Development of Retinal Layers in Prenatal Human Retina

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• PURPOSE: **To determine the developmental sequence of retinal layers to provide information on where in utero pathologic events might affect retinal development.**
• DESIGN: **Qualitative and quantitative descriptive research.**
• METHODS: **A histology collection of human eyes from fetal week (Fwk) 8 to postnatal (P) 10 weeks was analyzed. The length of the nasal and temporal retina was measured along the horizontal meridian in 20 eyes. The location of the inner plexiform layer (IPL) and outer plexiform layer (OPL) was identified at each age, and its length measured.**
• RESULTS: **The human eye retinal length increased from 5.19 mm at Fwk 8 to 20.92 mm at midgestation to 32.88 mm just after birth. The IPL appeared in the presumptive fovea at Fwk 8, reached the eccentricity of the optic nerve by Fwk 12, and was present to both nasal and temporal peripheral edges by Fwk 18–21. By contrast, the OPL developed slowly. A short OPL was first present in the Fwk 11 fovea and did not reach the eccentricity of the optic nerve until midgestation. The OPL reached the retinal edges by Fwk 30. Laminar development of both IPL and OPL occurred before vascular formation.**
• CONCLUSIONS: **In human fetal retina, the IPL reached the far peripheral edge of the retina by midgestation and the OPL by late gestation. Only very early in utero events could affect IPL lamination in the central retina, but events occurring after Fwk 20 in the peripheral retina would overlap OPL laminar development in outer retina.** **(Am J Ophthalmol 2016;161:29–35. © 2016 by Elsevier Inc. All rights reserved.)**

THE PRIMATE RETINA DEVELOPS OVER MANY MONTHS both in utero and postnatally. Moreover, it has a prominent foveal-to-peripheral gradient such that points on the retina only 2 mm apart may be at strikingly different stages of development.[1–4] This marked gradient is not mentioned or not recognized in many older papers, making it difficult to interpret the data presented. Moreover, having accurate timelines of human retinal development at known retinal loci is important for medicolegal issues as well as for knowledge of what regions and layers of the retina may be impacted by *in utero* events. The widespread use of optical coherence tomography to visualize the prenatal and postnatal human retina has greatly expanded our knowledge of foveal development,[5–7] but there is little systematic information on laminar development outside of the fovea in humans. We have analyzed a large collection of human retinas from embryonic to infant, which provides well-preserved material from which such an assessment can be made morphologically.

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METHODS

QUALITATIVE AND QUANTITATIVE DESCRIPTIVE ANALYSIS was done on 2 groups of human eyes. These were obtained with Human Subjects approval (0447-E/A07). Eyes from fetal week (Fwk) 6–22 were sourced from aborted fetuses obtained after consent by the Human Tissue Laboratory, University of Washington (UW), Seattle. Fetuses containing obvious abnormalities were excluded. Eyes from Fwk 24–40 and postnatal (P) infants were obtained through the support of the UW Neonatal Intensive Care Nursery and the Lions Eye Bank, Seattle. Infants containing obvious abnormalities or congenital conditions that might affect the eye, or eyes that had poor retinal structure, were not used in this study. Fetal age was determined by crown-rump and foot length, and should be taken to be ±1 week.

Fetal eyes <Fwk 16 were fixed whole by immersion in 4% paraformaldehyde in phosphate buffer pH 7.4; in older eyes, the cornea and lens were removed before fixation. Eyes were fixed overnight, washed in phosphate buffer, and measured using micrometer calipers for axial length and diameter. For the best morphology, the horizontal meridian containing fovea and optic nerve was embedded in glycol methacrylate, and serially sectioned at 2 μm using glass knives. For frozen sections to be used in other studies, the horizontal meridian was cryoprotected in 30% sucrose in phosphate buffer, and serially frozen sectioned at 12 μm. In both series, every 10th slide was stained with 1% azure II/methylene blue in pH 10.5 borax buffer to identify the fovea, and then additional sections within the fovea were stained for analysis.

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A stained section was selected from 1 well-preserved eye of infants from Fwk 8 to P 1 week; an example is shown in Figure 1. The section contained optic nerve, fovea, and the entire retina, including both nasal and temporal far peripheral edges. The far peripheral edge of the retina is called "edge" in the text and is indicated by arrows in Figure 1. The length of the nasal retina from optic nerve to edge; the nasal retina from optic nerve to foveal center; and the temporal retina from foveal center to edge was measured using a microscope ocular micrometer (Table 1). In selected sections, the inner plexiform layer (IPL) containing bipolar cell and amacrine synapses and outer plexiform layer (OPL) containing cone and rod synapses were identified and the distance from the center of the developing fovea was measured (Table 2). The amount of retina containing IPL and OPL was divided by the total length and expressed as "% coverage" for a given age (Table 2). Selected regions of well-preserved retinas were imaged digitally using a Nikon E1000 wide-field digital microscope. These images were processed in Adobe Photoshop CS5 for size, color balance, sharpness, and contrast.

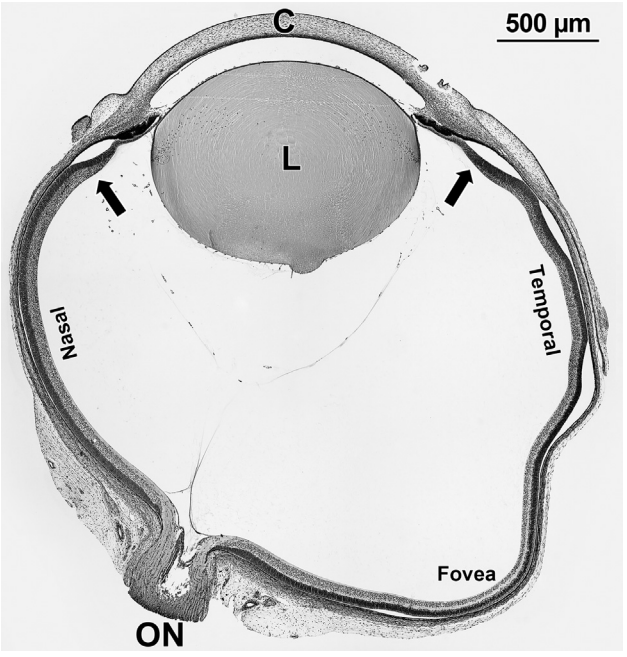

FIGURE 1. Section through the horizontal meridian of a fetal week (Fwk) 12 human eye. The cornea (C), lens (L), optic nerve (ON), and fovea are indicated. The peripheral edge of the retina, called "edge" in the text, is marked with arrows.

RESULTS

• **EYE GROWTH:** Both axial length and eye diameter almost doubled between Fwk 7 and Fwk 11, doubled again by Fwk 14, and increased another 75% by the end of our measurements at Fwk 27 (Figure 2, Left). Retinal growth along the horizontal meridian from Fwk 8 to P 1 week is depicted schematically in Figure 2, Right. Note the bulge where the fovea is developing in young fetal eyes (Figure 2, Right, asterisks). After midgestation, elongation of the pars plana (Figure 2, Right, double arrows) occurs as part of the eye growth process. Measurements are given in Table 1. At Fwk 8 the human retina averages slightly more than 5 mm long, but by Fwk 11 it has almost doubled in length to 9 mm. At Fwk 11 the retina nasal to the optic nerve is only ~3 mm long while the retina temporal to the optic nerve is twice as long. This is the first age when a fovea can be reliably identified because it is the only retinal region with 5 layers (Figure 4, Left).

After Fwk 11 the nasal retina peripheral to the optic nerve grows steadily, increasing to 6 mm by Fwk 15, 8 mm by Fwk 21, 13 mm by Fwk 26, and 14 mm by birth (Table 1). By contrast, the nasal retina between fovea and optic nerve is never more than 4 mm long and seems to stabilize around 3 mm shortly before birth (Table 1). Up to Fwk 15, the temporal retina from fovea to peripheral edge is longer than from nasal optic nerve to retinal edge (~7 mm vs ~6 mm), and it remains so until late gestation (Figure 2, Right).

• **MORPHOLOGIC RESULTS:** At Fwk 8 the first layers appear in the incipient fovea ~2 mm temporal to the optic nerve (Figure 3, Left). This region is marked by a "bulge" (Figure 1, fovea; Figure 2, Right, asterisks) in eyes before midgestation. A narrow IPL separates the pale ganglion cell layer from the dense thick basophilic outer neuroblastic layer, which later will become the inner nuclear layer and photoreceptor layer. These outer layers are not obvious at this age, although a single layer of large pale cones is present at the outer retina (indicated by "c" in Figure 3, Left). The Fwk 8 IPL covers 1.6 mm of foveal retina and extends farther into nasal than temporal retina, with 32% coverage (Table 2). Most of the remaining retina is divided into an inner layer composed of large pale neurons and an outer layer composed of smaller, densely basophilic neurons with no obvious separating zone (Figure 3, Middle) and many mitotic figures at the outer edge (Figure 3, Middle, right; indicated by "m"). There is a nerve fiber layer (NFL) on the temporal side of the Fwk 8 optic nerve (Figure 3, Middle), suggesting that by this age foveal ganglion cells have extended their axons centrally.

After Fwk 8 the IPL extends rapidly away from the fovea so that by Fwk 11–12 a distinct IPL is present nasal to the optic nerve. By Fwk 15 it extends 6 mm into both nasal and temporal retina with a coverage of 91% (Figure 3, Right; Table 2). By Fwk 18–21 the IPL is close to the far peripheral edges of both nasal and temporal retina with a coverage of 96%. Thus the IPL covers most of the fetal retina in the 10 weeks between Fwk 8 and Fwk 18.