

## Age-related changes in human vitreous structure \*

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**Abstract.** Changes in vitreous structure that occur with aging are important in the pathogenesis of vitreous liquefaction (synchysis senilis), vitreous detachment, and retinal disease. Vitreous morphology was studied in 59 human eyes post-mortem using dark-field horizontal slit illumination of the entire dissected vitreous. In many individuals younger than 30 years, the vitreous was homogeneous in structure. Middle-aged individuals had macroscopic fibers in the central vitreous, which coursed anteroposteriorly and inserted into the vitreous base and the vitreous cortex, posteriorly. During senescence, the vitreous volume was reduced, the vitreous body was collapsed (syneresis), and the fibers were thickened, tortuous, and surrounded by liquid vitreous. This sequence of age-related changes probably results from a progressive reorganization of the hyaluronic acid and collagen molecular networks. Characterization of the molecular events underlying these changes will elucidate the mechanisms of the phenomena of synchysis, syneresis, and detachment, and may provide methods with which to prevent or induce vitreous detachment prophylactically.

### Introduction

Histologic studies performed during the eighteenth and nineteenth centuries resulted in four different theories of vitreous structure: the Alveolar Theory of Demours, the Lamellar Theory of Zinn, the Radial Sector Theory of Hannover, and the Fibrillar Theory of Retzius (Sebag and Balazs 1985). Recent studies by Eisner (1973 a, b, 1984), Balazs (1984), and Foos and Wheeler (1982) have formed our current concepts of vitreous structure and the phenomenon of vitreous detachment. It is known that detachment of the vitreous plays an important role in the pathogenesis of rhegmatogenous retinal detachment (Schepens 1983; Chaine et al. 1983), macular-hole formation (Avila et al. 1983), neovascularization (Jalkh et al. 1982; Faulborn and Bowald 1985), and cystoid macular edema in pars planitis (Hirokawa et al. 1985) as well as, perhaps, aphakia (Schepens et al. 1984; Sebag and Balazs 1984). It is not known what structural changes and underlying molecular events

cause detachment of the posterior vitreous. There is, however, an increased incidence of posterior vitreous detachment with increasing amounts of synchysis (O'Malley 1976; Foos and Wheeler 1982). Furthermore, Larsson and Osterlin (1985) have shown that the extent of vitreous detachment increases with increasing amounts of liquid vitreous.

The present investigation was an attempt to learn more about vitreous synchysis (liquefaction) and detachment by studying the structural changes that occur in normal human vitreous throughout life. The observations document a progressive reorganization of the vitreous and suggest directions for continued research into the underlying molecular events.

### Materials and methods

Fifty-nine eyes from 31 individuals (18 males, 13 females; age range, 33 weeks of gestation to 94 years) were obtained through the courtesy of the New England Eye Bank and the New York Bank for Sight Restoration. None of the donors had a history of ocular disease or trauma, ophthalmic surgery, or diabetes mellitus.

The eyes were kept in moist chambers at 4° C and were examined within 12-24 h of donor deaths. Specimens were prepared by dissecting the sclera, choroid, and retina away from the vitreous from the ora serrata to the posterior pole. Sutures were placed through the limbus, and the entire vitreous (still attached to the anterior segment) was mounted on a frame in approximately the in vivo position and then placed in a chamber containing an isotonic or balanced salt solution.

The vitreous was illuminated from the side with a horizontal slit-lamp beam and was examined from above. The image created in this way represents a horizontal optical section through the vitreous. The vertical location of this section and the thickness of the illuminated portion could be adjusted as desired. Photographs were taken in a dark room with a black background using a 55-mm macro lens and Ilford XP1-400 film.

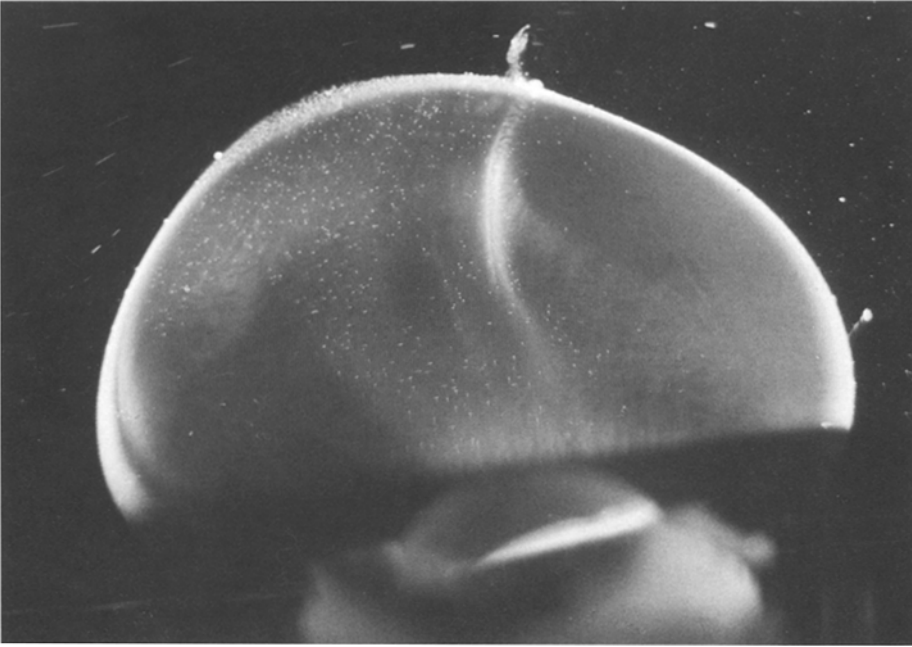
### Results

The findings are presented in three age categories, i.e., young, middle aged, and senescent. Allowing for a certain amount of individual variability, the descriptions given below are representative for these three groups. Table 1 presents a generalized description of the observed changes in vitreous structure that occur throughout life.

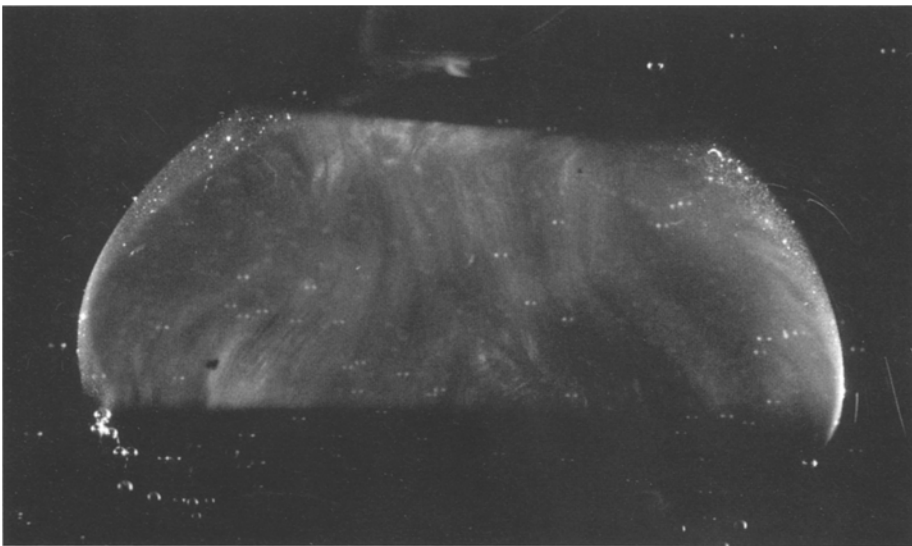
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**Fig. 1.** Vitreous of a 33-week-old human embryo. The anterior segment is below, and the posterior pole is above. The vitreous is homogeneous in structure except for the vitreous cortex and Cloquet's canal.  $\times 5.4$



**Fig. 2.** Vitreous of a 6-year-old human. The anterior segment is below, and the posterior pole is above. No macroscopic structures are visible within the vitreous except for remnants of Cloquet's canal.  $\times 4$



**Fig. 3.** Vitreous of a 59-year-old human. Only the central and posterior vitreous are shown, with the posterior pole above. Fine, parallel, linear fibers course anteroposteriorly in the central vitreous, these being surrounded by less dense light-scattering media (Sebag and Balazs 1985).  $\times 8.3$



**Fig. 4.** Special dissection of a human vitreous. The sclera, choroid, and retina surrounding the optic nerve were left attached to the vitreous, while these layers were dissected from the peripheral, equatorial, and retroequatorial vitreous. There was no extrusion of vitreous into the retrohyaloid space. Macroscopic linear fibers course anteroposteriorly and are oriented toward the macula.  $\times 2.9$



**Fig. 5.** Vitreous of an 88-year-old human. The anterior segment is below, and the posterior pole is above. The size of the vitreous is reduced, and the shape is collapsed, as evidenced by the irregular contour of the vitreous cortex. Fibers are present throughout the vitreous. The fibers are thickened, tortuous, and intertwined. Adjacent to the fibers, there are spaces devoid of any structure (Sebag and Balazs 1985).  $\times 2.7$

#### *Young (perinatal to 29 years of age)*

Twenty-three eyes from 12 donors in this age range were examined; 18 of the eyes were obtained from donors aged 14 years or less. The vitreous exhibited a remarkably homogeneous appearance. The only discernible structures were the vitreous cortex and, in younger individuals, a distinct canal of Cloquet (Fig. 1). During childhood, this canal became less prominent, although still visible. In 15 of the 18 eyes from donors aged 14 years or less, no prominent fibrous structures were present within the vitreous (Fig. 2).

#### *Adult (30–69 years of age)*

Twenty-three eyes from 12 individuals in this age group were examined. There were substantial differences in com-

parison to the findings for the vitreous of young individuals. Within the central vitreous, there were macroscopic, linear fibers oriented anteroposteriorly (Fig. 3). Anteriorly, the fibers inserted into the vitreous base, both anterior and posterior to the ora serrata (Sebag et al. 1987). There was a distinct curvilinear or ‘anterior loop’ configuration to the fibers inserting anterior to the ora serrata. In the posterior vitreous, most fibers were oriented toward the macula, and a few inserted into the premacular vitreous cortex (Sebag and Balazs 1984). Raising or lowering the level of the horizontal plane of the slit illumination beam did not give the impression that these structures were vertical membranes being illuminated in horizontal cross-section. Rather, different fibers were seen at different levels in the central vitreous.

**Table 1.** Human vitreous structure throughout life

Age range (years)	Number of eyes	Observations		
		Fibers	Liquefaction	Others
0-9	12	None	None	Cloquet's canal
10-19	8	Occasional	None	Cloquet's canal (occasional)
20-39	8	Early fibers	Slight central changes	Cloquet's canal (one case)
40-59	18	Prominent, parallel	Honeycomb pockets near cortex	Onset of PVD
60-79	5	Prominent; at times, irregular	Present near cortex and centrally	Increasing incidence of PVD
80-99	8	Thickened, tortuous, no longer parallel	Prominent near cortex and centrally	Reduced vitreous volume; collapsed PVD (syneresis)

PVD = Posterior Vitreous Detachment

The possibility that these fibers resulted from sagittal (anteroposterior) traction on the central vitreous by posterior vitreous extrusion through the two 'holes' in the posterior vitreous cortex (Sebag and Balazs 1984) was investigated in special preparations. In 3 eyes, the sclera, choroid, and retina were left attached to the posterior vitreous. The results demonstrated that, even in the absence of any posterior vitreous extrusion, there were linear fibers coursing anteroposteriorly and oriented toward the macula (Fig. 4).

#### *Senescent (over 70 years of age)*

The 13 eyes in this age group showed degeneration in the vitreous structure. Figure 5 illustrates the most advanced case of degeneration. The vitreous size was reduced, and the overall shape was collapsed, as evidenced by the irregular contour of the vitreous cortex. Fibers were no longer only present in the central vitreous, but were distributed throughout the vitreous. The fibers were not fine, straight and grouped in parallel bundles, but were thickened and tortuous. Adjacent to the degenerated fibers, there were spaces that contained liquid vitreous. In the subcortical region (anterior to the vitreous cortex), these spaces were larger and corresponded to 'lacunae'.

#### **Discussion**

There are four major difficulties inherent in the study of vitreous structure: artifacts that result from the use of histologic tissue fixatives; the absence of intravitreal landmarks that results in loss of orientation when the vitreous is removed from the eye; distortion of the shape of the vitreous when examined *in vitro*; and the transparency of the vitreous when viewed under diffuse light. In the present study, these difficulties were avoided as follows: by examining fresh, unfixed specimens; by leaving the vitreous attached to the anterior segment and mounting the specimen in the *in vivo* position; by immersing the specimen in solution to minimize shape distortion; and by examining the vitreous

with dark-field slit illumination using an illumination-observation angle of 90°, which maximizes the Tyndall effect (Eisner 1984).

This approach enables effective visualization of human vitreous structure. The results of the present study document progressive changes in normal vitreous throughout life. The presence of a homogeneous structure during childhood is consistent with the predominantly gel state of the vitreous in youth (Balazs and Flood 1978). This gel vitreous contains thin fibrils of a special type-II collagen organized in a random network (Snowden and Swann 1980). The fibrils are separated by large molecules of hyaluronic acid that stabilize the gel state (Fessler 1960; Balazs 1961; Snowden 1982). This arrangement prevents crosslinking of collagen filaments, minimizes light scattering, and maintains the optical transparency of the vitreous. In adults, macroscopic fibers first appear in the central vitreous. Sebag et al. (1987) have shown, using transmission electron microscopy, that these fibers are composed of packed bundles of parallel collagen fibrils.

The process of fiber formation occurs at about the same age as the onset of vitreous liquefaction (Balazs and Flood 1978) and at the same location as the earliest manifestations of liquefaction, i.e., the central vitreous (Eisner 1973a, b). Therefore, both synchysis and fiber formation probably result from the same underlying molecular events. During old age, the vitreous volume is reduced and the shape is collapsed. The fibers degenerate and are located adjacent to lacunae of the liquid vitreous. These observations are consistent with the finding of Balazs and Flood (1978) that, by the age of 80-90 years, one-half of the vitreous is in a liquid state. This probably represents the advanced stage of a molecular rearrangement that begins during youth and accelerates during the fifth and sixth decades of life.

It is likely that some alteration in the collagen/hyaluronic acid complex results simultaneously in vitreous synchysis and fiber formation. Although there is, at present, no evidence for the existence of an actual chemical bond between hyaluronic acid and collagen (Balazs 1984), it has been suggested that sulfated glycosaminoglycans act as a 'glue' between hyaluronate molecules and collagen (Asakura 1985). A more established interaction occurs on a physicochemical level, with hyaluronic acid being 'bound' to collagen by electrostatic forces (Comper and Laurent 1978). Gelman and Blackwell (1974) have shown that this charge interaction can influence the conformational state of proteins.

The "excluded volume effect" (Ogston and Phelps 1961) also influences the conformational state of these macromolecules by favoring more compact tertiary and quaternary structures (Comper and Laurent 1978). Thus, changes in the physicochemical interactions between hyaluronic acid and collagen may be the initiating events that lead to the structural changes observed in the present study. Evidence in support of this hypothesis can be found in recent work (Armand and Chakrabarti 1987) demonstrating conformational differences between the hyaluronic acid molecules present in liquid vitreous and those located in gel vitreous. Further work must be undertaken to identify the exact nature of the hyaluronic-acid/collagen interaction occurring in the vitreous as well as in other connective tissues and extracellular matrices. A better understanding of this interaction and the changes that occur during synchysis senilis and vitreous detachment could result in the development

of prophylactic therapeutic interventions intended either to induce or to inhibit liquefaction and vitreous detachment, depending upon the clinical circumstances.

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