables the molecule to assume a proteoglycan form. Electron microscopy of vitreous stained with cationic dyes enables visualization of the chondroitin sulfate chains of type IX collagen. In some of these studies sulfated glycosaminoglycans are found distributed regularly along the surface of vitreous collagen fibrils²⁴ and often bridge between neighboring collagen fibrils. Duplexing of glycosaminoglycan chains from adjacent collagen fibrils may result in a ladder-like configuration.²⁵

Type V/XI Collagen

Ten percent of vitreous collagen is a hybrid V/XI collagen, which is believed to comprise the central core of the major collagen fibrils of

vitreous.²⁶ Type V/XI is a heterotrimer that contains $\alpha 1$ (XI) and $\alpha 2$ (V) in two chains, but the nature of the third chain is presently not known.¹⁵ Along with type II collagen, type V/XI is a fibril-forming collagen. Although the interaction of the fibril with other extracellular matrix components is probably influenced by a retained N-propeptide that protrudes from the surface of the fibril in cartilage,²⁶ it is not known whether this is the case in vitreous.²⁷

Type VI Collagen

Although there are only small amounts of type VI collagen in vitreous, the ability of this molecule to bind both type II collagen and hyaluronan (HA) suggests that it could be important in organizing and maintaining the supramolecular structure of vitreous gel.

GLYCOSAMINOGLYCANS

Glycosaminoglycans are polysaccharides of repeating disaccharide units, each consisting of hexosamine (usually N-acetyl glucosamine or N-acetyl galactosamine) glycosidically linked to either uronic (glucuronic or iduronic) acid or galactose. The nature of the predominant repeating unit is characteristic for each glycosaminoglycan and the relative amount, molecular size, and type of glycosaminoglycan are said to be tissue specific.²⁸ Glycosaminoglycans do not normally occur as free polymers *in vivo* but are covalently linked to a protein core; the ensemble is called a proteoglycan. A sulfated group is attached to oxygen or nitrogen in all glycosaminoglycans except HA. Balazs²⁹ first documented the presence of sulfated galactosamine-containing glycosaminoglycans in bovine vitreous (less than 5% of total vitreous glycosaminoglycans), and others^{30,31} identified these as chondroitin-4-sulfate and undersulfated heparan sulfate. Studies in the rabbit³² found a total vitreous glycosaminoglycans content of 58 ng with 13% chondroitin sulfate and 0.5% heparan sulfate.

Hyaluronan

Although HA is present throughout the body, it was first isolated from bovine vitreous. In humans, HA first appears after birth and then becomes the major vitreous glycosaminoglycan. Although it has been proposed that hyalocytes synthesize HA,¹⁸ other plausible candidates are the ciliary body and retinal Müller cells. Whereas the synthesis of HA seems to continue at a constant rate in the adult while no extracellular degradation occurs, HA levels are in a steady-state because the molecule escapes via the anterior segment of the eye.²⁹ Laurent and Fraser³³ showed that the passage of HA from the vitreous to the anterior segment is strongly molecular-weight dependent, indicating a diffusion-controlled process. In contrast, disappearance of HA from the anterior chamber is independent of molecular weight, suggesting that this is

controlled by bulk flow.

HA is a long, unbranched polymer of repeating glucuronic acid β -1,3-N,N-acetylglucosamine disaccharide moieties linked by β 1-4 bonds.⁵ It is a linear, left-handed, three-fold helix with a rise per disaccharide on the helix axis of 0.98 nm.³⁴ Rotary shadowing electron microscopy of human and bovine vitreous detected lateral aggregates of HA that form an anastomosing three-dimensional network.³⁵ This periodicity, however, can vary depending on whether the helix is in a compressed or extended configuration.³⁶ Changes in the degree of extension of HA could be important in the role vitreous plays in retinal disease. Indeed, the volume of the unhydrated HA molecule is about $0.66\text{cm}^3/\text{g}$, whereas the hydrated specific volume is 2000 to $3000\text{cm}^3/\text{g}$.²⁹ Thus, the degree of hydration has a significant influence on the size and configuration of the HA molecular network. Although there is no definitive evidence that adjacent HA chains bind to one another, Brewerton and Mayne³⁷ first proposed such an arrangement. Recent rotary shadowing electron microscopy studies³⁸ of bovine and human vitreous found lateral aggregates of HA that formed three-dimensional lattice-like networks. HA also interacts with the surrounding mobile ions and can undergo changes in its conformation that are induced by changes in the surrounding ionic milieu.³⁹ A decrease in surrounding ionic strength can cause the anionic charges on the polysaccharide backbone to repel one another, resulting in an extended configuration of the macromolecule. An increase can cause contraction of the molecule and, in turn, the entire vitreous body. As a result of HA's entanglement and immobilization within the vitreous collagen fibril matrix, this mechanical force can be transmitted by collagen fibrils to the retina, optic disc, and other structures such as neovascular complexes. In this way, changes in the ionic milieu of vitreous may be converted into mechanical energy via extension or contraction of the HA macromolecule. This can be important in certain pathologic conditions that feature fluctuations in ionic balance and hydration, such as diabetes.⁴⁰

The sodium salt of HA has a molecular weight of 3 to 4.5×10^6 Da in normal human vitreous.²⁹ Laurent and Granath⁴¹ used gel chromatography and found the average molecular weight of rabbit vitreous to be 2 to 3×10^6 Da and of bovine vitreous to be 0.5 to 0.8×10^6 Da. In these studies there were age-related differences in the bovine vitreous, in which HA molecular weight varied from 3×10^6 Da in the newborn calf to 0.5×10^6 Da in old cattle. Furthermore, there may be several species of HA within vitreous that have polysaccharide chains of different lengths⁴² with a variable distribution in different topographic regions within the corpus vitreous.⁴³

An important property of HA is steric exclusion.⁴⁴ With its flexible linear chains and random coil conformation, HA occupies a large

volume and resists the penetration of this volume by other molecules to a degree dependent on their size and shape.³⁶ This excluded volume effect can influence equilibria between different conformational states of macromolecules and alter the compactness or extension of these molecules.⁴⁴ Steric exclusion also causes an excess of osmotic pressure when such compounds as albumin and HA are mixed, because the resultant osmotic pressure is greater than the sum of the two components. This could be important in diabetes, in which vascular incompetence can increase vitreous concentrations of serum proteins such as albumin. These osmotic effects can induce contraction and expansion of the corpus vitreus similar to the foregoing description of hydration and ion-induced changes within vitreous and can similarly play an important role in neovascularization and diabetic vitreous hemorrhage.⁴⁰ An increase in the chemical activity of a compound because of steric exclusion can also cause its precipitation if the solubility limit is reached. This could be important in the formation of pathologic vitreous opacities, such as asteroid hyalosis and amyloid bodies.¹⁸

Chondroitin Sulfate

Vitreous contains two chondroitin sulfate proteoglycans. The minor type is actually type IX collagen, which was described above. Most vitreous chondroitin sulfate is in the form of versican.³⁸ This large proteoglycan has a globular N-terminal that binds HA via a 45 kDa link protein.³⁸ Thus, in human, but not bovine vitreous, versican is believed to form complexes with HA, as well as with microfibrillar proteins such as fibulin-1 and fibullin-2.⁴⁵

Heparin Sulfate

This sulfated proteoglycan is normally found in basement membranes and on cell surfaces throughout the body. It was first detected in bovine vitreous in 1977⁴⁶ and in chick vitreous (as agrin) in 1995.⁴⁷ However, it is not clear whether heparan sulfate is a true component of vitreous or a contaminant from adjacent basement membranes, such as the internal limiting lamina (ILL) of the retina.⁴⁸ As pointed out by Bishop⁴⁵ this may also be the case for nodogen-1, the aforementioned fibulins, and fibronectin.

NONCOLLAGENOUS STRUCTURAL PROTEINS

Fibrillins

Fibrillin-containing microfibrils are more abundant in vitreous than the type VI collagen microfibrils described above. They are found in vitreous gel, as well as in the zonules of the lens. This fact explains why in Marfan's syndrome the defects in the gene encoding fibrillin-1 (*FBN1* on chromosome 15q21) result in both ectopia lentis and vitreous liquefaction.⁴⁵ The latter probably plays

a role in the frequent occurrence of rhegmatogenous retinal detachment in these patients.

Opticin

The major noncollagenous protein of vitreous is a leucine-rich repeat (LRR) protein, which is bound to the surface of the heterotypic collagen fibrils, known as opticin.⁴⁹ Formerly called vitrican, opticin is believed to be important in collagen fibril assembly and in preventing the aggregation of adjacent collagen fibrils into bundles. Thus, a breakdown in this property or activity may play a role in age-related vitreous degeneration⁵⁰ (see later discussion below).

VIT 1

Another novel vitreous protein is VIT1, a collagen-binding macromolecule.⁵¹ Because of its propensity to bind collagen, this highly basic protein may play an important role in maintaining vitreous gel structure.

MISCELLANEOUS MOLECULAR COMPONENTS

Amino Acids and Proteins

Free amino acids are present in the vitreous, but at one fifth of the levels found in plasma.⁵² An amino acid concentration gradient is present within the vitreous body, with anterior vitreous concentrations greater than posterior levels. This may be due to uptake and utilization of amino acids by the retina, a consideration that led Reddy⁵³ to propose that the vitreous acts as a metabolic repository for retinal protein metabolism. Other studies⁵⁴ found that the soluble proteins of vitreous resemble serum proteins with isoelectric points less than 6.0, leading investigators to conclude that the soluble proteins of vitreous derive from plasma and are constantly renewed. Flood and Balazs⁵⁵ studied 920 human eyes and found the following age-related protein concentrations: ages 10 to 50, 400 to 600 µg/ml; ages 50 to 80, 700 to 800 µg/ml; older than 80 years of age, about 1000 µ/ml. These age-related findings may be due to increased leakage of plasma proteins from the intravascular compartment into the vitreous, resulting from decreased tight-junction integrity with aging of the retinal and ciliary body vasculature and epithelia.

Glycoproteins

Glycoproteins are heteropolysaccharide macromolecules, as opposed to the homogeneous, repeating disaccharide units of glycosaminoglycans that are mostly proteinaceous and contain only a minor carbohydrate component (5% to 10% by weight).

According to Balazs²⁹ the most important difference between vitreous and serum proteins is the high content of glycoproteins in the vitreous, because these constitute 20% of the total noncollagenous protein content of vitreous. Sialic acid-containing glycoproteins are believed to be synthesized by hyalocytes.⁵⁶ Other studies⁵⁷ have led to the postulate that the inner layer of the ciliary epithelium is responsible for vitreous glycoprotein synthesis. Cartilage oligomeric protein (COMP), an acidic glycoprotein with a characteristic five-armed structure, is present in vitreous, but its function is currently unknown.

Ascorbic Acid

Ascorbic acid concentrations in vitreous are about 0.43 mmol/kg.⁵⁸ Thus, the vitreous-plasma ratio for ascorbic acid is 9:1. Vitreous levels that are much higher than plasma concentrations are believed to be due to active transport by the ciliary body epithelium.⁵⁹ The purpose of having high concentrations of ascorbic acid in the vitreous may relate to the abilities of this compound to absorb ultraviolet light and to serve as a free-radical scavenger,⁶⁰ which may protect not only the retina and lens but also vitreous from the untoward effects of metabolic and light-induced⁶¹ singlet oxygen generation.

Lipids

Swann and coinvestigators⁶² found that the residual fraction of vitreous contained a significant quantity of lipid. Later studies⁶³ found evidence of active lipid metabolism in vitreous of dogs and humans, but in the latter species no significant changes in vitreous lipid composition between the ages of 37 and 82 years.

SUPRAMOLECULAR ORGANIZATION

As described by Mayne,⁶⁵ vitreous is organized in a dilute meshwork of collagen fibrils interspersed with extensive arrays of long HA molecules. The collagen fibrils provide a scaffold-like structure that is inflated by the hydrophilic HA. If collagen is removed, the remaining HA forms a viscous solution; if HA is removed, the gel shrinks³⁹ but is not destroyed. Early physiologic observations⁶⁶ suggested the existence of an interaction between HA and collagen that stabilizes collagen. Biomechanical studies⁶⁷ of vitreous viscoelasticity suggested similar effects on HA. Early investigators⁶⁸ observed reversible complexes of an electrostatic nature between solubilized collagen and various glycosaminoglycans and suggested that collagen-HA interaction occurs on a physicochemical rather than chemical level. Others⁶⁹ later demonstrated that the sulfate group of a glycosaminoglycan is largely responsible for such interactions with the guanidino groups of arginine and epsilon-amino groups of lysine in collagen. Comper and Laurent³⁹ proposed that in vitreous electrostatic binding occurs

between negatively charged polysaccharides and positively charged proteins. These authors extensively reviewed the existing data characterizing the electrostatic properties of glycosaminoglycans and the factors influencing their electrostatic interactions with different ions and molecules.

Balazs²⁹ hypothesized that the hydroxylysine amino acids of collagen mediate polysaccharide binding to the collagen chain via O-glycosidic linkages. These polar amino acids are present in clusters along the collagen molecule, which may explain why proteoglycans attach to collagen in a periodic pattern.²⁴ Hong and Davison⁷⁰ identified a type II procollagen in the soluble fraction of rabbit vitreous and proposed a possible role for this molecule in mediating collagen-HA interaction. In cartilage, "link glycoproteins" have been identified that interact with proteoglycans⁷¹ and HA.⁷² Supramolecular complexes of these glycoproteins are believed to occupy the interfibrillar spaces. In Asakura's²⁴ studies of bovine vitreous stained with ruthenium red there are amorphous structures located on collagen fibrils at 55 to 60 nm intervals along the fibrils and filaments connecting these amorphous masses to adjacent collagen fibrils. These filaments may represent "link" structures of either a glycoprotein or proteoglycan nature. HA is known to interact with link proteins, as well as with an HA-binding glycoprotein called *hyaluronectin*.⁷³ In the cornea, chondroitin sulfate and keratan sulfate bridge the interfibrillar spaces and keep the fibrils at specified distances to achieve transparency.⁷⁴ The protein cores of these proteoglycans could be the linkage sites to collagen fibrils.

Bishop²⁷ has proposed that an understanding how vitreous gel is organized and stabilized requires an understanding of what prevents collagen fibrils from aggregating and by what means the collagen fibrils are connected to maintain a stable gel structure. Studies⁷⁵ have shown that the chondroitin sulfate chains of type IX collagen bridge adjacent collagen fibrils in a ladder-like configuration spacing them apart. This arrangement might account for vitreous transparency, because keeping vitreous collagen fibrils separated by at least one wavelength of incident light would minimize light scattering, allowing unhindered transmission of light to the retina for photoreception. However, depolymerizing with chondroitinases does not destroy the gel, suggesting that chondroitin sulphate side chains are not essential for vitreous collagen spacing. Complexed with HA, however, the chondroitin sulfate side chains might space the collagen fibrils,^{25,74,75} apart but Bishop believes that this form of collagen-HA interaction is "very weak." Instead, he proposes that the LRR protein opticin is the predominant structural protein in short-range spacing of collagen fibrils. Concerning long-range spacing, Scott²⁵ and Mayne and coworkers⁷⁷ have claimed that HA plays a pivotal role in stabilizing the vitreous gel via this mechanism. However, studies⁷⁶ using HA lyase to digest vitreous HA demonstrated that the gel structure was not destroyed, suggesting that HA is not essential for the

maintenance of vitreous gel stability, leading to the proposal that collagen alone is responsible for the gel state of vitreous.²⁷

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STRUCTURE

HISTORICAL PERSPECTIVE

Duke-Elder⁷⁸ claimed that the first theories of vitreous structure proposed that the vitreous is composed of "loose and delicate filaments surrounded by fluid," as conceptualized in 1741 by Demours⁷⁹ who formulated the alveolar theory. In 1780, Zinn⁸⁰ proposed that the vitreous is arranged in a concentric, lamellar configuration similar to the layers of an onion. The dissections and histologic preparations of Von Pappenheim⁸¹ and Brucke⁸² provided evidence for the lamellar theory. The radial sector theory was proposed by Hannover in 1845.⁸³ Studying coronal sections at the equator, he described a multitude of sectors approximately radially oriented around the central anteroposterior core that contains Cloquet's canal. Hannover likened this structure to the appearance of a cut orange. In 1848 Bowman⁸⁴ introduced the fibrillar theory. Employing microscopy, he described fine fibrils that form bundles that Retzius⁸⁵ described as fibrous structures arising in the peripheral anterior vitreous that assume an undulating pattern similar to a "horse's tail" in the central vitreous but maintain a concentric configuration at the periphery. The elegant studies of Szent-Gyorgi⁸⁶ in 1917 supported the descriptions of Retzius and introduced the concept that vitreous structure changes with age. Eisner⁸⁷ studied dissections of human vitreous and found "membranelles," which he described as funnels packed into one another diverging outward and anteriorly from the prepapillary vitreous. Worst⁸⁸ has also studied preparations of dissected human vitreous and described that the tracts of Eisner constitute the walls of cisterns within the vitreous body. In Worst's studies these cisterns are visualized by filling with white India ink. Worst has also studied the premacular vitreous in great detail and has proposed the existence of a "bursa premacularis," which he described as a pear-shaped space that is connected to the cisternal system in front of the ciliary body. Kishi and Shimizu⁸⁹ recently described a "posterior vitreous pocket" similar to what Worst has reported, which they claim to be an anatomic structure. However, because more than 95% of their study population was age 65 years or older, this is likely to be an age-related phenomenon.⁹⁰

VITREOUS BODY (CORPUS VITREUS)

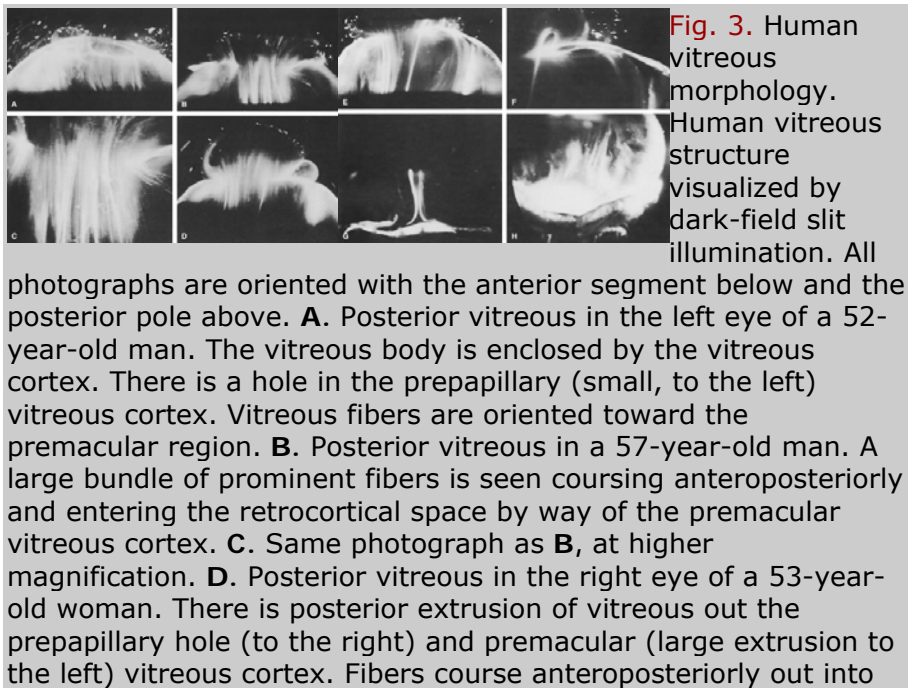
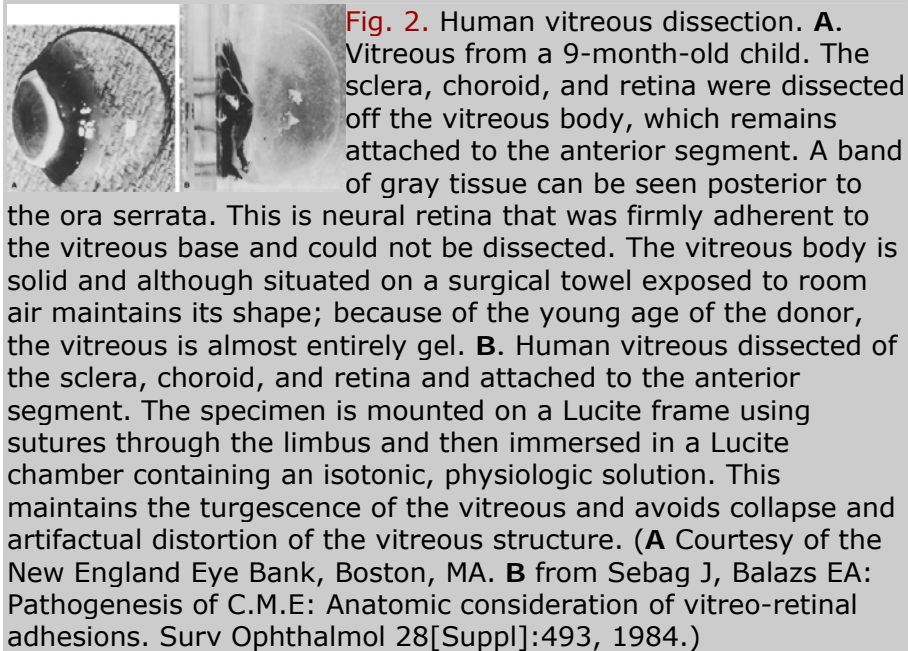
There is heterogeneity in the distribution of collagen throughout the vitreous body. Chemical^{91,92} and light-scattering^{93,185} studies have shown that the highest density of collagen fibrils is present in the vitreous base, followed by the posterior vitreous cortex anterior to the retina, and then by the anterior vitreous cortex behind the posterior chamber and lens. The lowest density is found in the

central vitreous and adjacent to the anterior cortical gel. HA molecules have a different distribution from collagen. They are most abundant in the posterior cortical gel with a gradient of decreasing concentration as one moves centrally and anteriorly.⁹⁴⁻⁹⁶ Balazs⁹⁶ has hypothesized that this is because vitreous HA is synthesized by hyalocytes in the posterior vitreous cortex and cannot traverse the ILL of the retina. HA leaves the vitreous to enter the posterior chamber by way of the annulus of the anterior vitreous cortex that is not adjacent to a basal lamina. Bound water (non-freezable) has a distribution within the vitreous similar to that of HA,⁹⁷ presumably resulting from binding by HA.

Laboratory investigations of vitreous structure have long been hampered by the absence of easily recognized landmarks within the vitreous body. Consequently, removal of the vitreous from the eye results in a loss of orientation. The transparency of the vitreous renders observation in conventional diffuse light unrewarding. Attempts to study vitreous structure with opaque dyes⁸⁸ do visualize the areas filled by dye but obscure the appearance of adjacent structures. The use of histologic contrast-enhancing techniques usually involves tissue fixation, which often includes dehydration of the tissue. Because vitreous is 98% water, dehydration induces profound alteration of the internal morphology. Consequently, any investigation of vitreous structure must overcome these difficulties.

The sclera, choroid, and retina can be dissected and the naked vitreous body can be maintained intact and attached to the anterior segment of the eye ([Fig. 2A](#)). This enables study of internal vitreous morphology without a loss of intraocular orientation. However, depending on the person's age and consequently the degree of vitreous liquefaction,⁵⁵ the dissected vitreous will remain solid and intact in young persons or will be flaccid and collapse in older individuals. Consequently, vitreous turgescence must be maintained to avoid distortion of intravitreal structure. Immersion of a dissected vitreous specimen that is still attached to the anterior segment into a physiologic solution maintains vitreous turgescence and avoids structural distortion (see [Fig. 2B](#)). The limitations induced by the transparency of the vitreous were overcome by Goedbloed,⁹⁸ Friedenwald and Stiehler,⁹⁹ and Eisner⁸⁷ who employed darkfield slit illumination of the vitreous body to achieve visualization of intravitreal morphology. Illumination with a slit-lamp beam directed into the vitreous body from the side and visualization of the illuminated portion from above produces an optical horizontal section of the vitreous body.¹⁰⁰ The illumination/observation angle of 90 degrees that is achieved by using this technique maximizes the Tyndall effect and, thus, overcomes the limitations induced by vitreous transparency. Furthermore, the avoidance of any tissue fixation eliminates the introduction of many of the artifacts that flawed earlier investigations. Recent studies have used these techniques to investigate human vitreous structure. Within the adult human

vitreous there are fine, parallel fibers coursing in an anteroposterior direction^{18,100-102} (Figs. 3B and C and 4). The fibers arise from the vitreous base (see Figs. 3H and 4) where they insert anterior and posterior to the ora serrata (see Fig. 3H). As the peripheral fibers course posteriorly they are circumferential with the vitreous cortex, whereas central fibers undulate in a configuration parallel with Cloquet's canal.⁸⁵ The fibers are continuous and do not branch. Posteriorly, these fibers insert into the vitreous cortex (see Fig. 3E and E).



the retrocortical space. **E.** Horizontal optical section of the same specimen as **D**, at a different level. A large fiber courses posteriorly from the central vitreous and inserts into the premacular vitreous cortex. **F.** Same view as **E**, at higher magnification. The large fiber has a curvilinear appearance because of traction by the vitreous extruding into the retrocortical space (see **D**). However, because of its attachment to the posterior vitreous cortex, the fiber arcs back to its point of insertion. **G.** Anterior and central vitreous in a 33-year-old woman. Cloquet's canal is seen forming the retrolental space of Berger. **H.** Anterior and peripheral vitreous in a 57-year-old man. The specimen is tilted forward to enable visualization of the posterior aspect of the lens and the peripheral anterior vitreous. To the right of the lens there are fibers coursing anteroposteriorly that insert into the vitreous base. These fibers "splay out" to insert anterior and posterior to the ora serrata. (**A**, **E**, and **F** from Sebag J, Balazs EA: Pathogenesis of C.M.E.: Anatomic consideration of vitreo-retinal adhesions. *Surv Ophthalmol* 28[Suppl]:493, 1984. **B**, **C** from Sebag J, Balazs EA: Morphology and ultrastructure of human vitreous fibers. *Invest Ophthalmol Vis Sci* 30:187, 1989. **D**, **G**, and **H** from Sebag J: *The Vitreous: Structure, Function and Pathobiology*. New York, Springer-Verlag, 1989. Specimens were courtesy of the New York Bank for Sight and Restoration, New York, NY.)



Fig. 4. A. Vitreous base morphology. Vitreous structure in a 58-year-old woman. Fibers course anteroposteriorly in the central and peripheral vitreous.

Posteriorly, fibers orient to the premacular region. Anteriorly, the fibers "splay out" to insert into the vitreous base. **B.** Fibers of the peripheral anterior vitreous forming the anterior loop. This configuration can provide the scaffold for cell migration and proliferation in the pathophysiology of anterior proliferative vitreoretinopathy. (**A** from Sebag J, Balazs EA: Pathogenesis of C.M.E.: Anatomic consideration of vitreo-retinal adhesions. *Surv Ophthalmol* 28[Suppl]:493, 1984. **B** from Sebag J: *The Vitreous: Structure, Function and Pathobiology*. New York, Springer-Verlag, 1989. Specimen in **A** courtesy of the New England Eye Bank, Boston, MA.)

Ultrastructural studies¹⁰³ have demonstrated that collagen fibrils are the only microscopic structures that could correspond to these fibers. These studies also detected the presence of bundles of packed, parallel collagen fibrils ([Fig. 5](#)). It has been hypothesized that visible vitreous fibers form when HA molecules no longer separate microscopic collagen fibrils, resulting in the aggregation of collagen fibrils into bundles from which HA molecules are excluded. Eventually the aggregates of collagen fibrils attain sufficiently large proportions and can be visualized *in vitro* (see [Figs. 3](#) and [4](#)) and clinically. The areas adjacent to these large fibers have a low

therefore, do not scatter light as intensely as the larger bundles of aggregated collagen fibrils. These adjacent channels probably offer relatively less resistance to bulk flow through the vitreous body and are the areas visualized in studies^{88,104} using India ink to fill the channels. There are changes occurring in these fibrous structures throughout life,^{50,100} which probably result from age-related biochemical alterations in the composition and organization of the molecular components that simultaneously result in vitreous liquefaction and fiber formation.

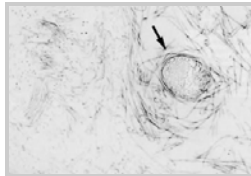


Fig. 5. Ultrastructure of human vitreous. Although specimens were centrifuged to concentrate structural elements, there were no membranes or membranous elements. Only collagen fibrils were detected. There were also bundles of parallel collagen fibrils such as the one shown here in cross section (*arrow*). (From Sebag J, Balazs EA: Morphology and ultrastructure of human vitreous fibers. Invest Ophthalmol Vis Sci 30:187, 1989.)

VITREOUS BASE

The vitreous base is a three-dimensional zone. It extends 1.5 to 2 mm anterior to the ora serrata, 1 to 3 mm posterior to the ora serrata,¹⁰⁵ and several millimeters into the vitreous body.¹⁰⁶ The posterior extent of the posterior border of the vitreous base varies with age.^{50,107} Vitreous fibers enter the vitreous base by splaying out (see Fig. 4A) to insert anterior and posterior to the ora serrata (see Fig. 3H). The anterior-most fibers form the anterior loop of the vitreous base (see Fig. 4B), a structure that is important in the pathophysiology of anterior proliferative vitreoretinopathy.^{18,108-110} In the posterior portion of the vitreous base, vitreous fibers are closer together than elsewhere. Gartner¹¹¹ has found that in humans the diameters of collagen fibrils in the vitreous base range from 10.8 to 12.4 nm, with a major period of cross-striations of 50 to 54 nm. Hogan¹²⁷ demonstrated that just posterior to the ora serrata, heavy bundles of vitreous fibrils attach to the basal laminae of retinal glial cells. Studies by Gloor⁶⁴ and Daicker¹¹² showed that cords of vitreous collagen insert into gaps between the neuroglia of the peripheral retina. They likened this structure to Velcro (a self-adhesive nylon material) and proposed that this would explain the strong vitreoretinal adhesion at this site. In the anterior vitreous base, fibrils interdigitate with a reticular complex of fibrillar basement membrane material between the crevices of the nonpigmented ciliary epithelium.¹¹³ The vitreous base also contains intact cells that are fibroblast-like anterior to the ora serrata and macrophage-like posteriorly.¹¹³ Damaged cells in different stages of involution and fragments of basal laminae, presumed to be remnants of the embryonic hyaloid vascular system, are also present in the vitreous base.¹¹³

VITREOUS CORTEX

The vitreous cortex is the peripheral shell of the vitreous body that courses forward and inward from the anterior vitreous base to form the anterior vitreous cortex and posteriorly from the posterior border of the vitreous base to form the posterior vitreous cortex. The anterior vitreous cortex, clinically referred to as the *anterior hyaloid face*, begins about 1.5 mm anterior to the ora serrata. Fine and Tousimis¹¹⁴ described that in this region the collagen fibrils are parallel to the surface of the cortex. Studies by Faulborn and Bowald¹¹⁵ detected dense packing of collagen fibrils in the anterior cortex with looser collagen fibril packing in the subjacent vitreous, giving the impression of lamellae. Rhodes¹¹⁶ studied mouse vitreous and found that the anterior vitreous cortex varied in thickness from 800 to 2000 nm. He also found that there are connections between the loose fibrils in the anterior vitreous and the anterior vitreous cortex.

The posterior vitreous cortex is 100 to 110 μm thick¹¹⁷ and consists of densely packed collagen fibrils (Fig. 6). There is no vitreous cortex over the optic disc (Figs. 3A and 7), and the cortex is thin over the macula because of rarefaction of the collagen fibrils.¹¹⁷ The prepapillary hole in the vitreous cortex can sometimes be visualized clinically when the posterior vitreous is detached from the retina. If peripapillary glial tissue is torn away during posterior vitreous detachment (PVD) and remains attached to the vitreous cortex around the prepapillary hole, it is referred to as Vogt's or Weiss's ring. Vitreous can extrude through the prepapillary hole in the vitreous cortex (see Fig. 3A) but does so to a much lesser extent than through the premacular vitreous cortex (see Figs. 2B and D and 6). Jaffe¹¹⁸ has described how vitreous can extrude into the retrocortical space created following PVD and has proposed that persistent attachment to the macula can produce traction and certain forms of maculopathy.^{119,120} Although there are no direct connections between the posterior vitreous and the retina, the posterior vitreous cortex is adherent to the ILL of the retina, which is actually the basal lamina of retinal Müller's cells. The exact nature of this adhesion between the posterior vitreous cortex and the ILL is not known but probably results from extracellular matrix molecules.¹²¹⁻¹²³

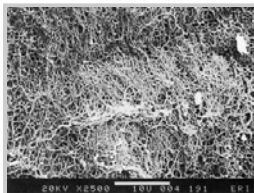


Fig. 6. Ultrastructure of human vitreous cortex. Scanning electron microscopy demonstrates the dense packing of collagen fibrils in the vitreous cortex. To some extent this arrangement is exaggerated by the dehydration that occurs during specimen preparation for scanning electron microscopy.

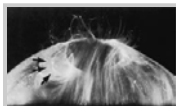


Fig. 7. Posterior human vitreous structure. Posterior vitreous in the left eye of a 59-year-old man. The vitreous cortex envelops the vitreous body and multiple, small, very bright points, which are hyalocytes (see Figs. 8, 9), are seen to scatter light intensely. There are two areas in the posterior vitreous cortex through which vitreous extrudes into the retrohyaloid space. The prepapillary hole is smaller (to the left, see *arrows* at nasal edge) and has a small amount of extruding vitreous. Larger amounts of vitreous extrude by way of the premacular vitreous cortex and fibers

the observer's pupil is imaged reaches that pupil. Thus, the fundus can be seen only where the observed and the illuminated areas overlap and where the light source and the observer's pupil are aligned optically. This restricts the extent of the examined area and also because of a limited depth of field, this method is rarely used to assess vitreous structure.

Indirect Ophthalmoscopy

Indirect ophthalmoscopy extends the field of view by using an intermediate lens to gather rays of light from a wider area of the fundus. Although binocularity provides stereopsis, the image size is considerably smaller than with direct ophthalmoscopy and only significant alterations in vitreous structure, such as the hole in the prepapillary posterior vitreous cortex (see [Figs. 3D](#) and [7](#)), vitreous hemorrhage, or asteroid hyalosis, are reliably diagnosed by indirect ophthalmoscopy. There is also difficulty in examining the peripheral vitreous resulting from a loss of stereopsis because when examining the periphery the circular pupil becomes an elliptic aperture, making it difficult to obtain an adequate view with both of the observer's eyes. This is more of a problem in the horizontal meridians than in the vertical, because the examiner's two eyes are positioned horizontally. Scleral indentation during indirect ophthalmoscopy decreases this difficulty, but more so for peripheral fundus examination than for the vitreous.

Slit-Lamp Biomicroscopy

The anterior vitreous is easily examined at the slit lamp without preset or contact lenses. In the absence of a crystalline or artificial intraocular lens, vitreous prolapse into the anterior chamber could be important in vitreocorneal touch and the risks of corneal endothelial cell dysfunction.¹⁶¹ Vitreous adhesions to a cataract wound or to the iris may be important in the pathogenesis of postoperative cystoid macular edema.¹⁶² Particulate opacities in the anterior vitreous can be seen at the slit lamp and can give important clues as to the possible presence of posterior pathology, such as retinitis pigmentosa.¹⁶³ Cells can be the augury of retinal infection, inflammation, tears, and/or detachments. Lacqua and Machemer¹⁶⁴ described that an increase in the number and size of pigmented cells in the vitreous of patients with retinal detachment (preoperatively or postoperatively) heralds the development of proliferative vitreoretinopathy. Bleeding can be associated with red blood cells in the anterior vitreous. Various neoplastic diseases, for example, endophytic retinoblastoma, choroidal melanoma, and reticulum cell sarcoma, can result in anterior vitreous cells. Anterior vitreous structures such as Mittendorf's dot, a remnant of embryonic hyaloid vessel regression, can be seen at the slit lamp and should alert the examiner to the possibility of other developmental disorders, such as persistent hyperplastic primary vitreous in the fellow eye.¹⁶⁵ Effectively using slit-lamp

biomicroscopy to overcome vitreous transparency necessitates maximizing the Tyndall effect. Although this can be achieved *in vitro*, as described previously (and for the anterior vitreous), there are limitations to the illumination/observation angle that can be achieved clinically in the mid- and posterior vitreous. This is even more troublesome in the presence of meiosis, corneal and/or lenticular opacities, and limited patient cooperation. Pupil dilation in the patient should be maximized to achieve an adequate Tyndall effect, because the Tyndall effect increases with an increasingly subtended angle between the plane of illumination and the line of observation (up to a maximum of 90 degrees) and dark adaptation in the examiner. Some observers purport that green light enhances the Tyndall effect, although this has never been explained or tested scientifically.

Preset lens biomicroscopy attempts to increase the available illumination/observation angle, offers dynamic inspection of vitreous *in vivo*, and provides the capability of recording the findings in real-time.¹⁶⁶ Initially introduced on a wide scale for use with a Hruby lens and currently practiced by using a hand-held 90.00 diopter (D) lens at the slit lamp, this technique can be performed either with a plano concave lens (e.g., -55.00 to -58.60 D Hruby lenses) or various convex lenses (e.g., +32.00, +58.60, +60.00, or +90.00 D). Plano concave lenses produce a highly magnified, narrow-field, erect image and enable visualization of the posterior pole, although it is difficult to achieve an adequate illumination/observation angle to examine the posterior vitreous. Peripheral examination can be performed only by varying the position of the fixation point of the eye(s), and the quality of the image is reduced by optical distortions. El-Bayadi¹⁶⁷ first proposed the use of a +55.00 D preset lens and advised maintaining at least a 10 degree illumination/observation angle. The resultant image is inverted and can be photographed. The advantage offered by this form of posterior vitreous biomicroscopy is that the avoidance of a contact lens facilitates the "ascension/descension" examination technique,¹⁶⁸ whereby eye movements are used to displace the vitreous. This can be helpful in visualizing structures such as an operculum or Vogt's (or Weiss's) ring, which may have descended inferiorly in the presence of a PVD with vitreous syneresis.¹⁰⁸ It is principally this feature that makes the approach superior to contact lens systems for examination of the posterior vitreous.¹⁶⁹ A +90.00 D double aspheric lens can similarly be used in a "preset" manner, also with photographic capabilities.¹⁷⁰ To examine the peripheral vitreous, Schepens¹⁶⁸ suggests reducing the illumination/observation angle, rotating the slit beam to the axis of the meridian being observed, and reducing the interpupillary distance of the slit-lamp eyepieces as ways of minimizing the loss of stereopsis.

Peripheral vitreous examination has been traditionally performed with the various mirrors of the Goldmann lens. Both Schepens¹⁶⁸ and Jaffe¹⁷¹ give excellent detailed accounts of the procedure to be

followed for peripheral vitreous examination. Each describes the use of the "oscillation" technique of "rocking" the slit-lamp joystick to alternate between direct and retroillumination for visualization of particulate or cellular opacities in the posterior vitreous.

Schepens¹⁶⁸ further describes the use of a tilted slit-lamp column to enhance visualization of the peripheral vitreous. Eisner⁸⁷ has devised a cone-shaped apparatus that fits onto a three-mirror Goldmann lens and enables peripheral scleral indentation during slit-lamp biomicroscopy with a contact lens. Binocular indirect ophthalmoscopy with scleral indentation can also permit such examination. The recent development of inverted-image contact lenses with various-size fields has greatly enhanced stereoscopic examination of the vitreous and fundus, and they are now routinely used for laser photocoagulation therapy of the fundus. The Mainster retina laser lens¹⁷² is a +61.00 D convex aspheric contact lens that produces a real inverted image of about 45 degrees of the posterior fundus with excellent stereopsis. The "panfundoscopic" lens is a +85.00 D convex spheric lens that provides less magnification and image clarity but offers a wider angle view. It is useful for peripheral examination to 60 or 70 degrees with a 15-degree tilt.

DIAGNOSTIC TECHNOLOGIES

Scanning Laser Ophthalmoscopy

The Scanning laser ophthalmoscope (SLO) was developed at the Schepens Eye Research Institute in Boston. This technique features tremendous depth-of-field and offers real-time recording of findings. Monochromatic green, as well as other wavelengths of light, are available for illumination.¹⁷³ To date, however, the SLO has really only improved the ability to visualize details in the prepapillary posterior vitreous, such as Weiss's ring (see [Figs. 3D](#) and [7](#)). Unfortunately, despite the dramatic depth-of-field possible with this technique, SLO does not adequately image the vitreous body and an attached posterior vitreous cortex, probably because of limitations in the level of resolution. Thus, PVD, by far the most common diagnosis to be entertained when imaging vitreous clinically, is not reliably determined by SLO. Indeed, there is an increasing awareness among vitreous surgeons that the reliability of the clinical diagnosis of total PVD by any existing technique is woefully inadequate. This awareness arises because vitreous surgery following clinical examination often reveals findings that are contradictory to preoperative assessments.

Ultrasonography

When examination of the vitreous is made difficult by opacification of the cornea, lens, or vitreous, there can, nevertheless, be worthwhile information garnered from careful study. As pointed out by Charles,¹⁷⁴ much can be learned from studying the geometric configuration of an opaque or semi-opaque vitreous. When

opacification is advanced, however, ultrasonography can be helpful in defining the nature of the opacification, the three-dimensional configuration of the opaque vitreous, and the presence or absence of structural pathology behind the vitreous.

Ultrasound is an inaudible acoustic wave that has a frequency of more than 20 kHz. The frequencies used in ophthalmology are generally in the range 8 to 10 MHz. Although these very high frequencies produce wavelengths as short as 0.2 mm, these are not short enough to adequately assess normal internal vitreous structure such as the fibers described previously. Even the posterior vitreous cortex, about 100 μm at its thickest point in the normal state, is below the level of resolution of conventional ultrasonography. The utility of this technique results from strong echoes produced at acoustic interfaces found at the junctions of media with different densities and sound velocities, and the greater the difference in density between the two media that create the acoustic interface, the more prominent the echo. Thus, age-related or pathologic phase alterations within the vitreous body are detectable by ultrasonography.

Oksala, among the first to employ B-scan ultrasonography to image vitreous in the late 1950s and early 1960s, summarized his findings of aging changes in 1978.¹⁷⁵ In that report of 444 "normal" subjects, Oksala defined the presence of acoustic interfaces within the vitreous body as evidence of vitreous aging and determined that the incidence of such interfaces was 5% between the ages of 21 to 40 years and was 80% in individuals older than 60 years of age. In clinical practice, however, only profound entities such as asteroid hyalosis, vitreous hemorrhage, and intravitreal foreign bodies (if sufficiently large) are imaged by ultrasonography. At the vitreoretinal interface, the presence of a PVD is often suspected on the basis of B-scan ultrasonography but can never be definitively established, because the level of resolution is not sufficient to reliably image the posterior vitreous cortex, which is only a little more than 100 μm thick at its thickest portion. However, recent studies have successfully used this technique to determine the presence of a split ([Fig. 11](#)) in the posterior vitreous cortex, called vitreoschisis, in patients with proliferative diabetic vitreoretinopathy.¹⁷⁶ The success achieved in using ultrasound to identify this important pathologic entity probably results because in advanced cases this tissue is significantly thickened by nonenzymatic glycation of vitreous collagen and other proteins¹⁷⁷ in the posterior vitreous cortex. Future studies should determine if vitreoschisis can be identified by ultrasound in other conditions, especially premacular membranes with pucker and macular holes. However, it may turn out that the thickness of these tissue planes is below the level of resolution presently available with ultrasonography. Thus, it follows that the diagnosis of complete PVD cannot be reliably established by ultrasonography.



Fig. 11. Vitreoschisis. B-scan ultrasonography of vitreoschisis demonstrates splitting of the vitreous cortex (*arrow*) that can mimic posterior vitreous detachment. In diabetic patients, blood can be present in the vitreoschisis cavity.¹⁷⁶ (I, inner wall; P, posterior wall of vitreoschisis cavity within the posterior vitreous cortex) (Photograph courtesy of Dr. Ronald Green. From Green RL, Byrne SF: Diagnostic ophthalmic ultrasound. In Ryan SJ (ed). Retina. St Louis: CV Mosby, 1989.)

Optical Coherence Tomography

Introduced in 1991, optical coherence tomography (OCT) is a new technique for high-resolution cross-sectional imaging of ocular structure.¹⁷⁸ OCT is based on the principle of low-coherence interferometry, in which the distances between and sizes of structures in the eye are determined by measuring the “echo” time it takes for light to be backscattered from the different structures at various axial distances. The resolution of all echo-based instrumentation (such as ultrasound and OCT) is based on the ratio of the speed of the incident wave to that of the reflected wave. As described previously, clinical ultrasonography is performed with a frequency of approximately 10 MHz and has a 150 μm resolution. Although recently introduced ultrasound biomicroscopy has increased the frequency (up to 100 MHz), and, thus, has a spatial resolution of 20 microns, penetration into the eye is no more than 4 to 5 mm. Light-based devices, such as the OCT, use an incident wavelength of 800 nm and have increased axial resolution to 10 μm , providing excellent imaging of retinal architecture, although vitreous applications are less useful. The limitations of OCT include the inability to obtain high-quality images through media opacities such as dense cataract or vitreous hemorrhage. The use of OCT is also limited to cooperative patients who are able to maintain fixation for the full acquisition time of 2.5 seconds per section. Thus, to date OCT has primarily been used to image, and to some extent quantitate, retinal laminar structure. Some vitreous applications have been useful, especially those that involve imaging the vitreomacular interface in patients with macular holes.¹⁷⁹ However, the exact nature and molecular composition of these preretinal tissue planes cannot be definitively deduced using OCT, and little information can be garnered about structures within the vitreous body.

Spectroscopy

NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY.

The nuclear magnetic resonance (NMR) phenomenon is based on the fact that, when placed in a magnetic field, magnetic nuclei such

as water protons tend to orient their magnetic vectors along the direction of the magnetic field. The time constant for this orientation, known as the longitudinal relaxation time T_1 , reflects the thermal interactions of protons with their molecular environment. Magnetic vectors that have previously been induced to be in phase with each other undergo a dephasing relaxation process that is measured by the transverse relaxation time T_2 . It is the transverse relaxation time T_2 that reflects inhomogeneities within the population of protons. Protons oriented by a magnetic field absorb radio waves of the appropriate frequency to induce transitions between their two orientations. This absorption is the basis of the NMR signal used to index relaxation times. Relaxation times in biologic tissues vary with the concentration and mobility of water within the tissue. Because the latter is influenced by the interaction of water molecules with macromolecules in the tissue, this noninvasive measure can assess the gel to liquid transformation that occurs in vitreous during aging¹⁸⁰ and in disease states, such as diabetic vitreopathy.^{50,181} These considerations led Aguayo et al¹⁸² to use NMR in studying the effects of pharmacologic vitreolysis¹⁵³ of bovine and human vitreous specimens and intact bovine eyes *in vitro*. Collagenase induced measurable vitreous liquefaction, more so than hyaluronidase. Thus, this noninvasive method could be used to evaluate age and disease-induced synchysis (liquefaction) of the vitreous body, although it is not clear that this technique would adequately evaluate the vitreoretinal interface. There have, curiously, been few recent studies that have employed NMR spectroscopy in research or clinical applications on vitreous.

RAMAN SPECTROSCOPY.

This form of spectroscopy was first described in 1928 by C.V. Raman in India. Raman spectroscopy is an inelastic light scattering technique wherein the vibrational mode of molecules in the study specimen absorb energy from incident photons, causing a downward frequency shift, which is called the Raman shift. Because the signal is relatively weak, current techniques employ laser-induced stimulation with gradual increases in the wavelength of the stimulating laser, to detect the points at which the Raman signal becomes apparent as peaks superimposed on the broad background fluorescence. The wavelengths at which these peaks are elicited are characteristic of the chemical bonds, such as aliphatic C-H (2939 cm^{-1}), water O-H (3350 cm^{-1}), and C-C and C-H stretching vibrations in π -conjugated and aromatic molecules (1604 cm^{-1} and 3057 cm^{-1}). To date, most applications of this technique in the eye have been for analysis of lens structure and pathology.¹⁸³ The use of near-infrared (IR) excitation wavelengths is particularly effective in the lens, because these wavelengths have better penetration through cataractous lenses.

Studies¹⁸⁴ of samples of excised human vitreous obtained during surgery used near-IR excitation at 1064 nm provided by a diode-

pumped neodymium:yttrium-aluminum-garnet (Nd:YAG) CW laser with a diameter of 0.1 mm and a power setting of 300 mW. Back-scattering geometry with an optical lens collected scattered light that was passed through a Rayleigh light rejection filter into a spectrophotometer. The results showed that this technique was able to detect peaks at 1604 cm^{-1} and 3057 cm^{-1} in vitreous of diabetic patients that were not present in controls. Further research and development is needed to reliably interpret such results and refine the methodology for noninvasive use *in situ*. Although this has already been achieved in the lens, it is not clear that this will be possible for vitreous applications.

Dynamic Light Scattering

Dynamic light scattering (DLS) is an established laboratory technique to measure average size (or size distribution) of microscopic particles as small as 3 nm in diameter that are suspended in a fluid medium in which they undergo random Brownian motion. Light scattered by a laser beam passing through such a dispersion will have intensity fluctuations in proportion to the Brownian motion of the particles. Because the size of the particles influences their Brownian motion, analysis of the scattered light intensity yields a distribution of the size(s) of the suspended particles. Visible light from a laser diode (power 50 μW) is focused into a small scattering volume inside the specimen (excised lens or vitreous, autopsy or living eye). The detected signal is processed via a digital correlator to yield a time autocorrelation function (TCF). For dilute dispersions of spherical particles, the slope of the TCF provides a quick and accurate determination of the particle's translational diffusion coefficient, which can be related to its size via a Stokes-Einstein equation, provided that the viscosity of the suspending fluid, its temperature, and its refractive index are known. For the lens and vitreous, a viscosity of $\eta = 0.8904$ centipoise, a refractive index of $n = 1.333$, and a temperature of 25°C for *in vitro* studies and 37°C for *in vivo* studies were used to determine macromolecule sizes.

Studies¹⁸⁵⁻¹⁸⁷ in the lens and vitreous employ DLS instrumentation that was developed by Dr. Rafat Ansari at National Aeronautics and Space Administration (NASA) to conduct fluid physics experiments on-board the space shuttle and space station orbiters. The input beam from a semiconductor laser (670-nm wavelength) at 50 μW power was projected into the specimens, and the scattered signal was collected by the DLS probe for a duration of 10 seconds. The signal was then detected by an avalanche photodiode detector system. A TCF was constructed using a digital correlator card. The slope of the TCF provides a measure of particle sizes in the selected measurement sites (volume = $50\text{ }\mu\text{m}^3$). Studies¹⁸⁶ in the lens found that DLS was significantly more sensitive than the Scheimpflug photography in detecting early changes in the lens. When the DLS probe was used to obtain measurements from the entire vitreous body, scanning was performed in conjunction with a

micropositioning assembly, which controlled detector position in the X, Y, and Z planes. This enabled semi-automated measurements from a sufficient number of sites within the bovine vitreous body to create a three-dimensional map of the distribution of the average particle sizes of vitreous macromolecules. Furthermore, in studies¹⁸⁷ of autopsy human eyes, DLS was able to detect the structural changes¹⁸¹ resulting from diabetic vitreopathy.⁴⁰

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