Vitreous Biochemistry, Morphology, and Clinical Examination*

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BIOCHEMISTRY

COLLAGEN

Collagen is an important structural protein in the vitreous. Gross\(^1\) initially claimed that the collagen fibrils of the vitreous were morphologically distinct from collagen in other connective tissues. Yet, Swann and co-workers\(^2\) demonstrated that the amino acid composition of the insoluble residue of vitreous is similar to that of cartilage collagen and later identified that the composition is most similar to cartilage collagen composed of alpha 1, type II chains.\(^3\) Comparisons of the arthritogenic and immunologic properties of collagens from bovine articular cartilage (type II) and vitreous showed that the two were indistinguishable by these assays.\(^4\) However, subsequent studies\(^5\) demonstrated that although vitreous collagen contained an alpha 1, type II chain similar to cartilage collagen, there was a lower alanine content. Furthermore, these studies found that vitreous collagen had additional peptides that were present as uncleaved extension chains containing an amino acid composition that was 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 collaborators\(^6\) measured in vivo synthesis of types I and II collagen in chick embryo vitreous by radioimmunoprecipitation after tritiated proline labeling and found that over 90% of the labeled material in the vitreous was type II collagen. Snowden\(^7\) provided further physicochemical evidence in support of the similarities between vitreous and cartilage collagens.

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.\(^8\) Swann and Sotman\(^9\) 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 Chakrabarti\(^10\) 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 others\(^11\) proposed that vitreous collagen should be considered a "special" type II collagen. Schmut and associates\(^11\) 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.

Gloor\(^12\) has pointed out that the collagen content is highest where the vitreous is a gel (vitreous cortex and vitreous base). Ayad and Weiss\(^13\) 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

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collagen chains were interpreted by Van der Rest to represent type IX collagen, although Eyre and colleagues felt that there was evidence to indicate the presence of type V collagen in the 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-PS1 to C-PS2 in both was 1:1, suggesting that in both tissues these collagens are components of a larger molecule. Other studies demonstrated that these disulfide-linked triple-helix fragments were actually derivatives of type IX collagen. In this regard vitreous is once again similar to cartilage insofar as both contain type IX collagen, although the two tissues differ in terms of the sizes of type IX collagen chains.

Hong and Davison 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. Once again it appears that although vitreous collagen could be considered a type II collagen, there are unique features that distinguish it from other collagens in the same class. These distinctive characteristics are possibly related to the unique physiologic roles of the vitreous, in particular, its mechanical function.

HYALURONIC ACID

Hyaluronic acid (HA) is one of many known glycosaminoglycans. Glycosaminoglycans are polysaccharides composed 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 glycosaminoglucan and the relative amount, molecular size, and type of glycosaminoglycan are said to be tissue-specific. A sulfated group is attached to oxygen or nitrogen in all glycosaminoglycans except HA. Glycosaminoglycans do not normally occur in vivo as free polymers, but are covalently linked to a protein core, the ensemble called a proteoglycan. Balazs and co-workers documented the presence of sulfated galactosamine-containing glycosaminoglycans in bovine vitreous (less than 5% of total vitreous glycosaminoglycans). Others identified these as chondroitin-4-sulfate and undersulfated heparan sulfate. Studies in the rabbit found a total vitreous glycosaminoglycan content of 58 ng with 13% chondroitin sulfate and 0.5% heparan sulfate.

HA is the major glycosaminoglycan present in the vitreous. In humans, HA first appears after birth and is believed to be synthesized primarily by hyalocytes. The synthesis of HA seems to stabilize at a constant level in the adult and there does not appear to be any extracellular degradation. HA levels remain constant due to escape of HA molecules from the vitreous by way of the anterior segment of the eye and because of reuptake by hyalocytes. Laurent and Fraser showed that the escape 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. The values ascribed to the molecular weight of vitreous HA in different studies range widely depending on analytic methodologies, species variations, and age-related differences. Balazs and Denlinger have stated that, in general, the sodium salt of HA has a molecular weight of 3 to 4.5 × 10⁶ in all normal human and animal tissues investigated and in all parts of the vitreous. Laurent and Granath used gel chromatography and found the average molecular weight of rabbit vitreous to be 2 to 3 × 10⁶ and of bovine vitreous to be 0.5 to 0.8 × 10⁶. In these studies there were age-related differences in the bovine vitreous, in which HA molecular weight varied from 3 × 10⁶ in the newborn calf to 0.5 × 10⁶ in old cattle. Furthermore, there may be several species of HA within the vitreous that have polysaccharide chains of different lengths. Topographic studies have identified five HA fractions in the cortical vitreous (intrinsic viscosities 420 to 1800 ml/g) and seven in the central vitreous (intrinsic viscosities 510 to 3320 ml/g).

The volume of the unhydrated HA molecule is about 0.66 ml/g, whereas the hydrated specific volume is 2000 to 3000 ml/g. Thus the degree of hydration and any pathologic condition that alters hydration (see chapter 39 on vitreous pathobiology, this volume) can have a significant influence on the size and configuration of the molecular network in the vitreous. Because the solution domains are so large, the long unbranched HA chains form widely open coils that at concentrations greater than 1 mg/ml become highly entangled. The large domain of HA spreads the anionic charge of the molecule over a wide space. Due to its entanglement and immobilization in tissue, HA can act much like an ion-exchange resin,
with an electrostatic interaction occurring between the small charges of mobile ions in the tissue and the electrostatic envelope of the stationary polyelectrolyte. This electrostatic interaction forms the basis for various properties of HA including its influence on osmotic pressure, on ion transport and distribution, and on electric potentials within the vitreous.\textsuperscript{32}

X-ray diffractograms have determined that HA is a linear helix.\textsuperscript{33} Further studies\textsuperscript{34} have demonstrated a left-handed, threefold helix with a rise per disaccharide on the helix axis of 0.98 nm. As described in a detailed review by Chakrabarti and Park,\textsuperscript{35} this periodicity can vary depending on whether the helix is in a "compressed" or "extended" configuration. The existence of the molecule in either of these two states can greatly influence the interactions of an HA molecule with its neighboring molecules. A compressed HA chain has extensive "interdigitation" to interact with its nearest antiparallel as well as parallel neighbors (totaling eight molecules), whereas extended forms interact with only three antiparallel neighbors.\textsuperscript{35} It is known that changes in the microenvironment and in the types of surrounding counterions can cause changes in the conformation of the HA polyanion.\textsuperscript{32} For example, a decrease in ionic strength can cause the anionic charges on the polysaccharide backbone to repel one another and result in an extended configuration of the macromolecule. Thus, changes in the ionic milieu surrounding vitreous HA may be converted to mechanical energy on extension or contraction of the HA macromolecule and, in turn, swelling or shrinkage of the entire vitreous. This can be important in certain pathologic conditions, such as diabetes (see chapter 39 on vitreous pathobiology, this volume). Early studies by Christiansson\textsuperscript{36} showed that alloxan-induced experimental diabetes in rabbits resulted in an increase in glucosamine content and viscosity of vitreous and a decrease in vitreous volume. More recent studies\textsuperscript{37} showed a slight increase in the tonicity of human diabetic vitreous (324 ± 23 mOsm versus 316 ± 21 mOsm in controls). In diabetes mellitus, there can be significant fluctuations in the systemic concentrations of a variety of molecules which can alter the ionic milieu of the vitreous. Such shifts could induce ultrastructural and volumetric changes in the vitreous. Furthermore, shifts in systemic metabolism, and in turn, osmolarity and hydration of the vitreous, could result in periodic swelling and contraction of the entire vitreous with resultant traction upon structures attached to the vitreous cortex, such as new blood vessels. These events could influence the course of diabetic retinopathy by contributing to the proliferation of neovascular fronds\textsuperscript{38} and perhaps even by inducing rupture of new vessels and causing vitreous hemorrhage. Indeed, Tasman\textsuperscript{39} has found that in 53 cases of vitreous hemorrhage due to proliferative diabetic retinopathy, 62.3% of bleeding episodes occurred between midnight and 6 AM while in the remaining parts of the day there was only an 11 to 13% incidence. Although he speculated that this could be due to nocturnal hypoglycemia, other metabolic or hormonal fluctuations could be influencing the vitreous in the ways described above and lead to vitreous hemorrhage.

Another important property of HA is that of steric exclusion.\textsuperscript{40} HA, with its flexible linear chains and entangled coil conformation, occupies a large volume and resists the penetration of this volume by other molecules to a degree dependent upon their size and shape.\textsuperscript{35} The excluded volume effect can influence equilibria between different conformational states of macromolecules and alter the compactness or extension of these molecules. Although there is evidence to suggest that steric exclusion by collagen is more important than HA,\textsuperscript{32} the relative contribution to this activity by these two components in the vitreous is not known. Steric exclusion occurs on a molecular level and is an entropic phenomenon not directly producing heat effects between interactive components. Comper and Laurent\textsuperscript{32} extensively reviewed the influence that steric exclusion can have on chemical phenomena within the vitreous, emphasizing the effects on osmotic pressure and enzyme activity. Steric exclusion causes an excess of osmotic pressure when such compounds as albumin and HA are mixed, since the resultant osmotic pressure is greater than the sum of the two components. This could be important in diabetes where vascular incompetence can increase vitreous levels of serum proteins such as albumin. In this way osmotic effects can induce contraction or expansion of the vitreous, which can in turn play an important role in neovascularization and vitreous hemorrhage. Enzyme reactions can be affected since steric exclusion alters the Michaelis-Menten constant as well as the inhibitor constant. An increase in the chemical activity of a compound due to steric exclusion can 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 amyloidosis (see chapter 39 on vitreous pathobiology, this volume).\textsuperscript{41}
COLLAGEN-Hyaluronic Acid Interaction

The vitreous is composed of interpenetrating networks of HA molecules and collagen fibrils. The collagen fibrils provide a solid consistency to the vitreous, which is "inflated" by the hydrophilic contribution of HA. Comper and Laurent\textsuperscript{12} found that if collagen is removed from the vitreous, the remaining HA forms a viscous solution; if HA is removed, the gel shrinks. There is substantial evidence to support the concept that there exists some sort of interaction between HA and collagen, and that the structure and function of these macromolecules are influenced by this interaction. Physiologic observations\textsuperscript{42,43} of changes in the denaturation-temperature profile induced by the addition of glycosaminoglycans to collagen support this hypothesis. Biomechanical studies\textsuperscript{44} of vitreous viscoelasticity also support the concept of collagen-HA interaction, but the nature of this interaction is not clearly understood. It has been hypothesized that in cartilage the hydroxylsine amino acids of collagen mediate polysaccharide binding to the collagen chain via O-glycosidic linkages. Thus the number of hydroxylsine residues per alpha chain should be proportional to the amount of polysaccharide bound. This was substantiated by the finding that type II collagen, which in cartilage is found in a matrix with significant amounts of proteoglycan, contains four to nine times more hydroxylsine than collagen types I and III.\textsuperscript{45} Furthermore, these polar amino acids are present in clusters along the collagen molecule, explaining why proteoglycans attach to collagen with a periodic pattern.\textsuperscript{46} Hong and Davison\textsuperscript{47} have identified a type II procollagen in the soluble fraction of rabbit vitreous and have raised the question of a possible role for this molecule in mediating collagen-HA interaction. Indeed, collagen-HA interaction in the vitreous may be mediated by a third molecule. Swann and co-workers\textsuperscript{48} have demonstrated large amounts of noncollagenous protein associated with collagen in the insoluble residue fraction of vitreous. In cartilage, "link glycoproteins" have been identified which interact with proteoglycans\textsuperscript{49} and HA.\textsuperscript{49} Supramolecular complexes of these glycoproteins are believed to occupy the interfibrillar spaces. Asakura\textsuperscript{50} has studied bovine vitreous by ruthenium red staining and demonstrated the presence of amorphous structures on collagen fibrils at 55- to 60-nm intervals along the fibrils that they believed to be HA. There are filaments connecting the collagen fibrils and these amorphous masses. 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, hyaluronectin.\textsuperscript{50} In the cornea, chondroitin sulfate and keratan sulfate bridge the interfibrillar spaces and keep the fibrils at specific distances to achieve transparency.\textsuperscript{51} The protein cores of these proteoglycans are the linkage sites to collagen fibrils.

Many investigators believe that collagen-HA interaction occurs on a physicochemical rather than chemical level. Mathews\textsuperscript{52} observed reversible formation of complexes of an electrostatic nature between solubilized collagen and various glycosaminoglycans. Both the sulfate and carboxyl groups of a glycosaminoglycan could be the binding sites for cations.\textsuperscript{53} Podraźsky and colleagues\textsuperscript{53} demonstrated that the sulfate group of a glycosaminoglycan was largely responsible for interactions with the guanidino groups of arginine and epsilon-aminogroups of lysine in collagen. Comper and Laurent\textsuperscript{52} proposed that electrostatic binding occurs in the vitreous between negatively charged polysaccharides and positively charged proteins. These authors extensively reviewed the existing data characterizing the electrostatic properties of glycosaminoglycans and the factors, including steric exclusion, influencing their electrostatic interactions with different ions and molecules.

Other Molecular Components

Free amino acids are present in the vitreous but at levels about one fifth that of plasma.\textsuperscript{54} Within the vitreous body there exists a concentration gradient, with anterior vitreous concentrations being greater than posterior levels. This may be due to uptake and utilization by the retina, a consideration that led Reddy\textsuperscript{55} to propose that the vitreous acts as a metabolic repository for retinal protein metabolism. Chen and Chen\textsuperscript{56} found that the soluble proteins of the vitreous resemble the serum proteins of isoelectric points less than 6.0, and these investigators concluded that the soluble proteins of the vitreous derive from plasma and are constantly renewed. Studies\textsuperscript{57} by Flood and Ballazs of 920 human eyes 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; greater than 80 years of age, about 1000 μg/ml. These age-related findings may be due to increased leakage of plasma proteins from the intravascular compartment into the vitreous that results from decreased tight-junction integrity with aging of the retinal and ciliary body vasculature and epithelia.
**Glycoproteins** are heteropolysaccharide macromolecules that are mostly proteinaceous and contain only a minor carbohydrate component (5 to 10% by weight). According to Balazs, the most important difference between vitreous and serum proteins is the high content of glycoproteins in the vitreous, since these constitute 20% of the total noncollagenous protein content of vitreous. Sialic acid-containing glycoproteins are believed to be synthesized by hyalocytes. Other studies have led to the consideration that the inner layer of the ciliary epithelium is responsible for vitreous glycoprotein synthesis.

**Ascorbic acid** concentrations in vitreous are about 0.43 mmol/kg. Thus the vitreous-plasma ratio for ascorbic acid is 9:1. Vitreous levels this much higher than plasma concentrations are believed to be due to active transport by the ciliary body epithelium. 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 serve as a free-radical scavenger, which protect the retina and lens from the untoward effects of metabolic and light-induced singlet oxygen generation.

Swann and co-investigators found that the residual fraction of vitreous contained a significant quantity of lipid. Reddy and associates found evidence to suggest active lipid metabolism in the vitreous of dogs and humans. Interestingly, these investigators found no significant changes in human vitreous lipid composition between the ages of 37 and 82 years.

Traces of strontium, barium, aluminum, molybdenum, manganese, iron, nickel, copper, zinc, and lead have been found in vitreous. Table 1 lists the vitreous concentrations of low molecular weight substances and other molecules in bovine and porcine eyes.

**TABLE 1. Chemical Analysis of Bovine and Porcine Vitreous Humors**

<table>
<thead>
<tr>
<th></th>
<th>Bovine (N = 120)</th>
<th>Porcine (N = 120)</th>
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<tbody>
<tr>
<td></td>
<td>Serum</td>
<td>Vitreous</td>
</tr>
<tr>
<td>Urea nitrogen (mg/dl)</td>
<td>12.6 ± 0.2</td>
<td>10.3 ± 6.4</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>1.3 ± 0.7</td>
<td>0.6 ± 0.3</td>
</tr>
<tr>
<td>Sodium (mEq/L)</td>
<td>145 ± 9</td>
<td>137 ± 9</td>
</tr>
<tr>
<td>Potassium (mEq/L)</td>
<td>6.3 ± 2.2</td>
<td>4.6 ± 1.6</td>
</tr>
<tr>
<td>Calcium (mg/dl)</td>
<td>9.9 ± 1.9</td>
<td>5.4 ± 2.5</td>
</tr>
<tr>
<td>Magnesium (mg/dl)</td>
<td>2.2 ± 0.7</td>
<td>2.3 ± 0.3</td>
</tr>
<tr>
<td>Chloride (mEq/L)</td>
<td>103 ± 14</td>
<td>120 ± 13.4</td>
</tr>
<tr>
<td>Phosphorus (mg/dl)</td>
<td>6.7 ± 2.6</td>
<td>1.0 ± 0.7</td>
</tr>
</tbody>
</table>

* All values are means ± SD.


**SPECIES VARIATIONS**

There are species variations in the relative concentrations of the major structural components of the vitreous, that is, HA and collagen. These differences account for variations in the rheologic (gel-liquid) state of the vitreous in different species, and are summarized in Table 2. The selection of an appropriate animal with which to model human disease for investigation must therefore take into consideration these species variations as well as age related differences.

**MORPHOLOGY**

**HISTORICAL PERSPECTIVE**

Duke-Elder 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 Demours who formulated the alveolar theory. In 1780, Zinn 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 Pappenheim and Brucke provided evidence for the lamellar theory. The radial sector theory was proposed by Hannover in 1845. 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 Bowman introduced the fibrillar theory. Employing microscopy, he described fine fibrils that form bundles that Reitzius described as fibrous structures arising in the peripheral anterior vitreous that assume an undulating pattern...
TABLE 2. Species Variations in the Relative Concentrations of the Major Structural Components of the Vitreous

<table>
<thead>
<tr>
<th>Component</th>
<th>Rheology</th>
<th>Range of HA Concentration (μg/ml)</th>
<th>Protein Concentration (μg/ml)</th>
<th>Collagen (% of Total Protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gel (% of Total)</td>
<td>Liquid (% of Total)</td>
<td>Insoluble</td>
<td>Soluble</td>
</tr>
<tr>
<td>Human</td>
<td>40-80</td>
<td>20-60</td>
<td>100-400</td>
<td>280-1360</td>
</tr>
<tr>
<td>Rhesus monkey</td>
<td>60</td>
<td>40</td>
<td>100</td>
<td>113-139</td>
</tr>
<tr>
<td>Owl monkey</td>
<td>2</td>
<td>98</td>
<td>300-600 (liquid)</td>
<td>91</td>
</tr>
<tr>
<td>Cow</td>
<td>100</td>
<td>0</td>
<td>800-900 (gel)</td>
<td>81</td>
</tr>
<tr>
<td>Sheep</td>
<td>100</td>
<td>0</td>
<td>100-1070</td>
<td>113</td>
</tr>
<tr>
<td>Dog</td>
<td>100</td>
<td>0</td>
<td>40-60</td>
<td>198</td>
</tr>
<tr>
<td>Cat</td>
<td>100</td>
<td>0</td>
<td>20-50</td>
<td></td>
</tr>
<tr>
<td>Rabbit</td>
<td>100</td>
<td>0</td>
<td>20-40</td>
<td></td>
</tr>
<tr>
<td>Guinea pig</td>
<td>100</td>
<td>0</td>
<td>10-20</td>
<td></td>
</tr>
<tr>
<td>Chicken, turkey</td>
<td>37</td>
<td>63</td>
<td>15-30</td>
<td></td>
</tr>
<tr>
<td>Owl</td>
<td>40</td>
<td>60</td>
<td>20-40</td>
<td></td>
</tr>
<tr>
<td>Carp</td>
<td>40</td>
<td>60*</td>
<td>600-700</td>
<td></td>
</tr>
<tr>
<td>Tuna</td>
<td>40</td>
<td>60*</td>
<td>200-700</td>
<td></td>
</tr>
<tr>
<td>Shark</td>
<td>40</td>
<td>60*</td>
<td>200-300</td>
<td></td>
</tr>
</tbody>
</table>

* Including the viscoelastic liquid of the anterior chamber.

+ Ichthyosan, a glucosamine- and galactosamine-containing glycosaminoglycan.


similar to a "horse's tail" in the central vitreous, but maintain a concentric configuration at the periphery. The elegant studies of Szent-Györgyi in 1917 supported the descriptions of Retzius and introduced the concept that vitreous structure changes with age.

Eisner studied dissections of human vitreous and found "membranelles," which he described as funnels packed into one another, diverging outward and anteriorly from the preapillary vitreous. Worst 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 Shimizu recently described a "posterior vitreous pocket" similar to what Worst has reported, which they claim to be an anatomic structure. However, as over 95% of their study population was age 65 or older, this is likely to be an age-related phenomenon (see chapter 39 on vitreous pathobiology, this volume).

MOLECULAR COMPONENTS

Collagen and HA are the major structural components of the vitreous. In humans, collagen is organized as thin fibrils 10 to 25 nm in diameter with cross-striations. Snowden and Swann identified a major period in the cross-striations of 62 nm (unfixed, dried bovine vitreous), while others describe a banding pattern with a periodicity of 12 to 25 nm. Vitreous collagen fibrils appear to be continuous and unbroken from the anterior peripheral vitreous to the posterior vitreous. This could be a consequence of either Müller cell synthesis of procollagen molecules that are added onto the most posterior end of existing fibrils or of fibroblast migration through the vitreous body leaving assembled collagen fibrils in their wake.

The distance between collagen fibrils in the rabbit vitreous was found to be 1.2 to 3.5 μm, which approximates estimates for the bovine vitreous and measurements in the albino rat. Vitreous collagen fibrils are unbranched and in the normal state are not cross-linked. This is supported by studies of the mechanical properties of the vitreous demonstrating a softening of the spring constant with increased elongation, suggesting that vitreous collagen is organized in a network in which fibrils can slip alongside each other. If so, this would be in contrast to the cornea where proteoglycans prevent collagen fibrils from moving independently along their axes. This may underly the elastic nature of the vitreous in contrast to the stiffness of the cornea.

There is heterogeneity in the distribution of collagen throughout the vitreous body. Chemical and light-scattering 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. Balazs has hypothesized that this is due to the fact that vitreous HA is synthesized by hyalocytes in the posterior vitreous cortex and cannot traverse the internal limiting lamina of the retina, but leaves the vitreous to enter the posterior chamber by way of the annulus of the anterior vitreous cortex which is not adjacent to a basal lamina. Bound water (nonfreezable) has a distribution within the vitreous similar to that of HA, presumably due to binding by HA. Balazs describes that HA molecules fill the spaces between the collagen fibrils and provide a "stabilizing effect" on the collagen network. In regard there are two important functions provided by this molecular arrangement. First, the large domains of the HA molecules spread apart the collagen fibrils as a result of "swelling pressure" and minimize light scattering by these structures, thereby contributing to the transparency of the vitreous. It is likely that the position of proteoglycans in the interfibrillar space is orderly, not random, and that these molecules provide lateral but not longitudinal stability. Second, the viscoelastic properties and mechanical functions of the vitreous result from the presence of both HA and collagen and are very likely related to their association on a molecular level. Adjacent collagen fibrils would tend to cross-link and alter these properties. Consequently, the presence of HA molecules that spread apart the collagen fibrils "stabilizes" the viscoelasticity of the vitreous. As described previously, there may be physicochemical binding sites for HA along the collagen fibrils that could explain the finding of an intimate association between collagen fibrils and HA. Alterations in the interaction between these structural components, for example with aging, could influence the physical properties of the vitreous.

**VITREOUS BODY (CORPUS VITREOUS)**

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 dyes 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. Since vitreous is 98% water, dehydration induces profound alteration of the internal morphology. Consequently, 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. 1A). 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, the dissected vitreous will remain solid and intact (young persons, Fig. 1A) or will be flaccid and collapse (older adults). 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 (Fig. 1B).

The limitations induced by the transparency of the vitreous were overcome by Goebel, Friedenwald and Stiehler, and Eisner 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. The illumination/observation angle of 90° that is achieved 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 as shown in Figure 2B and C and Figure 3. The fibers arise from the vitreous base (Figs. 2H and 3) where they insert anterior and posterior to the ora serrata (Fig. 2H). As the peripheral fibers course posteriorly they are circumferential with the vitreous cortex, while central fibers "undulate" in a
configuration parallel with Cloquet's canal.\textsuperscript{74} The fibers are continuous and do not branch. Posteriorly, these fibers insert into the vitreous cortex (Fig. 2E and F).

Ultrastructural studies\textsuperscript{99} 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. 4). 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. 2 and 3) and clinically. The areas adjacent to these large fibers have a low density of collagen fibrils separated by HA molecules and 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\textsuperscript{77,100} using india ink to fill the channels. There are changes that occur in these fibrous structures throughout life,\textsuperscript{27,96} 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. 2. Human vitreous morphology. Human vitreous structure visualized by darkfield slit illumination. All photographs are oriented with the anterior segment below and the posterior pole above. A. Posterior vitreous in the left eye of a 52-year-old man. The vitreous body is enclosed by the vitreous cortex. There is a "hole" in the preapillary (small, to the left) vitreous cortex. Vitreous fibers are oriented toward the premacular region. B. Posterior vitreous in a 57-year-old man. A large bundle of prominent fibers is seen coursing anteroposteriorly and entering the retrocortical space by way of the premacular vitreous cortex. C. Same photograph as B, at higher magnification. D. Posterior vitreous in the right eye of a 53-year-old woman. There is posterior extrusion of vitreous out the preapillary hole (to the right) and premacular (large extrusion to the left) vitreous cortex. Fibers course anteroposteriorly out into the retrocortical space.

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, and several millimeters into the vitreous body itself. The posterior extent of the posterior border of the vitreous base varies with age. Vitreous fibers enter the vitreous base by splaying out (see Fig. 3A) to insert anterior and posterior to the ora serrata (see Fig. 2H). The anteriormost fibers form the "anterior loop" of the vitreous base (Fig. 3B), a structure that is important in the pathophysiology of anterior proliferative vitreoretinopathy (see chapter 39 on vitreous pathobiology, this volume). In the posterior portion of the vitreous base, vitreous fibers are closer together than elsewhere. Gartner 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. Hogan demonstrated that just posterior to the ora serrata, heavy bundles of vitreous fibrils attach to the basal laminae of retinal glial cells. Studies by Gloor and Daicker showed that cords of vitreous collagen insert into gaps between the neuroglia of the peripheral ret-

Fig. 4. Ultrastructure of the 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). (Sebag J, Balazs EA: Morphology and ultrastructure of human vitreous fibers. Invest Ophthalmol Vis Sci 30:187, 1989.)
Fig. 5. 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.

VITREOUS CORTEX

The vitreous cortex is defined as 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 Tousimis\textsuperscript{107} described that in this region the collagen fibrils are parallel to the surface of the cortex. Studies by Faulborn and Bowd\textsuperscript{108} detected dense packing of collagen fibrils in the anterior cortex with looser collagen fibril packing in the subjacent vitreous, giving the impression of lamellae. Rhodes\textsuperscript{109} 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 thick\textsuperscript{103,110} and consists of densely packed collagen fibrils (Fig. 5). There is no vitreous cortex over the optic disc (Figs. 2A and 6), and the cortex is thin over the macula due to rarefaction of the collagen fibrils.\textsuperscript{110} 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 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

Fig. 6. Posterior human vitreous structure. Posterior vitreous in the left eye of a 59-year-old man. The vitreous cortex envelopes the vitreous body and multiple, small, highly refractile points are seen that scatter light intensely, likely hyalocytes (see Fig. 7). 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 course from the central vitreous into the retrohyaloid space. (Sebag J: Age-related differences in the human vitreoretinal interface. Arch Ophthalmol 109:966, 1991)
vitreous cortex (see Fig. 2A) but does so to a much lesser extent than through the premacular vitreous cortex (see Figs. 2B and D and 6). Jaffe\textsuperscript{111} has described how vitreous can extrude into the retrocortical space created following posterior vitreous detachment and has proposed that persistent attachment to the macula can produce traction and certain forms of maculopathy.\textsuperscript{112,113} Although there are no direct connections between the posterior vitreous and the retina, the posterior vitreous cortex is adherent to the internal limiting lamina 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 internal limiting lamina is not known, but probably results from extracellular matrix molecules.\textsuperscript{114}

**Hyalocytes**

Reeseer and Aaberg\textsuperscript{102} consider the vitreous cortex to be the "metabolic center" of the vitreous because of the presence of hyalocytes (Figs. 6, 7 and 8). These mononuclear cells are embedded in the vitreous cortex (Figs. 6 and 8A), widely spread apart in a single layer situated 20 to 50 \( \mu \text{m} \) from the internal limiting lamina of the retina posteriorly and the basal lamina of the ciliary epithelium at the pars plana and vitreous base. Quantitative studies of cell density in the bovine\textsuperscript{115} and rabbit\textsuperscript{116} vitreous found the highest density of hyalocytes in the region of the vitreous base, followed next by the posterior pole, with the lowest density at the equator. Hyalocytes are oval or spindle-shaped, 10 to 15 \( \mu \text{m} \) in diameter, and contain a lobulated nucleus, a well-developed Golgi complex, smooth and rough endoplasmic reticula, and many large periodic acid–Schiff (PAS)–positive lysosomal granules and phagosomes.\textsuperscript{110,117} Hogan and collaborators\textsuperscript{80} described that the posterior hyalocytes are flattened and spindle-shaped, whereas anterior hyalocytes are larger, rounder, and at times star-shaped. Saga and co-workers\textsuperscript{138} have described that different ultrastructural features can be present in different individual cells of the hyalocyte population in a single eye. Whether this relates to different origins for the different cells or different states of cell metabolism or activity is not clear. Balazs\textsuperscript{119} pointed out that hyalocytes are located in the region of highest HA concentration and suggested that these cells are responsible for vitreous HA synthesis. There is experimental evidence in support of this hypothesis.\textsuperscript{20,120-124} Swann\textsuperscript{3} claims that there is as yet no evidence that hyalocytes are responsible for the synthesis of vitreous HA. There is, however, evidence to suggest that hyalocytes maintain ongoing synthesis and metabolism of glycoproteins within the vitreous.\textsuperscript{125,126} Hyalocytes have also been shown to synthesize vitreous collagen\textsuperscript{127} and enzymes.\textsuperscript{128}

The phagocytic capacity of hyalocytes has been described in vivo\textsuperscript{129} and demonstrated in vitro.\textsuperscript{115,130} This activity is consistent with the presence of pinocytic vesicles and phagosomes\textsuperscript{117} and

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**Fig. 7.** Human hyalocytes. Phase-contrast microscopy of flat mount preparation of hyalocytes in the vitreous cortex from an 11-year-old girl. No stains or dyes were used in this preparation. These mononuclear cells are round and distributed in a single layer within the vitreous cortex, with pseudopodia in some cells. (Courtesy of New England Eye Bank, Boston, MA)
the presence of surface receptors that bind IgG and complement. Balazs has proposed that in their resting state, hyalocytes synthesize matrix glycosaminoglycans and glycoproteins and that the cells internalize and reutilize these macromolecules by means of pinocytosis. They become phagocytic cells in response to inducting stimuli and inflammation. HA may have a regulatory effect on hyalocyte phagocytic activity.

Fibroblasts

There is a second population of cells in the vitreous cortex which in some cases may be mistaken for hyalocytes. These cells constitute less than 10% of the total vitreous cell population. Several investigations have determined that these represent fibroblasts which are present in the vitreous cortex at the vitreous base, adjacent to the optic disc and ciliary processes. The argument for a role in normal vitreous collagen synthesis is mostly by analogy to studies of fibrillogenesis in tendon where investigations have found that secreted collagen molecules are assembled into fibrils within invaginations of secreting fibroblasts. The locations of fibroblasts in the anterior peripheral vitreous (vitreous base and near the ciliary processes) and posterior vitreous may explain how vitreous fibers become continuous structures spanning the distance between these locations.
Balazs and associates\textsuperscript{134} have found that near the pars plana vitreous fibroblasts decrease in number with age. Gartner\textsuperscript{135} has suggested that changes in these cells are responsible for aging changes in the collagen network of the vitreous base.

**VITREORETINAL INTERFACE**

The vitreous is situated adjacent to the retina posteriorly and behind the ciliary body and lens anteriorly. At all these sites the interface with adjacent tissues consists of a complex formed by the vitreous cortex and the basal laminae of the adjacent cells. These basal laminae are firmly attached to their cells\textsuperscript{115,135} and vitreous cortex collagen fibrils reportedly insert into the basal laminae. The only region not adjacent to a basal lamina is the annulus of the anterior vitreous cortex, which is directly exposed to the zonules and the aqueous humor of the posterior chamber. Balazs\textsuperscript{91} has pointed out the structural similarities between this zone and the surface of articular cartilage which in joints is exposed to synovial fluid. The significance of such an arrangement in the vitreous is not known, although it probably accounts for the ability of aqueous to enter the vitreous and the propensity of various substances (red blood cells, HA, growth factors, etc.) to exit the vitreous anteriorly.

The basal laminae surrounding the vitreous are composed of type IV collagen closely associated with glycoproteins.\textsuperscript{137} At the ciliary body the basal lamina of the pars plicata is a meshwork of lamina densa 0.05 to 0.1 μm thick and organized in a reticular, multilayered structure that is 2 to 6 μm thick and fills the spaces between the crevices of the ciliary epithelium. At the pars plana the basal lamina has a true lamina densa with insertions of vitreous collagen fibrils. The basal lamina posterior to the ora serrata is actually the basement membrane of retinal Müller’s cells, also called the internal limiting lamina (ILL) of the retina. Immediately adjacent to Müller’s cells is the lamina rara, which is 0.03 to 0.06 μm thick and demonstrates no species variations nor changes with topography or age. The lamina densa is thinnest at the fovea (0.01 to 0.02 μm). It is thicker elsewhere in the posterior pole (0.5 to 3.2 μm) than at the equator or vitreous base.

At the rim of the optic nerve head the retinal ILL ceases although the basement membrane continues as the “inner limiting membrane of Elschnig.”\textsuperscript{138} This membrane is 50 nm thick and is believed to be the basal lamina of the astroglia in the optic nerve head. At the centralmost portion of the optic disc the membrane thins to 20 nm, follows the irregularities of the underlying cells of the optic nerve head, and is composed only of glycosaminoglycans and no collagen.\textsuperscript{138} This structure is known as the “central meniscus of Kuhnt.” Balazs\textsuperscript{91} has stated that Müller’s cell basal lamina prevents the passage of cells as well as molecules larger than 15 to 20 nm. Consequently, the thinness and chemical composition of the central meniscus of Kuhnt and the membrane of Elschnig may account for, among other effects, the frequency with which abnormal cell proliferation arises from or near the optic nerve head (see chapter 39 on vitreous pathobiology, this volume).

Zimmerman and Straatsma\textsuperscript{139} showed the existence of fine, fibrillar attachments between the posterior vitreous cortex and the ILL and claimed that this results in an extremely intimate union between normal vitreous and retina. The composition of these fibrillar structures is not known. The vitreous is known to be most firmly attached at the vitreous base, the disc and macula, and over retinal blood vessels. In the posterior pole vitreoretinal adhesion is not focal but extends as a sheet encompassing the disc, peripapillary region, and macula.\textsuperscript{134} The thinness of the ILL and the purported presence of attachment plaques at the central macula\textsuperscript{40} could explain the predisposition of this region to changes induced by traction.\textsuperscript{97,111-113,141}

There is an unusual vitreoretinal interface overlying retinal blood vessels. Kuwabara and Cogan\textsuperscript{42} described “spider-like bodies” in the peripheral retina which coil around blood vessels and connect with the ILL. Pedler\textsuperscript{43} found that the ILL was thin over blood vessels, while Wolter\textsuperscript{44} noted the existence of pores in the ILL along blood vessels and found that vitreous strands inserted where the pores were located. Mutlu and Leopold\textsuperscript{45} described that these strands extend through the ILL to branch and surround vessels in what they termed “vitreoretinovascular bands.” Such structures could explain the strong adhesion between the vitreous and retinal blood vessels and account for the proliferative and hemorrhagic events associated with vitreous traction upon retinal blood vessels.

**CLINICAL EXAMINATION OF THE VITREOUS**

**CLINICAL MORPHOLOGY**

The vitreous body of an emmetropic human eye is approximately 16.5 mm in axial length with a depression anteriorly just behind the lens (patellar
fossa). Various structures and regions within the vitreous body are named after the anatomists and histologists who first described them (Fig. 9). The hyaloideocapsular ligament (of Wieger) is the annular region 1 to 2 mm in width and 8 to 9 mm in diameter where the vitreous is attached to the posterior aspect of the lens. Erggelet's or Berger's space is at the center of the hyaloideocapsular ligament. Arising from this space and coursing posteriorly through the central vitreous is the canal of Cloquet (Figs. 2G and 9), which is the former site of the hyaloid artery. Posteriorly, Cloquet's canal opens into a funnel-shaped region anterior to the optic disc known as the area of Martegiani.

CLINICAL EXAMINATION TECHNIQUES

Examination of the vitreous essentially consists of visualizing a structure intended to be virtually invisible. The clarity of the vitreous and its position within the inner recesses of the eye make clinical examination difficult. Optical transparency necessitates maximizing the Tyndall effect for visualization. Although this can be achieved in vitro (see section on Vitreous Body earlier in chapter) there are limitations to the illumination/observation angle that can be achieved clinically. This is even more troublesome in the presence of meiosis, corneal and lenticular opacities, and poor patient compliance. Essential to the success of achieving an adequate Tyndall effect are maximizing pupil dilation in the patient, since the Tyndall effect increases with an increasingly subtended angle between the axis of illumination and the line of observation (up to a maximum of 90°) and dark adaptation in the examiner. Some observers propose that green light further enhances the Tyndall effect. Prior to examining the individual parts of the vitreous, it is useful to consider the overall shape of the entire vitreous, as a clue to further characterizing vitreoretinal pathology. Charles has described a “problem-oriented” approach to preoperative vitreous examination wherein an appreciation of the configuration and mobility of the entire vitreous can guide the operative approach. For example, the identification of a “cone” in a partial posterior vitreous detachment and the characterization of the locations and number of apices in this cone can help in the accurate diagnosis of vitreoretinal traction and determine the best surgical approach.
Anterior Vitreous

The anterior vitreous is easily examined at the slit lamp with no preset or contact lens. It is practical to examine the anterior vitreous first, since this is readily done immediately after examination of the anterior segment and does not require the gel solutions used during contact lens examination of the posterior vitreous. In the absence of a crystalline or artificial intraocular lens, vitreous prolapse into the anterior chamber could be important in terms of vitreocorneal touch and the risks of corneal endothelial cell dysfunction.\(^1\) Vitreous adhesions to a cataract wound or to the iris may be important in the pathogenesis of postoperative cystoid macular edema. 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.\(^1\) Cells can be the augury of retinal infection, inflammation, tears, and/or detachments. Lacqua and Machemer\(^1\) described that an increase in the number and size of pigmented cells in the vitreous of patients with retinal detachment (proactively 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 Mitten-dorf'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.\(^1\)

Central and Posterior Vitreous

Examination of the central and posterior vitreous can rarely be achieved without the use of either a preset or contact lens. Preset lens biomicroscopy can be performed either with a plano concave lens (e.g., -55 to -58.6 D Hruby lenses) or various convex lenses (e.g., +32, +58.6, +60, or +90 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 only be performed by varying the position of the fixation point of the eye(s), and the quality of the image is reduced by optical distortions.

El-Bayadi\(^1\) first proposed the use of a +55 D preset lens and advised maintaining at least a 10° 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,\(^1\) 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 posterior vitreous detachment with vitreous syneresis (collapse) (see chapter 39 on vitreous pathobiology, this volume). It is principally this feature which makes the approach superior to contact lens systems for examination of the posterior vitreous.\(^1\)

A +90 D double aspheric lens can similarly be used in a "preset" manner, also with photographic capabilities.\(^1\)

Peripheral Vitreous

The major difficulty in examining the peripheral vitreous arises from a loss of stereopsis.\(^1\) This is due to the fact that 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 vertically, since the examiner's two eyes are positioned horizontally. Schepens\(^1\) 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 Jaffe\(^1\) and Schepens\(^1\) give excellent detailed accounts of the procedure to be followed for peripheral vitreous examination. Both describe 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. Schepens\(^1\) further describes the use of a tilted slit lamp column to enhance visualization of the peripheral vitreous. Eisner\(^1\) 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 lens[157] is a +61 D convex aspheric contact lens that produces a real inverted image of about 45° of the posterior fundus with excellent stereopsis. The “panfundusscopic” lens is a +85 D convex spheric lens which provides less magnification and image clarity but offers a wider angle view. It is useful for peripheral examination to 60 or 70° with a 15° tilt.

Opacified Vitreous

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,[148] 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. Green and Byrne[158] have described in detail how quantitative and qualitative echography can provide such information in the presence of vitreous opacification.

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