Chapter 1: Introduction
The vitreous body of the eye is a loose-meshed connective tissue, consisting mainly of extracellular matrix components. Human vitreous structure has been studied in some detail, but controversies with regard to its embryonic development, and the presence and course of intravitreal structures, viz. lamellae and channels still remain.

The human vitreous matrix is subject to age-related changes (liquefaction) which finally may result in posterior vitreous detachment (PVD), which in its turn may induce retinal pathology. The exact mechanisms underlying age-related changes and PVD have still to be clarified.

The rabbit is a much-used animal model to study pathobiological aspects of the vitreous matrix, but data on its structural organization are limited.

Because of its unique properties (high water content, lack of structural elements) studies on the structure of the vitreous meet with specific difficulties. This is one of the reasons why a specific and differentiated organization of the vitreous is frequently denied and why structures observed in the vitreous have often been explained in terms of artefacts of the fixation and preparation procedures.
Samenvatting

The aims of the present study are: (i) to resolve some of the controversies on human vitreous architecture, (ii) to give a detailed description of rabbit vitreous organization and (iii) to determine whether the rabbit may serve as a model to study age-related changes in vitreous matrix organization.

The thesis is subdivided into two parts. The first part (Ch. 2-5) describes aspects of rabbit vitreous structure, and the second part (Ch. 6,7) describes aspects of human vitreous organization.

PART I: The rabbit vitreous body

Chapter 2 describes rabbit vitreous anatomy studied by an ink-injection method and by anatomical dissection of ruthenium red-fixed specimens. A clearly defined central optico-lenticular canal of Cloquet is present in all specimens. A novel structure associated with this canal is found and named the 'alae canalis Cloqueti'. A series of dense lamellae are organized in a funnel-like pattern and run from the vitreous base towards the optic disc. The rabbit vitreous body is subject to age-related changes, consisting of an increase in the number and density of lamellae and signs of a localized liquefaction.

Chapter 3 describes rabbit vitreous structure by light microscopy (LM) and transmission electron microscopy (TEM). Regionally, a cortex, intermediate area and centre are identified. Specific matrix components include spider-like knots of collagen fibres, granular plaques and dots, and novel structures provisionally named IVS-1 and IVS-2 (IVS = intravitreal structure). During embryonic development, the hyaloid artery system transiently vascularizes the primary vitreous body. A regression of these blood vessels occurs in the perinatal period. We observed that lamellae in the avascular secondary vitreous develop at sites formerly occupied by hyaloid vessels. On aging, an increase in the length, density and thickness of lamellae is observed. The relationship between hyaloid vessels and lamellae is consistent with a theory that explains the formation of the primary vitreous theory which provides for the separation of the primary and the secondary vitreous.

Chapter 4 describes structures IVS-1 and IVS-2 throughout the vitreous body and explains why remnants of embryonic blood vessels persist in the adult vitreous matrix.

Chapter 5 explains the function and anti-collagen IV binding of hyaluronan and labelling and by the collagen IV residues.

Part II: The human vitreous body

Chapter 6 examines the human vitreous by anatomical dissection. The actual existence of the central canal and the funnel-like patterns of organization are confirmed. Variability is a common finding, and explains why different views are found in the literature.

Chapter 7 describes the development of the central conical canal in the human adult eyes. On aging, extensions are observed. A well-defined
formation of the secondary vitreous in terms of a gradual remodelling of the primary vitreous. It is in conflict with the generally accepted theory which proposes a strict spatial separation between the primary and the secondary vitreous.

Chapter 4 shows that by morphological criteria the novel structures IVS-1 and IVS-2 represent fragmented and non-fragmented remnants of embryonic blood vessels. The presence of IVS-1 and IVS-2 throughout the vitreous matrix strongly supports the alternative theory on vitreous development advanced in chapter 3.

Chapter 5 confirms the vascular origin of IVS-1 and IVS-2 by anti-collagen IV labelling and their arterial nature by anti-elastin labelling and by elastase digestion. Anchoring of vascular remnants to the vitreous matrix was demonstrated by labelling them with the hyaluronan marker and with anti-collagen II.

Part II: The human vitreous body

Chapter 6 unequivocally demonstrates lamellae in the human vitreous by anatomical dissection of ruthenium red-fixed specimens. The actual existence of lamellae is confirmed by LM, TEM and SEM (scanning electron microscopy). Lamellae are mainly organized in a funnel-like pattern and converge upon the optic disc. Alternative patterns of organization are locally observed in all specimens. Variability is an inherent aspect of human vitreous organization and explains why different views on vitreous organization exist in literature.

Chapter 7 evaluates the location and course of intravitreal channels and spaces by an ink-injection method and by anatomical dissection of ruthenium red-fixed specimens. A large loose-meshed central conical area (cilio-papillo-macular area) is demonstrated in adult eyes. On aging, the volume of this area increases and fingerlike extensions are noted to reach into the intermediate and cortical areas. A well-defined channel of Cloquet is not found. Instead, a variable
number of channel-like structures with ill-defined walls are observed in the central area. Therefore, we conclude that central channels in the adult human eye represent areas of early liquefaction rather than preformed anatomical spaces.

**Chapter 8: Discussion**

*Part A* concludes that the secondary vitreous is formed by a gradual remodelling of the primary vitreous. Comparative vitreous anatomy learns that intravitreal lamellae follow the courses outlined by the hyaloid blood vessels. Differences in the length of lamellae in human and rabbit eyes explain the stricter compartmentalization in the latter, and the absence of Cloquet's canal in the former. Differences in compartmentalization appear to be related to dissimilar patterns of vitreous liquefaction. A possible relationship between lamellae and retinal blood vessels is explained in terms of vascular complications of PVD. The rabbit eye is a valid model to study age-related changes in vitreous matrix organization, but not to evaluate mechanisms of PVD.

*Part B* discusses technical aspects of studies on vitreous structure. A reliable technique to evaluate vitreous anatomy is the combined use of an ink-injection method and anatomical dissection of ruthenium red-fixed specimens. Embedding of intact eyes in a suitable plastic gives minimal distortion of vitreous matrix organization. This makes it possible to evaluate specimens by LM, TEM, and immuno-TEM.