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Ageing of the Vitreous

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Summary
Changes that occur in the vitreous during ageing contribute to a variety of vitreo-retinal disorders. These age-related changes are rheologic, biochemical, and structural in nature. Our current knowledge of these ageing changes is reviewed. Hypotheses for the mechanisms of liquefaction (synchisis senilis) and posterior vitreous detachment are proposed.

A variety of vitreo-retinal disorders have a high incidence during the latter decades of life. Some diseases can be attributed to age-related changes within the vitreous. The events that contribute to the ageing changes in the vitreous are inter-related, but can be considered in three general categories: rheologic, biochemical and structural. Our current knowledge of the changes observed in these three areas support the postulate that liquefaction (synchisis senilis) and vitreous detachment can be understood in terms of a molecular re-arrangement of vitreous components.

This article reviews what is currently known about ageing of the vitreous. Particular emphasis will be placed upon the phenomena of synchisis senilis and posterior vitreous detachment. Based upon this information, a hypothesis for the mechanism of posterior vitreous detachment is proposed.

Rheology

Using slit lamp biomicroscopy in a clinical setting, Busacca[1] and Goldmann[2] observed that after the ages of 45–50 years there is a decrease in the gel volume and increase in the liquid volume of human vitreous. Eisner[3] qualitatively confirmed these findings in his post-mortem studies of dissected human eyes and pointed out that liquefaction begins in the central vitreous. In a large autopsy study of formalin-fixed human eyes, O'Malley[4] provided quantitative confirmation of these observations. He found that more than half of the vitreous was liquefied in 25 per cent of individuals aged 40–49 years and that this increased to 62 per cent of individuals aged 80–89 years. Oksala[5] used ultrasonography to detect echoes from gel-liquid interfaces in 444 normal human eyes, *in vivo*. He observed evidence of vitreous 'degeneration' in 5 per cent of individuals aged 21–40 years, 19 per cent of those aged 41–50 years, 63 per cent aged 51–60 years and in greater than 80 per cent of individuals over the age of 60 years. The vitreous was acoustically homogeneous in all individuals younger than 20 years and in 10 per cent of those older than 60 years.

However, liquefaction actually begins at a much younger age. There is evidence of liquid vitreous after the age of 4 years and by the time the eye reaches its adult size (ages 14–18 years), approximately 20 per cent of the total vitreous volume consists of liquid vitreous.[6]

In post-mortem studies of fresh, unfixed

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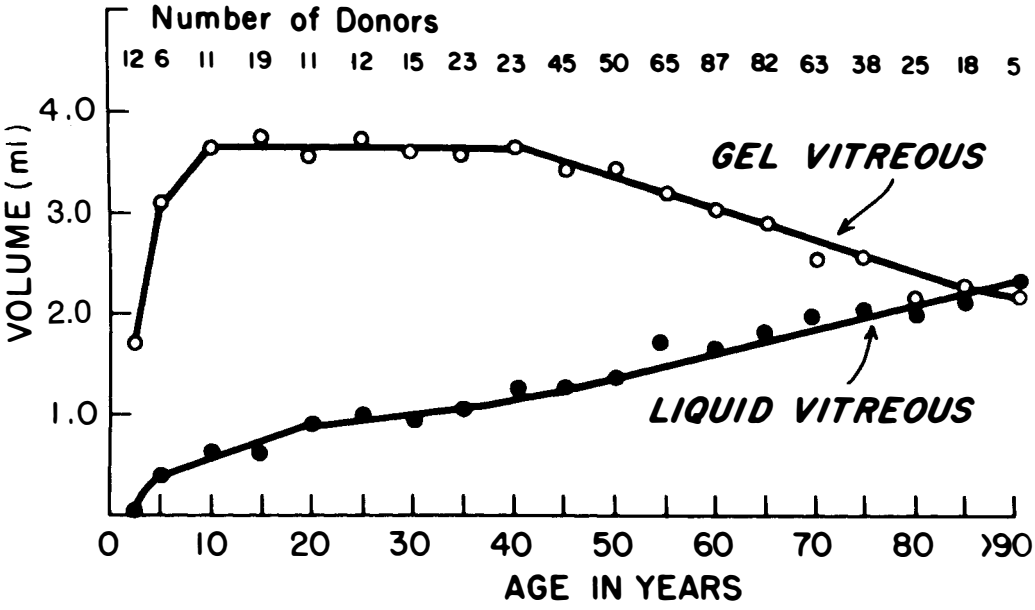

Fig. 1. *Gel-liquid transformation in human vitreous. The volumes of gel (open circles) and liquid (closed circles) vitreous are plotted with age (abscissa). Each point represents the mean value of measurements obtained from the number of eyes shown at the top of the graph. (Courtesy of Dr Endre A. Balazs.)*

human eyes it was observed that after the age of 40 years there is a steady increase in liquid vitreous which occurs simultaneously with a decrease in gel volume (Fig. 1). By the ages of 80–90 years more than half the vitreous is liquid. The central vitreous is the region noted to undergo liquefaction first, as determined clinically and in post-mortem studies.[3] The finding that it is in the central vitreous that fibres are first observed[7,8] is consistent with the concept that dissolution of the hyaluronic acid—collagen complex results in the simultaneous formation of liquid vitreous and aggregation of collagen fibrils into bundles of parallel fibrils seen as large fibres.[8]

In most mammals vitreous liquefaction does not occur[9] and only a few, if any, fibres develop.[10] Studies on rhesus monkeys have demonstrated that there exists an age-related process of synchisis similar to that in man.[11] However, there were no differences in protein or hyaluronic acid concentration between the ages of 6 and 21 (human age equivalent of 68 years), and no change in the size of the hyaluronic acid molecule.

The mechanism of vitreous liquefaction is poorly understood. Gel vitreous can be liquefied by removing collagen via filtration[12] and centrifugation[13] or by enzymatically destroying the collagen network.[14] It is unlikely, however, that such phenomena are at play *in vivo*. Chakrabarti and Park[15] claimed that the interaction between collagen and hyaluronic acid is dependent upon the conformational state of each macromolecule and that a change in the conformation of hyaluronic acid molecules could result in vitreous liquefaction and aggregation or cross-linking of collagen molecules. Differences in the tertiary structure of hyaluronic acid molecules have been found between gel and liquid vitreous,[16] suggesting that such conformational changes occurred during synchisis. Whether these changes are cause or effect is not known. However, Andley and Chapman[17] have demonstrated that singlet oxygen can induce conformational changes in the tertiary structure of hyaluronic acid molecules. Ueno *et al*[18] have suggested that free radicals generated by metabolic and photosensitised reactions could alter hyaluronic acid structure and trigger a dissociation of collagen hyaluronic acid molecules ultimately leading to synchisis. This is plausible since the cumulative effects of a life-

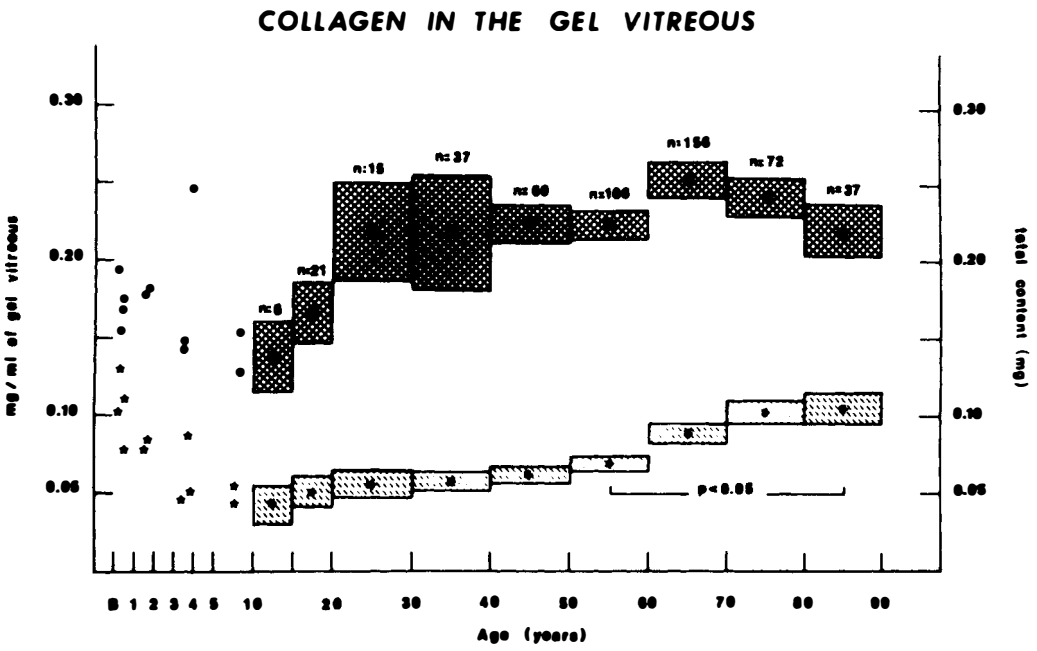

Fig. 2. *Collagen concentration (mg/ml) of human gel vitreous. The collagen concentration (mg/ml) of gel vitreous is represented by asterisks (individual samples) or by lightly hatched boxes with asterisks representing the means. The collagen content of the gel vitreous (mg) is represented by solid dots (individual samples) and by darkly hatched boxes with asterisks representing the means. The vertical sides of the boxes indicate the standard error of the means; the horizontal, the age range of individual cases included in the group. The number of samples in each group is indicated by 'n'. There is a significant increase in collagen concentration of the gel vitreous between the 50–60-year-old group and the groups of the next decades (p less than 0.05). (Courtesy of Dr Endre A. Balazs.)*

time of daily exposure to light may influence the liquefaction process by the proposed free radical mechanism. The importance of vitreous liquefaction in the pathogenesis of vitreous detachment is discussed below.

Biochemistry

The three major constituents of vitreous are collagen, hyaluronic acid and water. Total vitreous collagen content does not change after the third decade.[6,9] In a large series of normal human eyes studied at autopsy, the collagen concentration in the gel vitreous (Fig. 2) at the ages of 70–90 years (approximately 0.10 mg/ml) was found to be greater than the collagen concentration of the gel vitreous at ages 15–20 years (approximately 0.05 mg/ml; ($p<0.05$).[6,9] Since the total collagen content does not change, this finding is most likely due to the decrease in the volume of gel vitreous that occurs with ageing, raising the concentration of the collagen remaining in the gel. As will be described below, the collagen fibrils that remain in the gel become packed into bundles of parallel fibrils,[7] perhaps with cross-links between them.

Ageing of collagen throughout the body is associated with increased cross-linking as manifested by decreased solubility,[19] increased collagen stiffness,[20] and increased resistance to enzymatic degradation.[21] Although similar investigations have not been conducted on vitreous collagen, studies by Snowden *et al*[22] demonstrated that with ageing there was a decrease in the quantity of bovine vitreous collagen made soluble by heat alone. This could result from an increase in collagen cross-linking or, as suggested by Snowden *et al,* from changes in the surrounding glycoproteins and proteoglycans.[22]

Hyaluronic acid concentration increases until the ages of about 20–30 years when adult levels are attained (approximately 0.2 mg/ml).[6,9] The concentration of hyaluronic acid remains the same in both the liquid and gel vitreous from the ages of 20–70 years.[23,24]

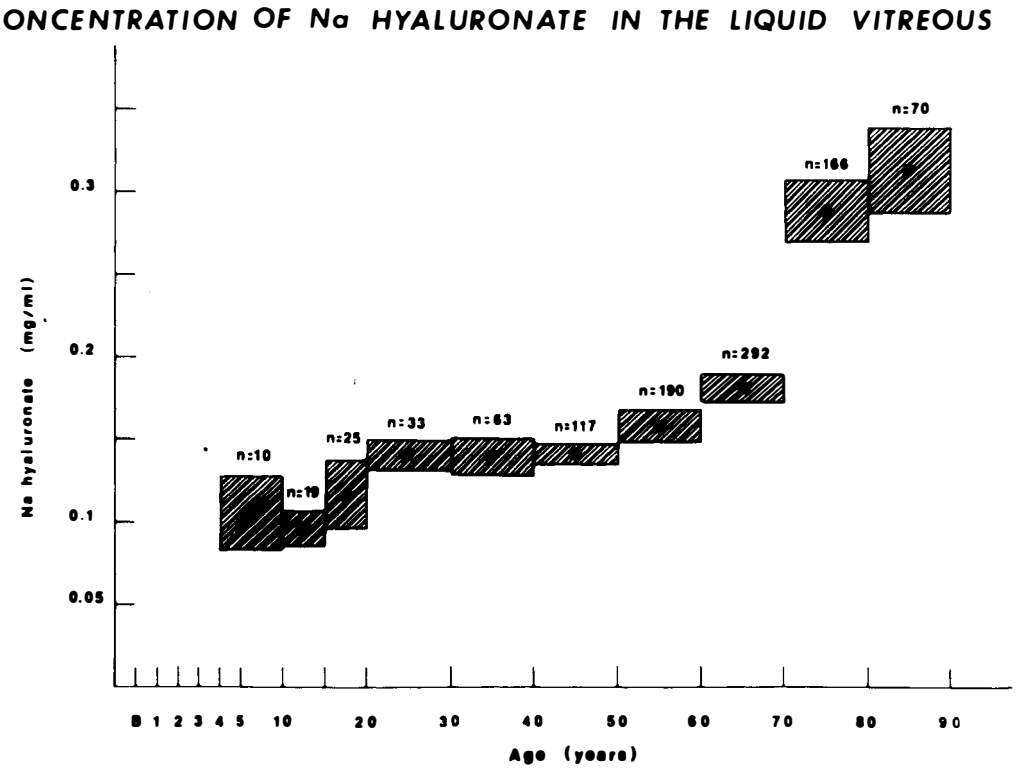

Fig. 3. *Concentration of hyaluronic acid in human liquid vitreous. Data for the first 4 years are missing because liquid vitreous does not exist at this time. (Courtesy of Dr Endre A. Balazs.)*

This suggests that there is an increase in the hyaluronic acid content of liquid vitreous and a decrease in the hyaluronic acid content of gel vitreous, since the amount of liquid vitreous increases and the amount of gel vitreous decreases with age. This is consistent with the concept that during synchisis senilis, there is a displacement of hyaluronic acid molecules from the gel to the liquid vitreous. Human studies have shown that after the age of 50 years there is an increase in the concentration of hyaluronic acid in the liquid vitreous[9] (Fig. 3).

Liquefaction has also been associated with changes in vitreous hyaluronic acid molecules. Studying post-mortem samples of human vitreous, Armand and Chakrabarti[16] found subtle but definite differences in the chromatographic elution profiles and optical properties of hyaluronic acid molecules isolated from gel as compared to liquid vitreous. These investigators suggested that the conformational differences between hyaluronic acid molecules in gel and liquid vitreous are likely to be at least partly responsible for the gel-liquid transformation observed during ageing.

Vitreous soluble protein concentrations also increase from 0.5–0.6 mg/ml at ages 13–50 years, to 0.7–0.9 mg/ml at ages 50–80 years, and 1.0 mg/ml above the age of 80 years.[23,24] This increase probably results from an age-related breakdown in the blood-ocular barriers at the retinal blood vessels, retinal pigment epithelium and ciliary body epithelium.

Structure

As in the other parts of the body[25] the basal laminae surrounding the vitreous thicken with age.[26] Hogan *et al*[27] stated that the thickening of the internal limiting lamina (ILL) of the retina occurs after it is initially laid down, probably as a result of synthesis by the subja-

Fig. 4. *Vitreous structure of a 33-week gestational age human[38]. The anterior segment is below and the posterior pole is above. A dense homogeneous appearance is present throughout the vitreous. The vitreous cortex and Cloquet's Canal can be seen (magnification 4×).*

cent Mueller cells. ILL thickening may play a role in the weakening of vitreo-retinal adhesion that contributes to the development of posterior vitreous detachment.

Teng and Chi[28] found that the vitreous base posterior to the ora serrata varied in width depending on the age of the individual. More than half of eyes from people older than 70 years had a posterior vitreous base wider than 1.0 mm and half of these were wider than 2.0 mm. With increasing age the width increased to nearly 3.0 mm, bringing it close to the equator.

This widening was also observed to be most prominent in the temporal portion of the globe. Posterior migration of the area of strongest vitreo-retinal adhesion plays an important role in the pathogenesis of retinal breaks and rhegmatogenous retinal detachment resulting from peripheral vitreo-retinal traction.

Within the vitreous body ageing is associated with substantial structural changes. During development there is a transition from a dense, highly light-scattering structure (Fig. 4) to a relatively transparent homogeneous body. In the adult, 'tracts', as observed by Eisner,[3] and fibres[7,8] form (Fig. 5). During the latter decades of life the fine parallel fibres in the central vitreous become thickened and tortuous (Fig. 6). Immediately adjacent to these coarse structures are areas with little or no light-scattering properties that are filled with liquid vitreous. When advanced, this vitreous degeneration forms large pools of liquid vitreous identified clinically as 'lacunae' (Fig. 6). When the posterior vitreous detaches from the retina there is an overall reduction in the size of the vitreous due to the collapse of the vitreous (syneresis) that occurs when liquid vitreous enters the retrohyaloid space anterior to the retina. This displacement of liquid vitreous occurs via the two 'holes' in the posterior vitreous cortex (Fig. 5) and is an important event in the pathogenesis of posterior vitreous detachment.

Posterior Vitreous Detachment

The most common age-related pathologic finding in the vitreous is posterior vitreous

Fig. 5. *Vitreous structure in a 58-year-old human[39]. Fibrous structures are present in the central vitreous. These fibres are fine, parallel and have an antero-posterior orientation with insertions at the vitreous base and posterior vitreous cortex (not shown) (magnification 8×).*

detachment (PVD). This can be defined as a clean separation between the posterior vitreous cortex and the internal limiting lamina (ILL) of the retina, as demonstrated by histopathologic study.[29,30] PVD can be localised, partial, or total (from the posterior pole up to the posterior border of the vitreous base). The incidence of PVD in clinical studies has been found to be 53 per cent in people older than 50 years, and 65 per cent over the age of 65 years.[31] Autopsy studies revealed an incidence of 27–51 per cent in the 7th decade and 63 per cent in the 8th decade.[32] PVD is more common in myopes, occurring 10 years earlier than in emmetropes and hyperopes.[31] Several studies have found a higher incidence of PVD in women.[32,33] There is evidence to suggest that the glycosaminoglycans composition of connective tissue is influenced by gonadal hormones. Thus, post-menopausal women may experience a biochemical disruption of the vitreous that contributes to PVD.

Kloti[34] has shown that experimental embolisation of the central retinal artery causes total PVD. In less severe cases, he found a distinct membranous layer adherent to the detached posterior vitreous which was identified as the ILL of the retina. Kloti concluded that embolisation resulted in Mueller cell ischemia and dysfunction allowing the ILL to detach along with the posterior vitreous. Presumably, more advanced ischemia caused dissolution of the ILL-cortical adhesion resulting in true PVD. This suggests that Mueller cell metabolism is at least partly responsible for vitreo-retinal separation at the ILL-cortical interface. Another form of vitreo-retinal separation which must be distinguished from true PVD occurs just anterior to the posterior vitreous cortex. Balazs[35] has termed this 'vitreoschisis' to denote liquefaction with cavitation in the posterior vitreous but persistent attachment of the posterior vitreous cortex to the ILL. Clinically this is often mistaken as true PVD and occurs in advanced synchisis.

The pathogenesis of PVD relates to the rheologic changes within the vitreous that lead to synchisis (liquefaction) as well as weakening

Fig. 6. *Vitreous structure in an 88-year-old human[8]. The vitreous is reduced in size and collapsed (syneresis). Within the vitreous the fibres are coarse, thickened and tortuous. Adjacent to the fibres are large collections of liquid vitreous (lacunae) (magnification 3×).*

of the cortical-ILL adhesion. Once liquid vitreous forms and the collagen network is destabilised, owing to the loss of association with hyaluronic acid molecules, collapse of the vitreous body (syneresis) can occur. Two possible mechanisms for syneresis are:

(1) Shortening and condensation of vitreous fibrils contracting the vitreous body and pulling the posterior vitreous forward;[36]
(2) Dissolution of the posterior vitreous cortex-ILL adhesion at the posterior pole allowing liquid vitreous to enter the retrocortical space via the prepapillary and prefoveal holes in the vitreous cortex and dissect a plane leading to true PVD.[31]

This volume displacement from the central vitreous to the pre-retinal space causes the observed syneresis of the vitreous body.

The latter mechanism is supported by the observations of Foos that PVD begins at the macula.[30] This could result from a predisposition or an increased stimulus for vitreous degeneration in the premacular region. Foos and Wheeler[32] proposed that synchisis here resulted from light toxicity to the premacular vitreous, since this is where the eye focuses most light, and/or some toxicity caused by metabolic waste products resulting from the high density of metabolically-active cells and neurons in the macula. Both light irradiation and metabolic processes can generate free radicals which could alter hyaluronic acid structure and disrupt the collagen-hyaluronate association.[18]

O'Malley[24] suggested that PVD was related to synchisis since both are correlated with age, although PVD had a later onset than synchisis. Foos and Wheeler[32] studied 4,492 eyes and found a statistically significant correlation between the degree of synchisis and the incidence of PVD. Larsson and Osterlin[33] studied 61 human eyes post-mortem and correlated the degree of vitreous liquefaction with the extent of PVD. In eyes with no PVD, about 10 per cent of the vitreous was liquid; with partial PVD 23 per cent of the vitreous was liquefied. These studies also showed that the concentration of hyaluronic acid in eyes with no PVD was higher than those with total PVD ($p<0.04$). This loss of hyaluronic acid mol-

ecules would further destabilise the vitreous and contribute to its collapse.[37]

Conclusions

The concept that arises from all these observations and findings is that PVD results from events within the vitreous and at the vitreoretinal interface. Whether due to age-related changes in collagen structure, hyaluronic acid configuration and/or concentration, light-induced or metabolically-derived free radicals, hormonal effects or combinations of all these factors, there is a disruption of the normal collagen-hyaluronic acid association transforming gel vitreous to liquid. Dissolution of the ILL-vitreous cortex adhesion at the macula allows this liquid vitreous to dissect the retrohyaloid plane and result in collapse of the vitreous body. Future studies on the nature of the hyaluronic acid-collagen interaction and the forces underlying posterior vitreous cortex-ILL adhesion should further identify the mechanisms by which PVD occurs. Elucidating the nature of these mechanisms may enable the development of prophylactic techniques by which liquefaction and PVD could be induced or prevented, depending upon the clinical circumstances.

References

[1] Busacca A. La structure biomicroscopique du corps vitre normal. *Ann. Ocul.* 1958; **91:** 477–99.

[2] Goldmann VH: Senescenz des glaskörpers. *Ophthalmologica* 1962; **143:** 253–79.

[3] Eisner G: Zur anatomie des glaskorpers. *A v Graef Arch. Klin Exp. Ophthal.* 1975; **193:** 33–56.

[4] O'Malley P: The pattern of vitreous syneresis—A study of 800 autopsy eyes. In Irvine AR & O'Malley C, eds. Advances in vitreous surgery. Springfield: Charles Thomas 1976; 17–33.

[5] Okasala A: Ultrasonic findings in the vitreous body at various ages. *A v Graef. Arch. Klin Exp. Ophthalmol.* 1978; **207:** 275–80.

[6] Balazs EA, Denlinger JL: The vitreous. In Dawson H, ed. *The Eye.* London: Academic Press 1984; Vol. 1a: 533–89.

[7] Sebag J, Balazs EA, Flood MT: The fibrous structure of human vitreous. *Ophthalmology* 1981; **88:** 62.

[8] Sebag J, Balazs EA: Human vitreous fibres and vitreoretinal disease. *Trans. Ophthalmol. Soc. U.K.* 1985; **104:** 123–8.

[9] Balazs EA, Denlinger JL: Aging changes in the vitreous. In Sekuler R, Kline D, Dismukes K, eds. *Aging and human visual function. Vol 2: Modern Aging Research.* New York: Alan R Liss 1982; 45–57.

[10] Eisner G, Bachman E: Verglichend morphologische spaltlampenuntersuchung des glaskorpers. *A v Graef Arch. Klin Exp. Ophthalmol.* 1974; **191:** 329–50.

[11] Denlinger JL, Eisner G, Balazs EA: Age-related changes in the vitreous and lens of rhesus monkeys macaca mulatta). *Exp. Eye Res.* 1980; **31:** 67–79.

[12] Morner CT: Untersuchung der proteinsubstanzin in der lichtbrechenden medien des auges. *Z. Physiol. Chem.* 1894; **18:** 233–40.

[13] Friedenwald JS, Stiehler RD: Structure of the vitreous. *Arch. Ophthalmol.* 1935; **14:** 789–908.

[14] Pirie A, Schmidt G, Waters JW: Ox vitreous humour. I. The residual protein. *Br. J. Ophthalmol.* 1948; **32:** 321–9.

[15] Chakrabarti B, Park JW: Glycosaminoglycans structure and interaction. *CRC. Crit. Rev. Biochem.* 1980; **8:** 255–313.

[16] Armand G, Chakrabarti B: Conformational differences between hyaluronates of gel and liquid human vitreous—fractionation and circular dichroism studies *Cur. Eye Res.*(In Press).

[17] Andley UP, Chapman SF: Effect of oxidation on the conformation of hyaluronic acid. *Invest. Ophthalmol. Vis. Sci.* 1984, **25:** 318.

[18] Ueno N, Sebag J, Hirokawa H, Chakrabarti B: Effects of visible light irradiation on vitreous structure in the presence of a photosensitizer. 1987 *Exp. Eye Res.* (In Press).

[19] Schnider SL, Kohn RR: Effects of age and diabetes mellitus on the solubility of collagen from human skin, tracheal cartilage and dura mater. *Exp. Gerontol.* 1982; **17:** 185–94.

[20] Kohn RR, Rollerson E: Relationship of age to swelling properties of human diaphragm tendon in acid and alkaline solutions. *J. Gerontol.* 1958; **13:** 241–7.

[21] Hamlin CR, Kohn RR: Evidence for progressive age-related structural changes in post-mature human collagen. *Biochem. Biophys. Acta* 1971; **236:** 458–67.

[22] Snowden JM, Eyre DR, Swann DA: Vitreous structure. VI. Age-related changes in thermal stability and crosslinks of vitreous, articular cartilage and tendon collagens. *Biochem. Biophys. Acta* 1982; **706:** 153–7.

[23] Berman ER, Michelson IC: The chemical composition of the human vitreous body as related to age and myopia. *Exp. Eye Res.* 1964; **3:** 9–15.

[24] Balazs EA: The molecular biology of the vitreous. In McPherson A ed. *New and controversial aspects of retinal detachment.* New York: Harper and Row 1968; 3–15.

[25] Caulfield JB: Medical progress in the application of the electron microscope to renal disease. *N. Engl. J. Med.* 1964, **270:** 183–94.

[26] Gartner J: Electron microscopic observations on the cilio-zonular border of the human eye with particular reference to the aging changes. *z. Anat. Entwicklung.* 1970; **131:** 263–73.

[27] Hogan MJ, Alvarado JA, Weddel JE: *Histology of*

the human eye: An atlas and textbook. Philadelphia, WB Saunders, 1971; 607.

[28] Teng CC, Chi HH: Vitreous changes and the mechanism of retinal detachment. *Am. J. Ophthalmol.* 1975; **44:** 335–56.

[29] Hogan MJ: The vitreous—its structure in relation to the ciliary body and retina. *Invest. Ophthalmol. Vis. Sci.* 1963; **2:** 418–45.

[30] Foos RY: Posterior vitreous detachment. *Trans. Am. Acad. Ophthalmol. Otolaryngol.* 1972; **76:** 480–96.

[31] Lindner B: Acute posterior vitreous detachment vitreous detachment and its retinal complications. *Acta Ophthalmol. (Kbh)* 1966; **87** (Suppl.): 1.

[32] Foos RY, Wheeler NC: Vitreoretinal juncture, synchisis senilis, and posterior vitreous detachment. *Ophthalmology* 1982; **89:** 1502–12.

[33] Larsson L, Osterlin S: Posterior vitreous detachment—a combined clinical and physiochemical study. *Graefe Arch. Clin Exp. Ophthalmol.* 1985; **223:** 92–95.

[34] Kloti R: Experimental occlusion of retinal and ciliary vessels in owl monkeys. I. Technique and clinical observations of selective embolism of the central retinal artery system. *Exp. Eye Res.* 1967; **6:** 393–9.

[35] Balazs EA: The vitreous. In Zinn K, ed. Ocular fine structure for the clinician. Int. Ophthalmol. Clin. Boston: Little, Brown and Co. 1973; Vol. 15: 53–63.

[36] Kuhn W, Hargitay B, Katchalsky M, Eisenberg H: Reversible dilation and contraction by changing the state of ionization of high polymer acid networks. *Nature* 1950; **165:** 514–6.

[37] Osterlin S: Changes in the vitreous with age. *Trans. Ophthalmol. Soc. U.K.* 1975, **95:** 372–7.

[38] Sebag J: Age-related changes in human vitreous structure. *Graefe Arch. Clin. Exp. Ophthalmol.* 1987 (In Press).

[39] Sebag J, Balazs EA: Pathogenesis of cystoid macular edema—An anatomic consideration of vitreoretinal adhesions. *Surv. Ophthalmol* 1984; **28** (Suppl): 493–8.