Development and Developmental Disorders of Vitreous

II.A.

Zabrina S. Kingston, Jan M. Provis,
and Michele C. Madigan

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Z.S. Kingston, MBBS • J.M. Provis, PhD, FARVO
ANU Medical School, John Curtin School of Medical Research,
The Australian National University, Canberra, ACT, Australia
e-mail: zabrina.kingston@gmail.com; jan.provis@anu.edu.au

M.C. Madigan, PhD (✉)
School of Optometry and Vision Science,
University of New South Wales, Sydney, NSW, Australia

Save Sight Institute, The University of Sydney,
Sydney, NSW, Australia
e-mail: m.madigan@unsw.edu.au

Keywords
Vitreous • Embryology • Primary vitreous • Secondary vitreous • Hyaloid vessels • Hyalocytes • Vascular regression • Vitreous congenital disorders

Key Concepts

1. Primary vitreous is the collective cellular vitreous that is an extension of the hyaloid vasculature adventitia during the first trimester, which later regresses during the second trimester as the secondary vitreous is formed.
2. Secondary vitreous is the acellular material comprised mainly of type II collagen, proteoglycans, and other macromolecules that fill the space between the retina and the lens.
3. Understanding the molecular processes involved in vitreous development and hyaloid regression may provide insights for the treatment and future management of various ocular diseases.

I. Introduction

Vitreous is a highly hydrated, avascular extracellular matrix located between the lens and retina that serves a variety of structural and physiological functions [1, 2]. Formation of the vitreous is a complex process that has been studied for over a century and still is not clearly understood. Two overlapping developmental phases have been described, the primary and secondary vitreous then followed by formation of the lens zonules previously referred to as tertiary vitreous [3], all closely tied to development of the retina, lens, and retinal vasculature. For this reason, errors in ocular development are often associated with severe congenital disorders

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that are reflected in the vitreous. In this chapter we provide a brief overview of the embryology of the eye and explore in detail vitreous development and its anomalies.

II. Overview of Eye Development

A. Embryonic Origins

By the third week of development, the embryonic plate comprises the three primary germ layers – the ectoderm, mesoderm, and endoderm [3]. Towards the end of gastrulation, the ectoderm anterior to the primitive streak differentiates into the columnar neural ectoderm, which forms of the neural plate, then neural groove, and, subsequently, the neural tube. By 20 days of gestation (DG), the first morphological sign of the developing eye, the optic sulcus, is evident in the anterior-most portion of the neural tube. The optic sulci become internalized when the neural tube closes at ~4 weeks of gestation (WG) and the embryo is entirely covered by surface ectoderm. Optic vesicles form when the optic primordia enlarge and evaginate outwards to make contact with the surface ectoderm (*se*) (Figure II.A-1a) [4–6]. As development progresses, the surface and neural ectoderm

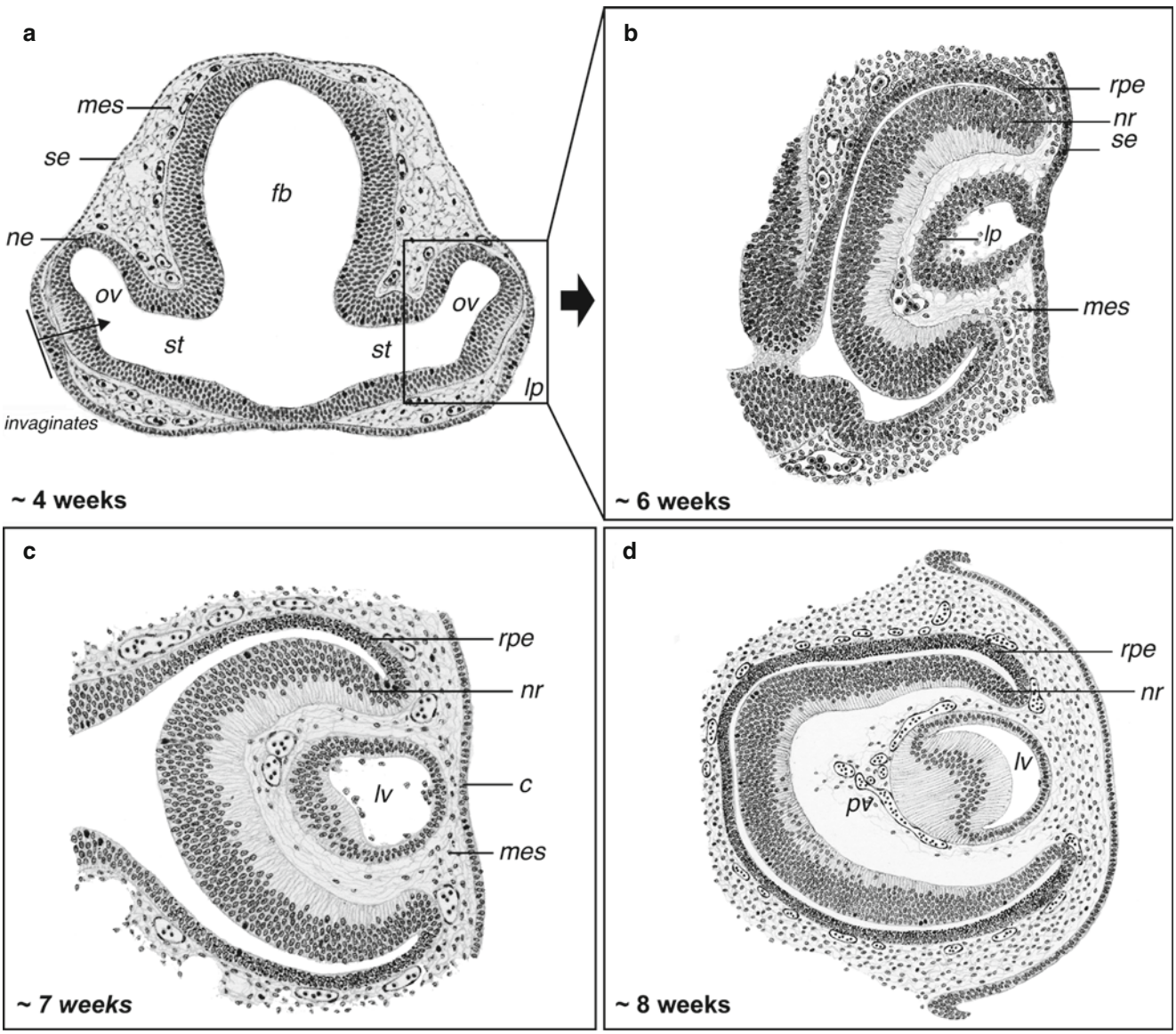

Figure II.A-1 Overview of ocular development. (**a**–**d**) Drawings of sections through the developing eye of human embryos ranging from approximately 4–8 weeks of gestation (WG). (**a**) At around 4 WG the germ layers involved in ocular development are evident, including neural ectoderm (*ne*), surface ectoderm (*se*), and mesoderm (*mes*). The early development of the forebrain (*fb*) is connected to evaginated optic vesicle (*ov*) by the optic stalk (*st*). (**b**) The lens placode (*lp*) pinches off from the surface ectoderm and invaginates to form the lens vesicle (*lv*). Invagination of the neural retina (*nr*) results in a double-layer optic cup comprising neural retina (*nr*) and retinal pigmented epithelium (*rpe*). (**c**) By around 7 WG the lens vesicle (*lv*) has separated from the surface ectoderm that will subsequently become the cornea (*c*). (**b**) By around 8 WG the primary vitreous (*pv*) is clearly discernable, between the lens vesicle and neural retina. *Abbreviations*: *c* cornea, *fb* forebrain, *lv* lens vesicle, *mes* mesenchyme, *ne* neuroepithelium, *nr* neural retina, *ov* optic vesicle, *pl* lens placode, *pv* primary vitreous, *rpe* retinal pigmented epithelium, *se* surface ectoderm, *st* optic stalk [132]

Table II.A-1 Embryonic derivation of ocular tissues

Embryonic origin	Ocular structure
Surface ectoderm	Lens
	Corneal epithelium
	Conjunctival epithelium
	Epithelium of eyelids and cilia
	Lacrimal apparatus
	Meibomian glands and glands of Zeis and Moll
Neural ectoderm	Neural retina
	Retina pigment epithelium
	Epithelium of ciliary body
	Epithelium of iris
	Iris sphincter and dilator muscles
	Optic nerve fibers
Neural crest	Corneal stroma and endothelium
	Stroma of iris and ciliary body
	Perivascular connective tissue and smooth muscle cells
	Sclera (except caudal region)
	Trabecular meshwork cells
	Ciliary muscles
	Vitreous
	Orbital cartilage and bone
Mesoderm	Extraocular muscles
	Endothelium of blood vessels
	Caudal region of sclera

(*ne*), neural crest, and mesoderm (*mes*) will each contribute to the formation of ocular structures, as summarized in Table II.A-1 [4, 7].

B. The Optic Vesicle, Cup, and Fissure

The optic vesicles remain connected to the lumen of the primitive forebrain by the optic stalk (*os*), which at first comprises a layer of neuroepithelium surrounding a narrowing lumen (Figure II.A-1a) [8]. Neural crest cells located between the vesicles and the surface ectoderm are modified as these layers come into closer proximity and subsequently will form the bulk of the connective tissues of the eye [4, 9]. Contact between the neural and surface ectoderm, at around 27 DG, triggers a cascade of induction signals that result in formation of the lens placode (*lp*), the precursor to the lens [10–12]. A double-layered optic cup is formed as the lens placode and neurectoderm together invaginate (Figure II.A-1a), partially obliterating the cavity of the optic vesicle (*ov*). This results in the first appearance of a structure recognizable as a mammalian eye (Figure II.A-1b) [13]. At around 33 DG the lens vesicle (*lv*) detaches from the surface ectoderm (Figure II.A-1c) under the influence of AP-2 transcription factors [14], and the separated surface ectoderm along with resident neural crest cells begin to form the cornea [15, 16]. Differentiation of the optic cup into the neurosensory retina (*nr*) (inner layer) and retinal pigmented epithelium (*rpe*) (outer layer) starts at around 47 DG (Figure II.A-1c, d). Both the ciliary body and iris are later derived from cells located at the junction of the neurosensory and RPE layers of the optic cup [17].

III. Embryology of the Vitreous Body

A. Primary Vitreous

Invagination of the optic cup occurs in an eccentric manner such that initially the cup is open inferiorly, at the optic fissure (*of*) (Figure II.A-2a) [8]. From around 4 WG mesodermal cells surrounding the optic fissure and cup differentiate into the hyaloid artery, marking the beginning of differentiation of the vascularized primary vitreous (Figure II.A-1d). The hyaloid vessels traverse the optic stalk then directly cross the optic cup to reach the lens vesicle, providing a transient blood supply to the developing anterior eye [3, 4].

As the neural ectoderm of the optic vesicle separates from the surface ectoderm (3–4 WG), a fibrillar meshwork of periodic acid-Schiff (PAS)-positive and Alcian-blue-positive material bridges the space [3, 4, 18]. This is the primordial vitreous – a collection of ectodermal and mesodermal-derived cells. The ectodermal fibrillar components are produced by the inner layer of the future neurosensory retina, as well as by cells on the posterior surface of the lens vesicle. The mesodermal cells enter the emerging eye cup inferiorly during invagination of the optic vesicle, via the patent optic fissure (4–5 WG), and are the anlage of the hyaloid vascular system (*hvs*), comprising the vasa hyaloidea propria (*vhp*), posterior tunica lentis (*tvl*), pupillary membrane (*pm*), and the hyaloid artery (Figure II.A-3) [3, 4]. They are surrounded by fibroblasts which, from the outset, synthesize a collagen that is similar to that found in the adult vitreous [19]. This collective cellular vitreous is considered the "primary vitreous" and conceptually can be thought of as an extension the adventitia of the hyaloid vasculature (Figure II.A-2a, orange shading) [18].

In addition to entering the eye posteriorly along with the hyaloid vessels, mesodermal cells also enter the eye cup anteriorly, through the space between the anterior rim of the optic cup and the lens vesicle [13, 20, 21]. These cells include the monocyte-lineage hyalocytes [19]. Recent studies suggest that some mesodermal cells entering the optic cup at this stage are hemangioblasts, which later differentiate into hyalocytes [22, 23]. By 6 WG the hyaline lens capsule has formed a barrier between the lens proper and the matrix of the optic cup, marking the end of lens ectodermal contributions to the vitreous [21].

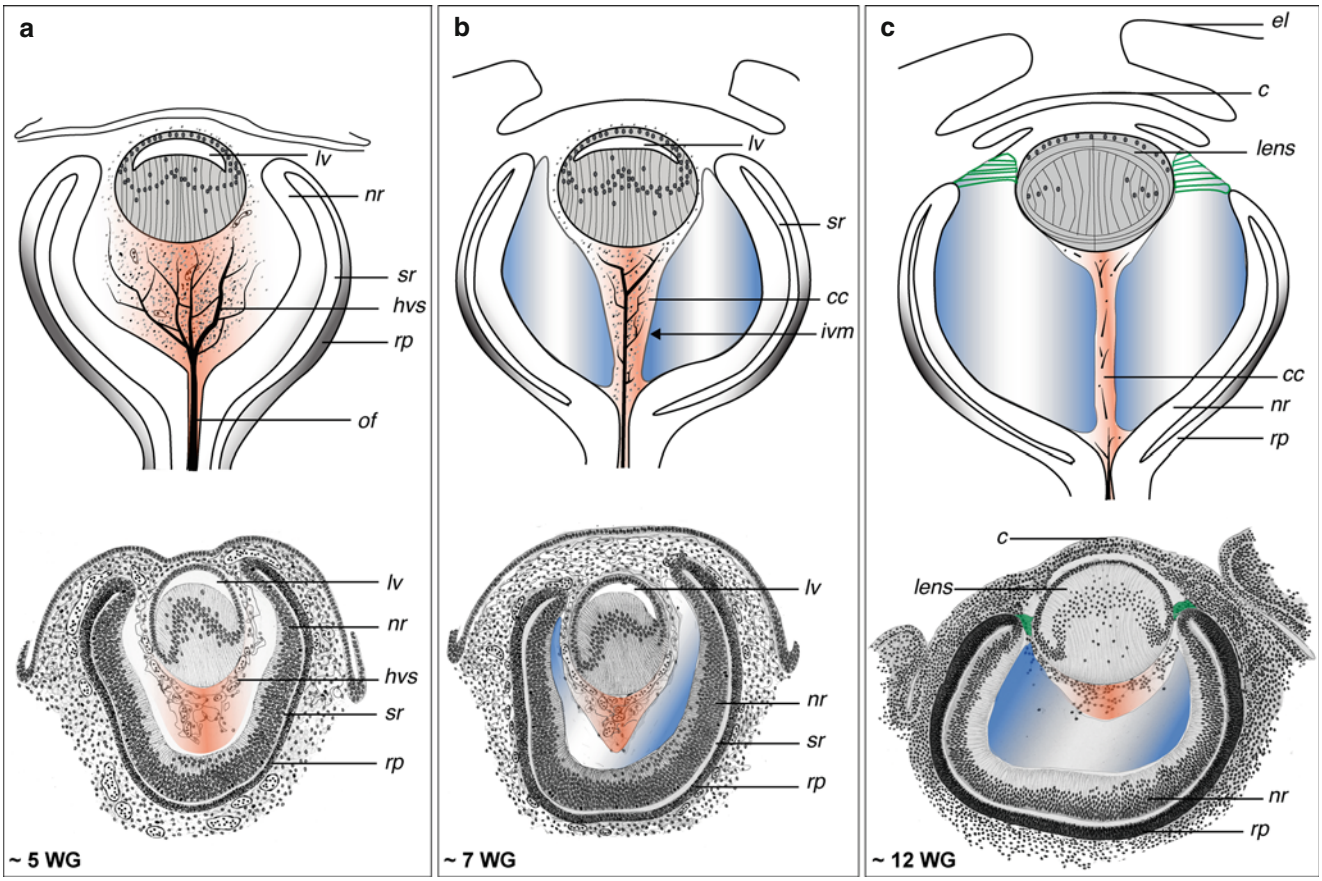

Figure II.A-2 Three stages in development of the vitreous. (**a**) The primary vitreous (*red*) may be considered to be an extension of the adventitia of the hyaloid vascular system (*hvs*) and comprises ectodermal and mesodermal cells that have invaded the developing eyecup. (**b**) By around 7 weeks of gestation (WG), the secondary vitreous (*blue*) has increased in volume and is separated from primary vitreous by the intravitreous membrane (*ivm*). (**c**) By around 12 WG vitreous continued deposition of secondary vitreous and *hvs* regression within Cloquet's canal (*cc*), concurrent development indicated by formation of the zonule fibers (*green*). *Abbreviations*: *c* cornea, *el* eyelids, *lv* lens vesicle, *nr* neural retina, *of* optic fissure, *rpe* retinal pigmented epithelium, *sr* subretinal space, *WG* weeks of gestation [25, 132]

Following closure of the optic fissure, the hyaloid artery rapidly extends anteriorly, ramifying within the primary vitreous to create the anastomotic *vhp*, which in turn anastomoses with the posterior portion of the *tvl* surrounding the posterior lens capsule (Figure II.A-4c) and the *pm* adherent to the anterior surface of the lens capsule and iris diaphragm (Figure II.A-3). By approximately 10 WG the *hvs* has reached its zenith, nourishing the growing lens and adjacent mesoderm (Figure II.A-4b). Thereafter, the hyaloid vasculature slowly regresses, in concert with the very early phases of retinal vessel development [24]. The early stages of regression of the hyaloid system are seen around 11–12 WG in the peripheral *vhp*, closely followed by the *tvl*. This regression is characterized by gradual shrinkage of vessel walls and reduction in lumen diameters, leaving behind thread-like acellular strands of tissue [24, 25]. The initiating and regulatory factors are not clearly understood. However, apoptosis is a significant feature in the early stages, and migration of hyalocytes into the adventitia of the hyaloid vessels is also part of the regression process and may involve cytolysis [24]. At 13–15 WG, there is clear morphological evidence of vessel regression, including thinning and narrowing of the *vhp* vessels, thinning and stretching of the interconnecting vessels of the *tvl*, and decreased tortuosity and loss of anastomoses in the pupillary membrane (Figure II.A-4a, d) [24]. End-stage changes in endothelial cells, pericytes, and macrophages progress, with complete regression of the hyaloid system and atrophy of the primary vitreous not complete until 35–36 WG (Figure II.A-4e, f) [21].

B. Secondary Vitreous

The secondary vitreous is derived entirely from neural crest cells and forms between 6 and 13 WG [25]. This acellular material fills the space between the retina and posterior *vhp* as the eye enlarges (Figure II.A-2b, blue shading) [3, 21, 26, 27]. By 9 WG it has increased significantly in volume, forcing the fully developed vascularized primary vitreous

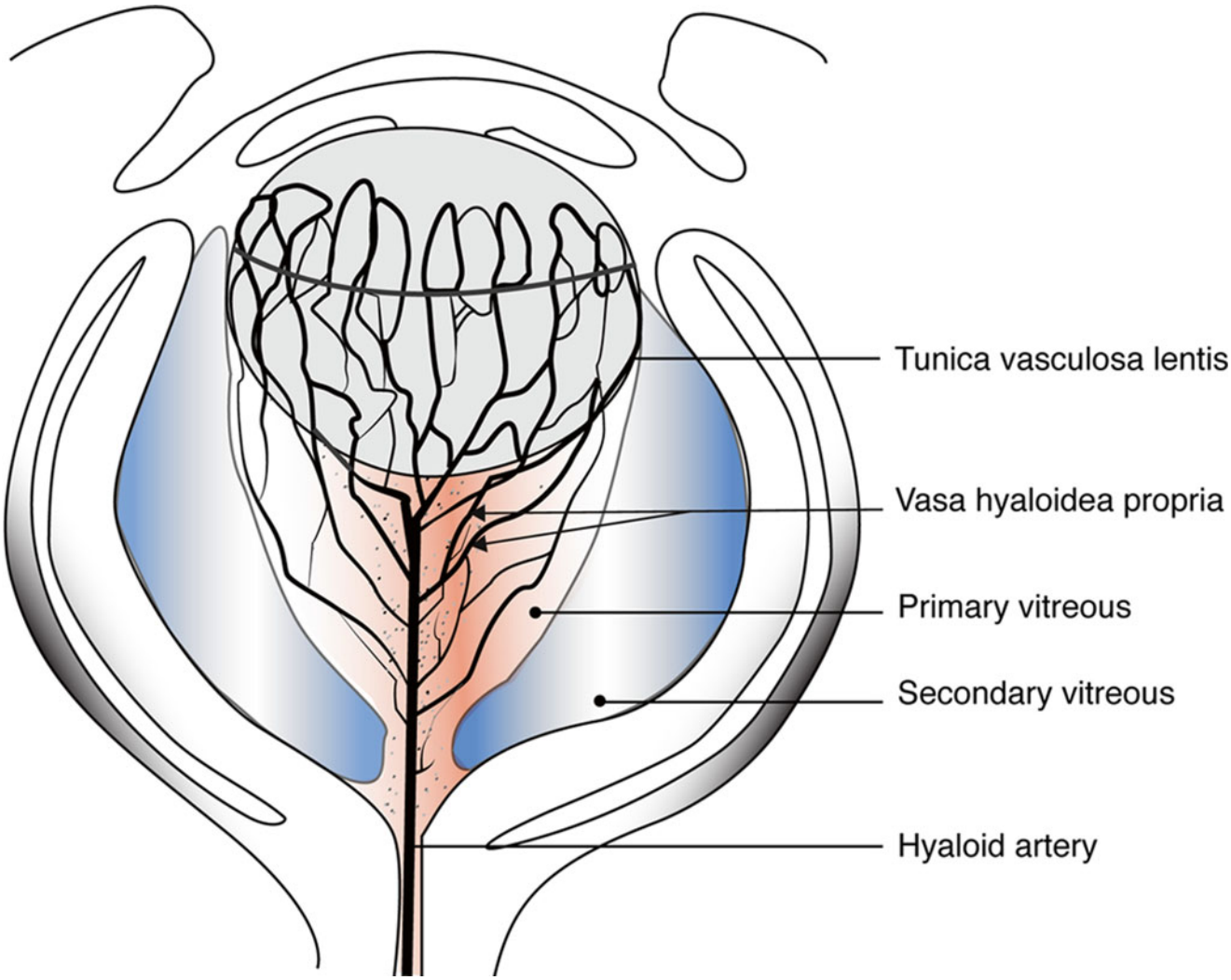

Figure II.A-3 Hyaloid vascular system. Following closure of the optic fissure, the hyaloid artery rapidly extends anteriorly, ramifying within the primary vitreous (*orange shading*) to create the anastomotic vasa hyaloidea propria, which in turn anastomoses with the posterior portion of the tunica vasculosa lentis surrounding the posterior lens capsule and the pupillary membrane adherent to the anterior surface of the lens capsule and iris diaphragm. Collectively the hyaloid vascular system has reached its zenith by 10 WG [25]

into a central position [3, 21, 25, 27, 28], and by 12 WG, a layer of condensed funnel-like fibers known as the "intravitreous membrane" (*ivm*) is apparent along the junction between the primary and secondary vitreous. The classical view is that this membrane forms the walls of Cloquet's canal (*cc*), a tubular structure running through the vitreous from the optic disc to the posterior lens capsule and which contains remnants of the hyaloid artery and primary vitreous (Figure II.A-2b, c). Recent morphological evidence suggests, however, that retracting hyaloid vessels act as a scaffold along which fibers of the secondary vitreous organize themselves [29–31]. Such a process – formation of the secondary vitreous by a method of continuous and gradual remodeling of the primary vitreous – has also been proposed by Jokl (1927) and Pau (1957) [31, 32]. In this latter view, the terms "primary" and "secondary" vitreous refer to the temporal transformation of vitreous materials, rather than to a process of replacement.

Secondary vitreous is essentially an extracellular matrix comprising a compact meshwork of type II collagen, proteoglycans, and other macromolecules [33–35]. Prenatally, hyaluronan content of the vitreous is quite low, but increases after birth [36]. The collagen fibers of the secondary vitreous are thought to be synthesized by, and continuous with, the footplates of retinal Müller cells, which are the radial glia of the mature retina and the end-stage cell type that differentiates from the retinal progenitor cells [21, 37, 38]. Around 10 WG, Müller cell processes vitreoretinal border at the posterior pole start to form lateral junctions with each other, comprising the inner limiting membrane (*ilm*) of the retina [21, 38, 39] [see chapter II.E. Vitreoretinal interface and inner limiting membrane].

Posteriorly, collagen fibrils of the vitreous cortex lie almost parallel to the *ilm*. In contrast, anteriorly the secondary vitreous fibers become thickened, forming the margins of the bundle of Druault or "*faisceau isthmique*" that extends from the anterior rim of the optic cup to the equator of the lens. A portion of the bundle of Druault will later define the anterior extent of the vitreous base. Neural crest-derived mesenchymal cells that accompany the hyaloid vessels may also contribute to the formation of the mature vitreous [21].

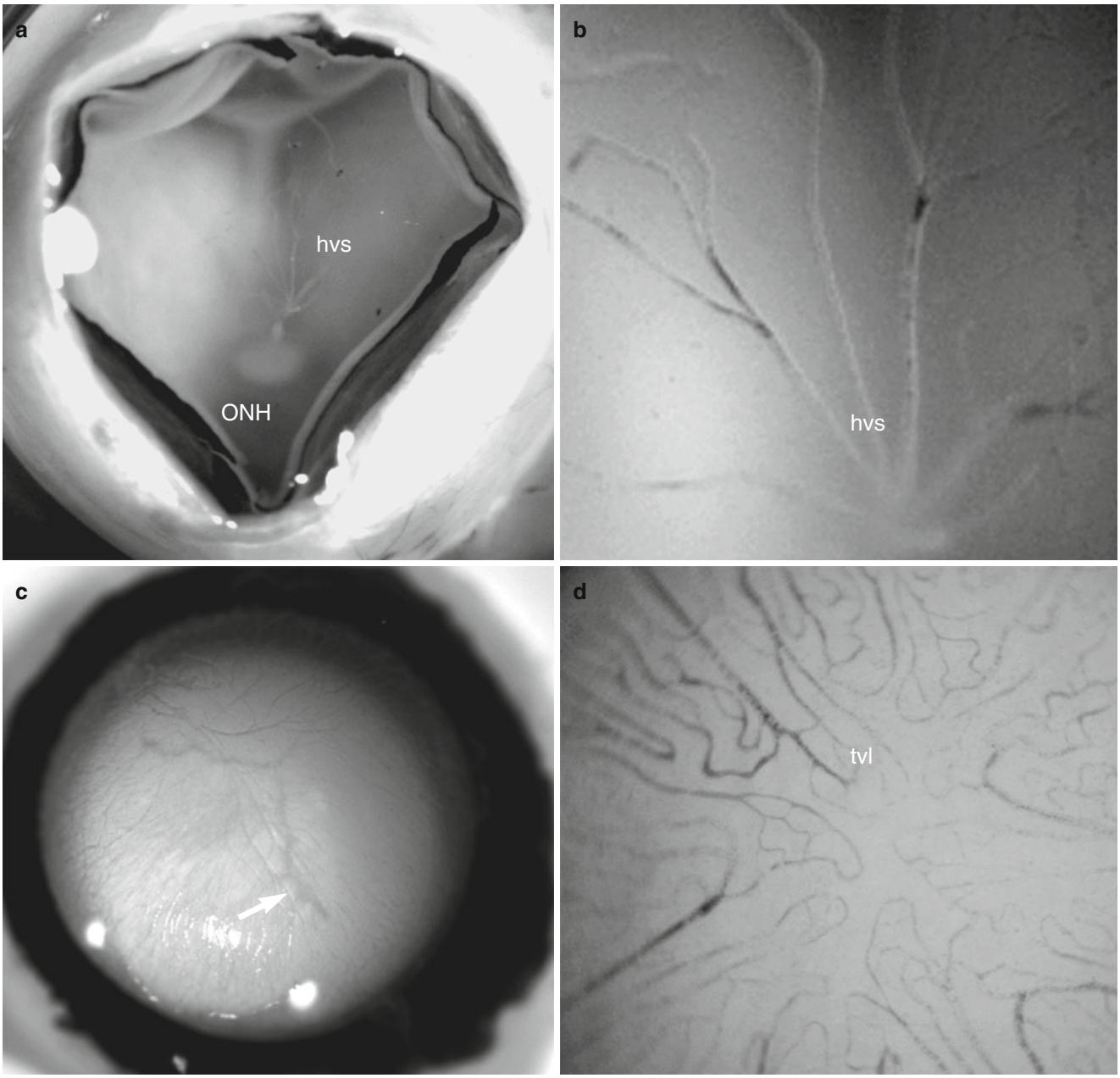

Figure II.A-4 Hyaloid vascular system, tunica vasculosa lentis, and hyalocytes. (**a**) A 17 weeks of gestation (WG) human fetal eyecup with the anterior segment, including the lens, removed. The hyaloid vascular system (*hvs*) is seen extending into the vitreous from the optic nerve head (*ONH*). (**b**) A high-power image of the hyaloid vascular system (*hvs*) showing branches from the hyaloid artery. Some vessel segments are filled with red blood cells (*arrow heads*). (**c**) Anterior segment of a ~17 WG human fetal eye showing the posterior lens surface with vessels of the *tunica vasculosa lentis* (*tvl*). Out-of-focus lens sutures (*small arrow*) can also be seen. (**d**) A high-power image showing the network of vessels on the posterior lens surface (*tvl*). (**e**, **f**) Electron micrographs from a ~18 to 20 WG human fetal eye, showing the typical morphological features of different types of hyalocytes. (**e**) A hyalocyte (*H*) with a bilobed nucleus, fimbriated cytoplasmic processes, and electron-dense cytoplasmic granules is shown (*arrow*) (Bar = 2 μm). (**f**) This hyalocyte (*H*) features a single-lobed nucleus, smooth cell surface, and electron-dense granules (*arrow*) (Bar = 2 μm)

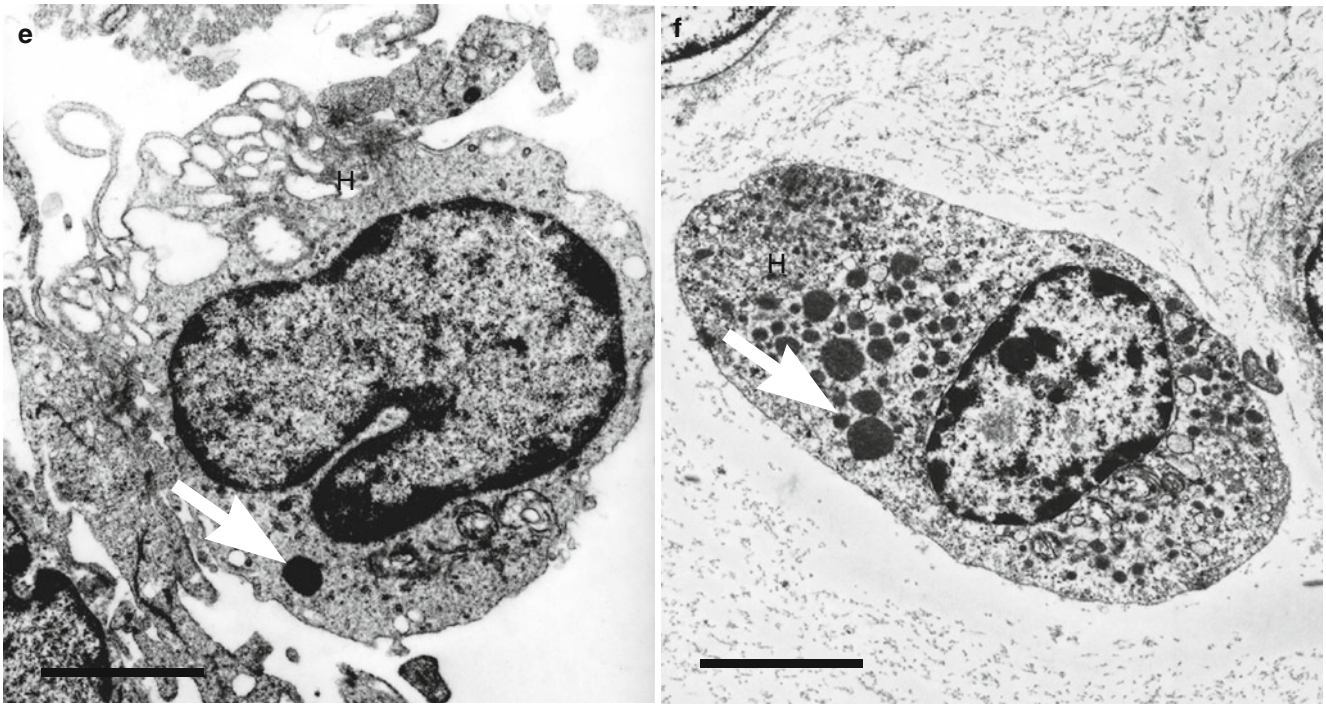

Figure II.A-4 (continued)

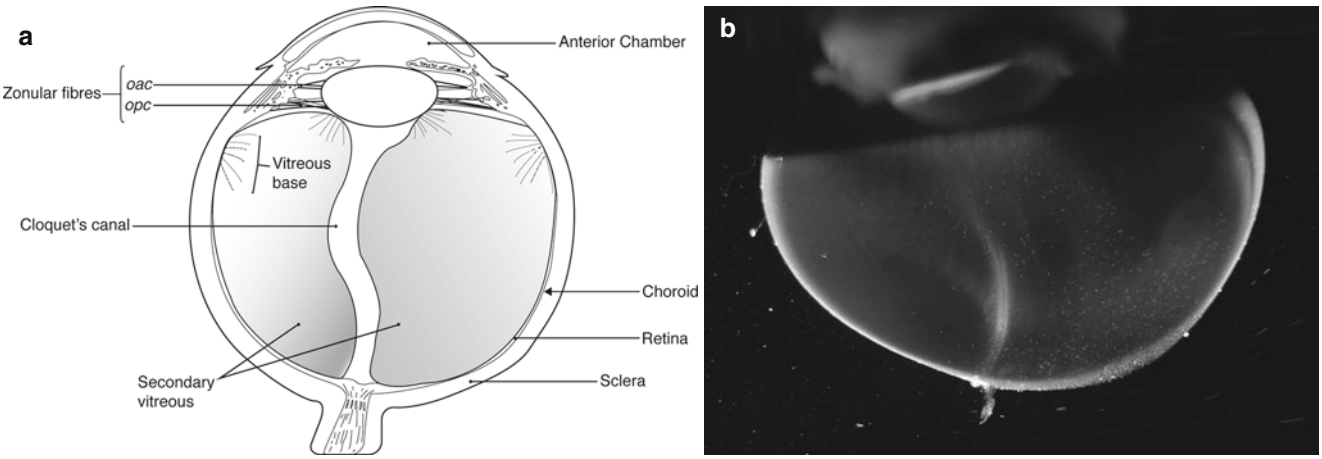

Figure II.A-5 Developed vitreous. (**a**) Formation of the vitreous is marked by the final stages of secondary vitreous development and the regression of the primary vitreous and hyaloid vasculature. This occurs concurrently with the growth of the lens zonular system, which extends from the *pars plana* of the ciliary body and to the equator of the lens. The zonular fibers form two distinct bundles, the orbiculo-anterocapsular and orbiculo-posterocapsular fibers [25]. (**b**) Appearance of the formed vitreous body in a 33 weeks of gestation (WG) human postmortem. The eye underwent dissection of the sclera, choroid, and retina exposing the vitreous body, which was left attached to the anterior segment. A slit lamp beam illuminated a horizontal section that was photographed from above at a 90° illumination-observation angle maximizing the Tyndall effect. The anterior segment is above and the posterior pole is below. The posterior aspect of the lens can be seen above. There is considerable light scattering from the peripheral vitreous cortex due to the dense collagen matrix at this location. Cloquet's canal is seen in the central and posterior vitreous oriented towards the prepapillary region (Courtesy of J. Sebag, MD)

Around 12–16 WG the zonular system is produced, and approximately two-thirds of the optic cup is filled with secondary vitreous and the primary vitreous is located centrally and axially (between the optic rim and lens) (Figure II.A-2c) [3]. The rim of the optic cup has grown forwards to form the cilary body, with the secondary vitreous occupying the area between the ciliary region and the lens [19]. During the final period of antenatal vitreous development (~7 month), the blood flow in the hyaloid artery ceases, followed by regression of hvs and the primary vitreous [20, 28, 40]. The vitreous finally acquires characteristics of fully developed vitreous (Figure II.A-5).

IV. Structural and Molecular Factors in Vitreous Development

A. Structure of the Hyaloid Vascular System

The hyaloid artery (Figure II.A-4b) fine structure is typical of an arteriole [41]. The *tunica intima* consists of flattened endothelial cells connected by tight junctions [24] with an underlying basement membrane. The *tunica media* includes concentric layers of smooth muscle with basement membranes around each contractile fiber [41], surrounded by an *adventitia* of fibroblasts and collagen [42]. Ultrastructural studies show that vessels of the *pm*, *tvl*, and *vhp* are similar in structure. These are small capillaries of the "A-1-alpha" structure [43] with a continuous, single layer of non-fenestrated endothelial cells, joined by intervening tight junctional complexes containing zonulae adherens, *macula adherens*, and possible *zonulae occludens* [24, 44]. They have a continuous basement membrane and an incomplete layer of pericytes [24]. Studies in zebra fish suggest that the *FoxC1* gene regulates integrity of this basement membrane and influences the morphology of the *hvs* [45].

B. Molecular Factors in Formation and Regression of the Hyaloid Vasculature

The genes and signaling pathways involved in the formation and regression of the hyaloid vasculature remain poorly defined. Much of the experimental work in this area is derived from rodent and zebra fish models, which provide a good basis for understanding eye development. Many studies have demonstrated the involvement of vascular endothelial growth factor (VEGF-A) as a key regulator of physiological angiogenesis in the eye, particularly in the retina. Changes in VEGF-A mRNA expression in the lens, in the proximity of the developing TVL and PM, suggest that VEGF-A may be one of the factors that trigger the growth of these two vascular networks [46–49]. Indeed, overexpression of VEGF-A in the lens of transgenic mice leads to vascular endothelial cell hyperplasia adjacent to the lens [50–52], and VEGF-A deletion from the developing lens results in failure of the hyaloid vessels to form adjacent to the lens capsule, although the capillary network in proximity of the retina is not affected [46]. A recent study of dark-reared mice demonstrated an association between increased levels of retinal VEGF-A, hyaloid persistence, and deregulated retinal angiogenesis during development [53]. This is consistent with retinal VEGF-A being critical for development of the hyaloid vasculature.

Several hypotheses have been proposed to explain regression of the hyaloid vessels. While the trigger for regression is not well understood, the most widely accepted hypothesis is that hyaloid vessel involution is macrophage dependent. Several studies in humans and other mammals now show that the ocular macrophages, or hyalocytes (Figure II.A-4e, f), are directly involved in apoptosis of the vascular endothelial cells in the pupillary membrane and the hyaloid vascular system [24, 54–57]. Recent studies in mice demonstrated that a particular class of macrophage (LYVE-1^{+}) is attached to the hyaloid vessels during regression [58] and secrete the protein, periostin, which enhances HVS regression by mediating and strengthening the adhesion of macrophages to the hyaloid vessels [59].

In the early stages of hyaloid regression, apoptosis is prevalent and knockout of proapoptotic genes attenuates hyaloid vascular regression [60, 61]. Some features of necrosis are also detected and cytolytic processes appear to be in effect in the later stages of regression [24, 62]. Other studies have shown that a decrease in capillary blood flow is correlated with an increase in programmed cell death in vascular endothelial cells, suggesting that a hemodynamic disadvantage may be a triggering factor [63, 64].

Throughout development both pro- and antiangiogenic proteins are detected in the vitreous with the balance moving towards an antiangiogenic environment in the later stages of development [65–67] [see chapter I.D. Vitreous proteomics and regression of the fetal hyaloid vasculature]. Antiangiogenic molecules including transforming growth factor-β (TGF-β) [68–72], pigment epithelium-derived factor (PEDF) [73–77], endostatin [78–80], thrombospondin-1 (TSP-1) [81–83], and opticin [84, 85] have been identified in the vitreous in rodents and humans, suggesting that these proteins play a role in hyaloid regression and inhibit angiogenesis in the normal adult vitreous. The canonical Wnt signaling pathway is also emerging as having a critical role in hyaloid regression. Optimal Wnt signaling requires the transmembrane Frizzled family receptors and the coreceptors Lrp5 and Lrp6. Studies in mice have shown that the production of Wnt7b by macrophages in the vitreous is a mediating factor [86]. Wnt7b is promoted by angiopoietin-2 (Ang-2), which may induce regression by inhibiting cell-survival signals [87]. In mice, both Wnt7b and Ang-2 are regulated by the production of Ninjurin-1 by resident vitreous macrophages [88]. Furthermore, mutation in the Wnt pathway coreceptor Lrp5 is linked with the syndrome *osteoporosis pseudoglioma*, which features persistence of the hyaloid vessel [89]. Phenotypes of the Fzd4 and Norrie disease protein (Ndp) mutant mice also include a persistent hyaloid, further implicating the canonical Wnt signaling pathway in hyaloid regression [90].

C. Cells in the Developing Vitreous

Vitreous normally contains relatively few cells of which hyalocytes represent approximately 90 % and fibroblasts the remainder [91]. In the postnatal eye, hyalocytes reside

in the peripheral or cortical region of the vitreous body abutting the inner surface of the retina (preretinal) [18, 92]. They are concentrated anteriorly in the vitreous base, surface of the ciliary processes, and posteriorly in the vicinity of the optic papilla, at an average distance of 50 µm from the inner retinal surface [19, 20, 93]. Hyalocytes vary in morphology depending on the stage of development and most likely are comprised of heterogeneous, distinct subpopulations of cells (Figure II.A-4e, f) [94]. Hyalocytes are more numerous in fetal than in adult vitreous, and while their function in the adult eye is unclear, they play a major role in regression of the hyaloid vasculature during fetal life. They are evident in the vitreous at an early embryonic stage, and several studies suggest that they secrete vitreous collagen and hyaluronan (HA) [95–97]. It has been suggested that hyalocytes are remnants of the primary vitreous [18], and studies in animals and humans indicate that hyalocytes may originate from mesenchymal cells in the optic cup, the embryonic fissure, or migrate from the hyaloid vessel wall [18, 98–100]. In the postnatal human eye, hyalocytes derive from blood monocytes and are replenished on the order of every several months [see chapter II.D. Hyalocytes: essential vitreous cells in vitreo-retinal health and disease].

Human hyalocytes express macrophage-like characteristics and leukocyte-associated antigens, CD45 and CD11a and CD64 (Fc receptor I), although, they do not express CD68 (a marker for a major subpopulation of macrophages) [101]. They do not express glial fibrillary acidic protein (GFAP), cellular retinaldehyde-binding protein, or cytokeratin, indicating that they are not of glial or RPE cell lineages [101–106]. Hyalocytes migrate into the vitreous cavity under physiological conditions, most likely from the bone marrow, and begin to accumulate in the cortical vitreous by the fifth month of gestation [107, 108].

While the exact role of hyalocytes is unclear, they are involved in modulation of intraocular immune responses, regression of the hyaloid vascular system, and the synthesis of extracellular matrix. The eye is an immune-privileged site, and hyalocytes have been shown to provide phagocytic defense against invading organisms with surface receptors for IgG and complement components [102, 109]. At the same time, however, hyalocytes are actively involved in the inhibition of immune reactions [110, 111] and enzymatic removal of fibrin and related products [103] to maintain vitreous transparency.

D. Molecular Changes During Vitreous Development

The vitreous body is subject to an ongoing process of matrix remodeling, starting in embryonic stages and continuing after birth. In very early development the majority of vitreous collagen is type III; however, at 8 WG this is replaced by type II collagen, which comprises approximately 75 % of the total vitreous [112]. The vitreous extracellular matrix is synthesized by numerous cells including retinal cells (most likely Müller cells), cells of the hyaloid vascular system, early lens cells, and presumably cells of the ciliary body [2, 35, 112–114]. In human eyes, there appears to be a high rate of collagen type II production during embryogenesis, which decreases within 2 years after birth [113, 114]. Some evidence suggests that a low level of postnatal synthesis of collagen occurs in cells in the peripheral human retina until adulthood [115]. Hyaluronan is the major GAG in all stages of vitreous development and throughout adulthood [112, 116]. Hyalocytes, Müller cells, and possibly cells associated with the hyaloid vessels secrete hyaluronic acid [95, 116]. Researchers recently hypothesized that collagens and GAGs are synthesized by the mesenchymal cells during the primary vitreous stage, by the retina during the secondary vitreous stage and by hyaloid vessels throughout both these stages [91]. Interestingly, analyses of primary and secondary vitreous failed to show differences in the types and distribution of GAGs and collagens; rather, they are distinguished by the presence of the hyaloid vascular system per se [112].

V. Disorders of the Developing Vitreous

A. Pathologies of the Primary Vitreous

1. Persistent Primary Vitreous

Regression of the primary vitreous occurs during normal eye development; however, its remnants including the anterior, posterior, or the entire hyaloid artery may persist in the adult eye. Anterior hyaloid artery remnants may be seen in the vitreous as small posterior lens opacities (Mittendorf's dot). Posterior remnants of the hyaloid artery may remain at the optic disc, extending into Cloquet's canal, associated with glial tissue known as Bergmeister's papilla (see descriptions below).

2. Persistent Hyperplastic Primary Vitreous Persistent Fetal Vasculature

Persistent hyperplastic primary vitreous (PHPV) is a rare congenital condition that results from a failure of the primary vitreous and the hyaloid vasculature to regress normally (Figure II.A-6). This is associated with hyperplasia of the primary vitreous, *tvl*, and hyaloid system. PHPV can occur in isolation or associated with other congenital disorders or syndromes, such as anterior segment dysgenesis disorders (including Axenfeld-Rieger syndrome), Aicardi syndrome, neurofibromatosis, and morning glory syndrome (reviewed Shastry, 2009) [117]. The term "persistent fetal vasculature (PFV)" was introduced in 1997 by Goldberg

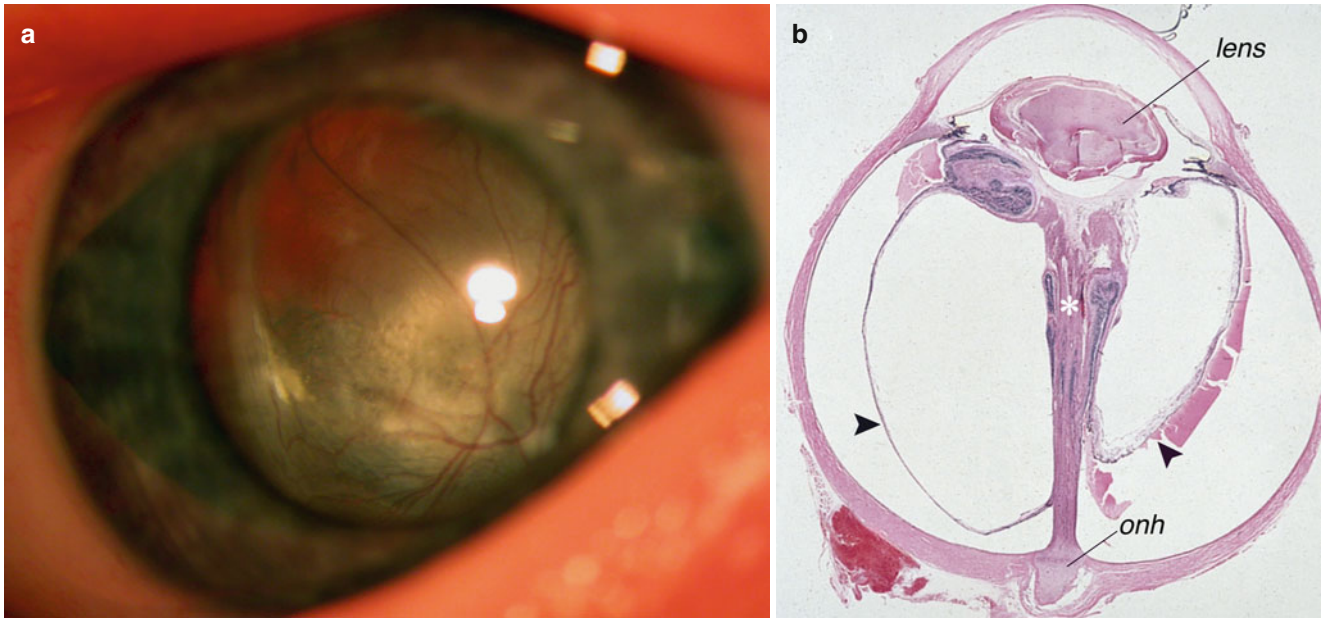

Figure II.A-6 Persistent hyperplastic primary vitreous. (**a**) A photograph of the right eye of a patient showing a persistent hyperplastic primary vitreous (Courtesy of John Grigg, MD, Save Sight Institute, Sydney Medical School). (**b**) Section of an eye with persistent hyperplastic primary vitreous showing a curved fold of detached dysplastic retina (*arrowheads*) that encircles the persistent hyaloid artery and primary vitreous (*asterisk*). The primary vitreous extends from the optic nerve head (*onh*) to the posterior lens, forming a fibrovascular mass (hematoxylin and eosin) (http://commons.wikimedia.org/wiki/Category:The_Armed_Forces_Institute_of_Pathology_Public_Domain_Images)

to provide an inclusive name for the various forms of congenital persistent anterior and posterior intraocular vasculature and associated clinical features, encompassing PHPV, intravitreal vascular remnants such as Mittendorf dot, persistent hyaloid artery and Bergmeister's papilla, persistent pupillary membrane, congenital retinal folds or dysplasia, or optic nerve dysplasia [118]. The clinical implications of these disorders for vision are significant. PHPV may be considered to refer more specifically to vitreous and hyaloid system-related hyperplasia; however, it continues to be used within contemporary literature, often interchangeably with PFV, to describe the various presentations of a complex and heterogeneous group of disorders. These varied presentations are usually classified as "anterior," "posterior," or "combined." Anterior PHPV is more common and presents as a posterior lens fibrovascular (retrolental) opacity, elongated ciliary processes, or cataract. Congenital fibrovascular pupillary membranes, extending from the posterior iris surface across the anterior lens surface, have also been reported as a variant of PHPV/PFV [119]. Posterior PHPV typically shows one or more features including elevated vitreous membranes or a stalk extending from the optic nerve (a remnant of Cloquet's canal), retinal folds or hypoplasia, retinal detachment, or optic nerve hypoplasia. Combined, they encompass features of both anterior and posterior PHPV. The molecular mechanisms involved in the pathogenesis of PHPV are not fully understood, although mutations in several candidate genes have been reported including *FZD4* and *NDP* [120, 121].

B. Pathologies of the Secondary Vitreous

1. Syndromic disorders

Several syndromic human disorders that feature congenital high myopia result from mutations in genes that encode ECM proteins that are prominent in the vitreous, ILM, the vitreoretinal border, and the sclera. In Stickler (and Wagner) syndromes, for example, the vitreous is more liquid than gel like, related to dominant mutations of collagen II and V/XI [122]. In particular, mutations in type II collagen gene COL2A1, COL2A, and the type XI collagen gene COLL11A1 have been shown to result in the membranous phenotype of Stickler syndrome [123, 124] [see chapter I.C. Hereditary vitreo-retinopathies]. Stickler syndrome is an autosomal dominant inherited congenital disorder that affects type II and XI fibrillar collagens in cartilage and vitreous. Sporadic cases and ocular-only variants are also reported. Numerous mutations in *COL2A1*, *COL2A*, and *COL11A1* genes have been characterized associated with Stickler syndrome [123, 124], and the systemic phenotype is widely variable. At least

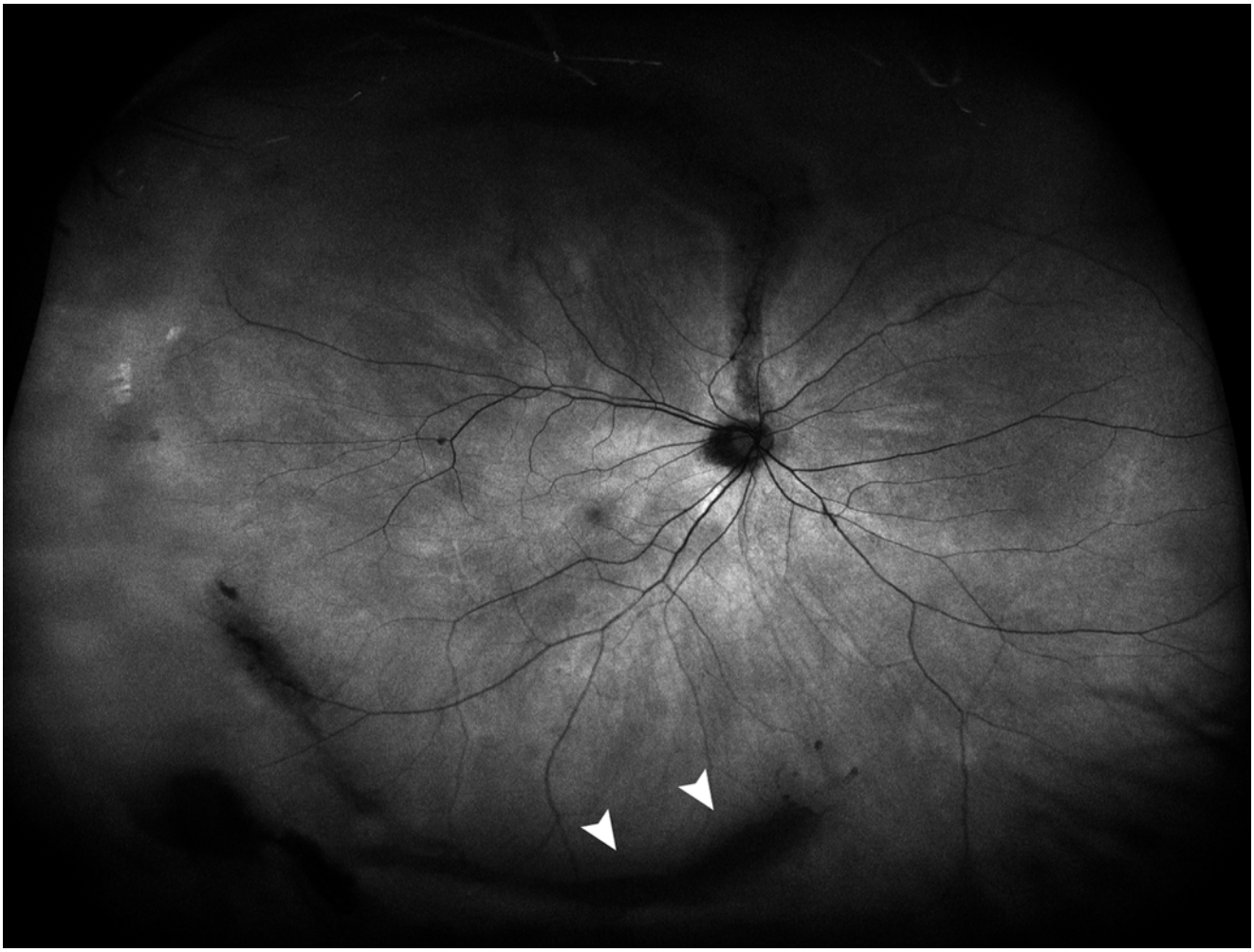

Figure II.A-7 Vitreous veils. A wide-field fundus image showing a vitreous veil (*arrowheads*) in a patient with Stickler syndrome (Courtesy of John Grigg, MD, Save Sight Institute, Sydney Medical School)

five subgroups or types of Stickler syndrome are currently recognized [125]. Vitreous phenotypes are considered a pathognomonic feature, particularly for type I (membranous vitreous) and type II (beaded vitreous). These phenotypes in particular are characterized by an optically empty posterior chamber with the exception of retrolenticular hypoplastic vitreous also known as "vitreous veils" (Figure II.A-7) or comprises sparse irregular, collagen lamellae associated with mutations in both *COL2A1* and *COL11A1* [122].

C. Hyaloid Vascular System

1. Persistent Hyaloid Artery

A persistent hyaloid artery presents as a single vessel that may (fully or in parts) extend from the optic nerve head, through Cloquet's canal, and to the point of attachment (Mittendorf's dot) at the posterior lens capsule. Persistent hyaloid artery, resulting from a failure of vessel regression during development, contrasts with the complex variants that comprise PHPV (Figure II.A-6).

2. Mittendorf's Dot

Mittendorf's dot is an embryologic remnant of the anterior attachment of the hyaloid artery to the posterior lens capsule, where the hyaloid artery joins the *tunica vasculosa lentis*. The dot or opacity is typically at an inferonasal location on the posterior pole of the lens. A stalk of hyaloid artery or fibrovascular tissue may sometimes be seen attached at Mittendorf's dot, floating freely in the anterior vitreous.

3. Bergmeister's Papilla

Remnants of the cone of posterior glial tissue, that envelops the central hyaloid artery – Bergmeister's papilla (BP) – can extend from the optic nerve head disc and may persist in the

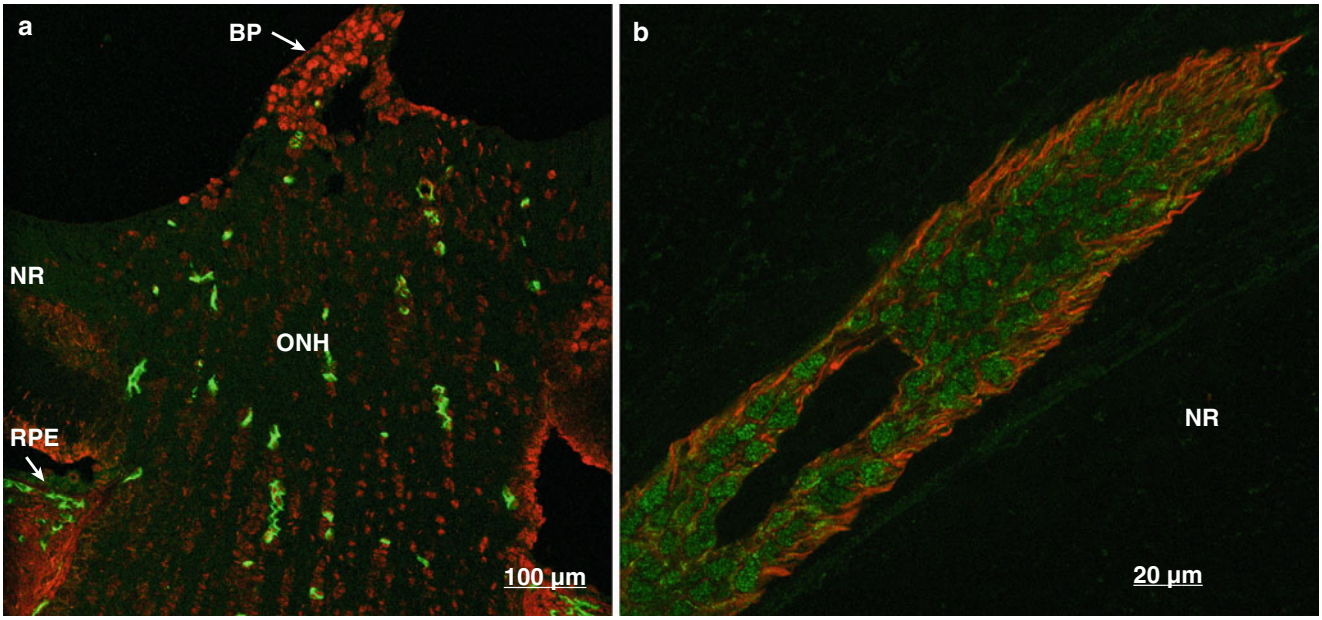

Figure II.A-8 Immunohistochemistry of a Bergmeister's Papilla. Confocal microscopy images of a developing macaque monkey eye at 55 days of gestation, equivalent to approximately 12 WG in the human. (**a**) Optic nerve head, showing endothelial cells (*green*) labeled with antibody to CD34 antigen among cells within the optic nerve. The base of Bergmeister's papilla (*BP*) extends vitread from the optic nerve head at this stage and includes cells immunoreactive for ephrinA1 (*red*). No endothelial cells are present in the retina (*NR*). (**b**) Section through Bergmeister's papilla of a 55-day-gestation macaque monkey. EphrinA1-positive cells of the papilla (*green*) are ensheathed with GFAP-immunoreactive astrocyte processes (*red*). *Abbreviations*: *BP* Bergmeister's papilla, *ONH* optic nerve head, *NR* neural retina, *RPE* retinal pigmented epithelium

vitreous of adult human eyes. BP and the glial sheath, but not the hyaloid artery, expressed intense GFAP immunoreactivity in fetal human eyes, indicating ensheathment by astrocytes derived from the optic nerve (Figure II.A-8) [106]. A recent study in mice has suggested that ensheathment of the hyaloid artery by abnormal astrocytes may limit normal macrophage-pericyte contact and interfere in the macrophage-mediated hyaloid vessel regression that normally occurs during development [126].

4. Persistent Pupillary Membranes

During embryogenesis, the anterior *tunica vasculosa lentis* forms the iris vasculature and pupillary membrane. Normally, involution of the pupillary membrane and vessels occurs during the third trimester (7–9 months of gestation) and is complete by 34 WG, forming the pupillary aperture. Failure to completely regress can produce persistent pupillary membranes (PPM) that can occur either sporadically or associated with other ocular or syndromic conditions. These membranes are attached at the iris collarette on one side and may extend across the anterior lens surface as free-floating pigmented strands or with focal attachments to the lens anterior capsule or to the iris on the opposite side; these often persist into adulthood (Figure II.A-9). Histopathology of surgically removed PPM showed evidence of thickened iris fibrocellular stroma and pigmented cells [127].

5. Vitreous Cyst

Primary (congenital) free-floating vitreous cysts are translucent, semitransparent, or pigmented spherical or oval forms that may also be multilobed and can vary in size from 0.15 to 12 mm diameter [128]. These rare benign cysts are thought to be the remnants of the primary hyaloid or Bergmeister's papilla (translucent cysts) or associated with iris, ciliary body, or retinal pigment epithelium. Cysts from Bergmeister's papilla are observed to be small, round, and located in posterior vitreous [129]. Several case reports showed connection of cysts and hyaloid remnants and cysts frequently located within Cloquet's canal, consistent with a primary hyaloid origin [130, 131]. One group has proposed that cysts with immature melanosomes (not seen in adults) provide evidence of congenital vitreous cysts being primary hyaloid choriostomas [129].

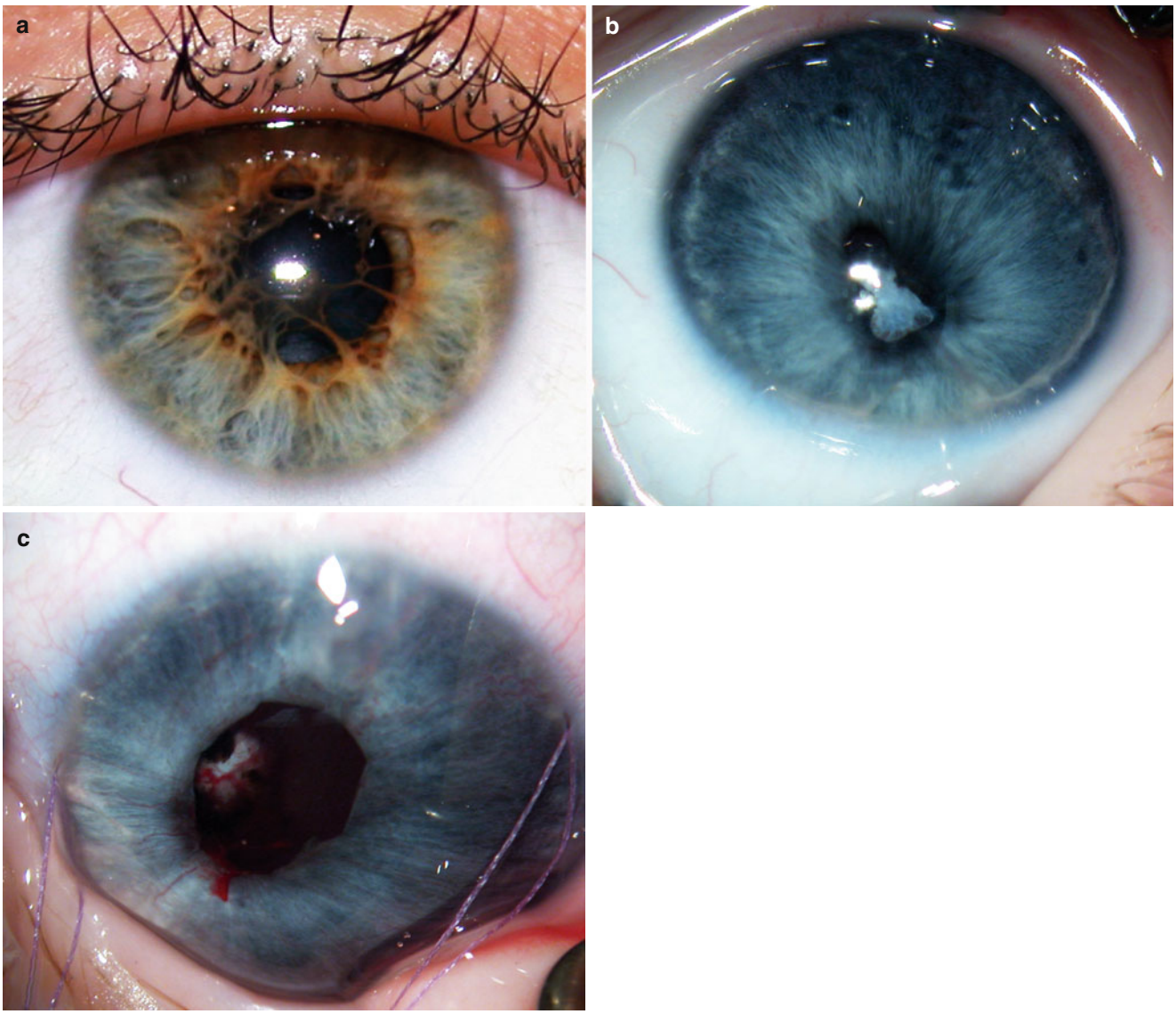

Figure II.A-9 Persistent pupillary membranes. (**a**) Anterior eye photograph of a patient with a persistent pupillary membrane (*ppm*), seen as fine iris strands bridging the pupil to form intricate webs with attachments to the lens. (**b**) A patient with a severe form of *ppm*. (**c**) This image shows the postoperative appearance following surgical management of the severe form of *ppm* (Courtesy of John Grigg MD, Save Sight Institute, Sydney Medical School)

Abbreviations

Ang-2	Angiopoietin-2
AP-2	Activating protein 2
cc	Cloquet's canal
COL	Collagen
DG	Days of gestation
ECM	Extracellular matrix
FoxC1	Forkhead box C1
Fzd	Frizzled protein
GAG	Glycosaminoglycan
GFAP	Glial fibrillary acidic protein
HA	Hyaluronan
hvs	Hyaloid vascular system
ilm	Inner limiting membrane
Ivm	Intravitreous membrane
lp	Lens placode
lv	Lens vesicle
LYVE-1	Lymphatic vessel endothelial receptor 1
mes	Mesoderm
Ndp	Norrie disease protein
ne	Neural ectoderm
nr	Neurosensory retina

of	Optic fissure
os	Optic stalk
PAS	Periodic acid-Schiff
PEDF	Pigment epithelium-derived factor
PFV	Persistent fetal vasculature
PHPV	Persistent hyperplastic primary vitreous
pm	Pupillary membrane
PPM	Persistent pupillary membranes
rpe	Retinal pigmented epithelium
se	Surface ectoderm
TGF-β	Transforming growth factor-β
TSP-1	Thrombospondin-1
tvl	*Posterior tunica lentis*
VEGF-A	Vascular endothelial growth factor
vhp	*Vasa hyaloidea propria*
WG	Weeks of gestation

Conclusion

Vitreous serves a variety of structural and physiological functions during development and throughout life. This is reflected in the transition from the primary fetal vascularized vitreous that supports the development of surrounding intraocular structures to the mature highly hydrated, optically clear, avascular extracellular matrix gel seen in adult eyes. Understanding the molecular processes involved in vitreous development and hyaloid regression may provide insights for the treatment and future management of various ocular diseases.

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