HUMAN VITREOUS FIBRES AND VITREORETINAL DISEASE

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Human Vitreous Fibres and Vitreoretinal Disease

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Summary

Adult human vitreous structure was studied by dark-field Slit Microscopy in fresh, unfixed preparations of vitreous dissected off the sclera, choroid and retina. Macросscopic fibres oriented in an antero-posterior direction were detected.

These fibres inserted at the vitreous base and oriented towards the macula. Significant alterations were observed in this vitreous structure with advanced age. The role of these structures in vitreo-retinal disease is discussed.

Duke-Elder cites Demours and Zinn as the first to ascribe any structure to the ‘clear’ vitreous.1 During the mid-eighteenth century these investigators proposed that the vitreous is a framework of loose and delicate structures enclosing spaces filled with ‘humour’. A great number of studies have since been undertaken to elucidate the morphology of this remarkable tissue.

During the eighteenth and nineteenth centuries no less than four different theories prevailed. In 1741 Demours formulated the ‘Alveolar Theory’.2 After freezing and slowly thawing the vitreous, he described a multitude of membranes oriented in all possible directions, enclosing compartments or alveoli containing the fluid portion of the vitreous. The studies of Von Haller3 and Virchow4 supported this concept. However, Zinn proposed that the vitreous was arranged in a concentric, lamellar configuration similar to the ‘layers of an onion’.5 The dissections and histologic preparations of von Pappenheim6 and Brucke7 provided evidence for the ‘Lamellar Theory’. Stillings8 and Iwanoff9 modified this theory by stating that only the peripheral third of the vitreous had a lamellar structure.

The ‘Radial Sector Theory’ was first proposed by Hannover in 1845.10 Studying coronal sections at the equator, he described a multitude of sectors approximately radially-oriented about the central antero-posterior core that contained Cloquet’s Canal. Hannover likened this structure to the appearance of a ‘cut orange’. For many years a controversy existed between proponents of the ‘Lamellar Theory’ and supporters of the ‘Radial Sector Theory’. Smith11 and Gerlach12 attempted to unify the two theories by stating that the peripheral vitreous had concentric lamellae, while the central vitreous had a radial sector structure.

In 1848, Bowman introduced the ‘Fibrillar Theory’. Employing microscopy, he described fine fibrils that formed bundles with ‘nuclear granules’.13 Blix proposed that the nuclear granules were actually the sites of intersection of fibres coursing in all directions.14

Virchow attempted to unify the ‘Alveolar’ and ‘Fibrillar’ theories by stating that the compartments or alveoli were separated by fibrils.4 Retzius described fibrous structures arising in the peripheral anterior vitreous that assumed an undulating pattern similar to a ‘horse’s tail’ in the central vitreous, but maintained a concentric configuration at the periphery.15

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The elegant studies of Szent-Gyorgi supported the descriptions of Retzius and introduced the concept that vitreous structure changes with age. He claimed that between the ages of 40 and 60 years the central vitreous undergoes dissolution and loss of structure, while in the periphery fibres fuse to form 'membranelles'.

The work of Baumann, Stroemberg and Redslob showed that these early studies were flawed by artefacts due to the use of tissue fixatives. Yet, the introduction of in vivo slit-lamp biomicroscopy spawned an equally varied set of descriptions. Gullstrand saw membranes made of a network of web-like structures. Koeppe described vertical and horizontal fibres arranged in regularly intercrossing systems. Baumann saw a grill-like pattern of darker and lighter bands resembling several layers of chain-linked fences.

Even the use of post-mortem dark-field microscopy resulted in various interpretations ranging from Goedbloed’s description of fibrillar structures to Friedenwald and Stiehler’s description of concentric sheets. Most recently Eisner used this technique to describe a system of ‘membranelles’ he has named ‘Tractae’. Our current understanding of the composition of human vitreous and its ultrastructural organisation derives in great part from the work of Balazs.

These studies have shown that fine collagen filaments are dispersed throughout the vitreous in association with large molecules of hyaluronic acid. These hydrophilic hyaluronic acid molecules serve to keep collagen filaments dispersed and prevent cross-linking, thereby maintaining the transparency of the vitreous.

This association of hyaluronic acid and collagen filaments imparts the characteristic gel quality to the vitreous. Yet it remained unclear how this ultrastructural organisation corresponded to macroscopic vitreous morphology.

Our aim is to define the macroscopic anatomy of human vitreous, to determine the changes in this structure that occur with age, and to integrate this information with our present understanding of vitreo-retinal pathophysiology.

Material and Methods
Human eyes from donors aged 53 to 88 years were obtained from the New England Eye Bank Boston, Mass. No individuals had a history of ocular disease or surgery. The time elapsed since death ranged from 9 to 24 hours and the eyes were kept at 4°C. The sclera, choroid and retina were dissected and the fresh vitreous, unfixed and still attached to the anterior segment, was mounted in a lucite chamber containing isotonic saline and sucrose (3.5 Gm/L).

As previously described, dark-field slit microscopy was performed using a slit-lamp beam illuminating a horizontal optical section of variable thickness. Photographs were taken at an angle of 90° to the plane of illumination, using a Nikon F3 camera with a 55 mm macro lens and Ilford XP-400 film.

Results
The vitreous was highly turgescent with little distortion in its spherical shape. Enclosing the vitreous was a thin, membrane-like structure that was continuous from the ora serrata to the posterior pole, corresponding to the vitreous cortex. In the posterior vitreous cortex there were two round ‘holes’ in the pre-papillary and premacular regions (Fig. 1). The premacular hole was always larger than the pre-papillary hole (approximately 1.25 mm and 5 mm in diameter, respectively).

![Fig. 1. Dark-Field Microscopy of the Posterior Vitreous. The two 'holes' in the posterior vitreous cortex correspond to the prepapillary (P) and premacular (M) regions. Vitreous can be seen extruding through these holes (black arrows and white arrow heads).]
Vitreous was seen extruding from these holes to varying degrees. Within the central and posterior vitreous, fibres were seen coursing in an antero-posterior direction (Fig. 2). The fibres were continuous, regular, of uniform calibre and linear.

Raising or lowering the level of the horizontal plane of illumination did not give the impression that these structures represented membranes being illuminated and observed in cross-section. Indeed at different levels other fibres that were previously not illuminated were visualised.

Anteriorly the fibres inserted into the vitreous base where they straddled the ora serrata inserting both anterior and posterior to this line. The posterior extent of the inserting fibres seemed greater at the temporal ora serrata than the nasal side. At the posterior pole most fibres coursed through the pre-macular hole in the posterior vitreous cortex (Fig. 2). On occasion, a fibre could be seen to insert into the posterior vitreous cortex at the rim of the pre-macular hole. Special preparations demonstrated that these fibres were not solely the result of traction induced by vitreous extrusion out of the holes in the posterior vitreous cortex and that their orientation to the macula was not an artefact.

In the mid-peripheral and equatorial regions the fibres condensed into bundles and inserted in the vitreous cortex (Fig. 3). Between these bundles there were fairly regular spaces that seemed devoid of structure.

No lamellar structures were observed in the peripheral vitreous. At no time could membranes, sheets or radial sectors be identified.

The vitreous of older individuals (80 to 90 years of age) demonstrated marked disruption of this fibrous structure (Fig. 4). The uniform appearance of the fibres was altered to one of substantial variability in calibre. The central and posterior vitreous fibres no longer had a regular and smooth appearance, but rather seemed knurled. The fibres were no longer linear and continuous but appeared 'broken' and curled upon themselves (Figs. 4 and 5). The highly-oriented, parallel organisation was altered to an intertwined mesh of seemingly random, irregular 'remnants' of
fibres. Large spaces devoid of any structure were present in the central (Fig. 4) and peripheral vitreous (Fig. 5).

In the equatorial and posterior periphery of the vitreous the regular areas between the bundles of fibres inserting into the vitreous cortex were enlarged into pockets or lacunae of variable size and shape (Fig. 5). Comparative studies of both eyes in an 88-year-old individual were performed to determine the effect of time elapsed since death on the appearance of vitreous structure. One eye was dissected 12 hours after death and the other 48 hours after death. No difference in vitreous structure could be appreciated (Figs. 4 and 5).

Discussion
This study demonstrates that the human vitreous contains fibres of macroscopic dimensions with an antero-posterior orientation. Anteriorly the fibres insert into the vitreous base, posteriorly they are oriented towards the macula, and in the peripheral vitreous condense into bundles and insert into the vitreous cortex. The factors enabling visualisation of these fibres are the maintenance of vitreous turgescence, sagittal tension placed upon intravitreal structures and the use of an illumination-observation angle of 90° which maximised the Tyndall effect.

In 1894 Morner\textsuperscript{30} and Young\textsuperscript{31} independently proposed that the human vitreous was, in part, composed of collagen.

Transmission electron microscopy studies have shown that there is no ultrastructural element in the human vitreous that could correspond to these fibres, other than collagen filaments.\textsuperscript{27} We believe that at birth there is a homogeneous distribution of collagen filaments that is stabilised by the association with large molecules of hyaluronic acid.\textsuperscript{26,27} During development and aging there is a dissociation of collagen filaments and hyaluronic acid, resulting in aggregates of collagen filaments and pools of hyaluronic acid.

The aggregates form fibrils, bundles of parallel fibrils, and ultimately macroscopic fibres. The collections of hyaluronic acid are at first roughly linear in shape, adjacent to the fibres that are forming. These areas of relatively high hyaluronic acid concentration may function as microchannels since they would offer little resistance to flow.

The existence of such channels has been suggested by the studies of Stilling\textsuperscript{12} and Worst.\textsuperscript{33} Such hyaluronic acid microchannels could be important in ocular physiology, influencing trans-vitreal flow of aqueous humour and systemic or topical drugs, the passage of fluorescein during fluorophotometry and the clearance of vitreous haemorrhage. In this regard it is interesting how much faster blood clears from the vitreous of adults than children. With advancing age and the continued dissociation of collagen and hyaluronic acid, degenerative changes become manifest. The pools of hyaluronic acid enlarge to form pockets and ultimately clinically recognisable lacunae.

The once turgescent vitreous seems to shrink and this syneresis induces vitreous detachment. Pockets of hyaluronic acid (‘liquid vitreous’) drain into the retro-vitreal space via the two holes in the vitreous cortex of the posterior pole, the first sites to detach.\textsuperscript{34} Complete vitreous detachment is also dependent upon factors at the vitreo-retinal interface.

With age, the increasingly destabilised vitreous collagen begins to degenerate and lose elasticity. At the sites of insertion of the fibres the risks of retinal tears increase. Myopia and aphakia augment the risk by exaggerating traction mediated by these fibres. Indeed routine slit-lamp examination of the anterior and central vitreous in myopic

Fig. 5. Senescent Human Vitreous Structure. The other eye of the patient shown in Fig. 4 was not dissected until 48 hours after death. No substantial differences in appearance are seen. Large Lacunae are present in the peripheral vitreous (arrows).
individuals reveals a prominent fibrous appearance rather early in life. The precocity of this appearance may be directly proportional to the degree of myopia. Concerning aphakia, it is well-known that the risks of retinal detachment increase following intracapsular cataract extraction.3

It is interesting to note that following experimental intracapsular lens extraction the hyaluronic acid concentration in vitreous decreases by 50 per cent.36 Such a significant alteration must disturb the collagen filament-hyaluronic acid association and may contribute to further fibre formation. In conjunction with anterior displacement of the vitreous, the increasingly prominent vitreous fibres pose a great risk of creating tears at the vitreous base and other sites of attachment. The lack of a decrease in hyaluronic acid concentration after experimental extracapsular lens extraction29 is consistent with the lower incidence of rhegmatogenous retinal detachment following extracapsular cataract extraction.

Vitreous attachments to the macula are in direct communication with the Mueller cells of the retina.29,37 Traction transmitted by vitreous fibres may damage the Mueller cells, predisposing the macula to oedematous or degenerative changes.29,38 The onset of cystoid macular oedema long after cataract extraction may be due to partial posterior vitreous detachment with persistent attachment of vitreous fibres to the macula.29 These fibres are probably also important in the pathogenesis of so-called ‘idiopathic’ macular holes.39

In short, the presence of these fibres is not to be perceived as an anatomic curiosity. Rather, understanding the process of their formation may be important in furthering our knowledge of vitreo-retinal pathophysiology and our ability to treat these disorders.

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