Regression of the tunica vasculosa lentis in
the postnatal rat

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Involution of the tunica vasculosa lentis (TVL) was examined in albino rats from 1 to 21 days after birth. Scanning electron microscopy demonstrated that as the lens increased in size, the larger vessels became straight and the small interconnecting vessels disappeared. Transmission electron microscopy revealed that the endothelial cells lost their close relationship with the posterior lens capsule. In the endothelial cells and pericytes the cytoplasm was dense, and many of the organelles became difficult to recognize. However, the cells appeared to remain intact far into regression, shrinking until a basement membrane-like remnant remained. Vitreal cells were close to the TVL throughout the period studied, but few contained phagosomes. During regression the TVL appeared to (1) change its shape in order to accommodate the enlarging ocular structures, (2) maintain separation of the vitreal and vascular compartments by remaining intact during regression, and (3) be associated with vitreal cells, which did not seem to play a prominent role in the involution of these vessels. (INVEST OPHTHALMOL VIS SCI 21:689-699, 1981.)

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The hyaloid vascular system (HVS) is a transitory network of vessels present during early development of the mammalian eye and important for growth and maturation of ocular structures. These vessels normally develop and mature prenatally, and in the rat most regress within 3 weeks after birth. The HVS in the rat is formed by two groups of vessels that radiate from the optic disc to reach the lens. On the lens surface these vessels ramify forming a complex network, the tunica vasculosa lentis (TVL).

Although the histology and ultrastructure of the HVS during development and maturity have been studied in the rabbit, calf, as well as rat, studies following the HVS into regression have been limited. The purpose of the present study was to investigate the sequential morphological changes that occurred in the small blood vessels of the HVS during regression and to quantitate the rate at which these vessels involuted. In order to compare similar groups of vessels at different stages, we were primarily concerned with changes seen in the TVL of the HVS and in particular those vessels associated with the posterior pole of the lens. The mechanism of involution may be similar for the rest of the vessels surrounding the lens.

Methods

Sprague Dawley rats were sacrificed daily through day 21 after birth. On each day the animals were divided into two groups.

Group I. Six to eight pups were sacrificed on each day. The animals were decapitated, and one eye was immersed in phosphate-buffered 3% paraformaldehyde-glutaraldehyde fixative for 4 hr.
Fig. 1. A, Changes in the surface area of the posterior lens during the postnatal period. B, Changes in the area of the posterior TVL during the postnatal period. Each point is the mean of 8 to 10 lenses, and the vertical bars indicate ±1 S.D.

Fig. 2. Scanning electron micrographs of the TVL adjacent to the posterior pole of the lens. (All ×300.) A, One-day-old rat. The vessels are seen branching and forming a complex vascular network. The walls of the larger vessels appear tortuous and are linked by many small interconnecting vessels (arrow). B, Four-day-old rat. The vessels are long, straight channels of approximately the same width. They appear farther apart, with only a few small interconnecting vessels (arrow). C, Nine-day-old rat. Some of the vessels resemble those seen in Fig. 4, B, (broad arrow), and others are decreased in width (arrow) the full length of the vessel. D, Eighteen-day-old rat. The vessels are farther apart than in the earlier stages. Some of the larger vessels appear collapsed (black arrow), whereas others are reduced to threadlike strands (white arrow).
Fig. 2. For legend see facing page.

Fig. 3. Transmission electron micrograph of a vessel from a 1-day-old rat. The capillary is adjacent to the lens capsule (LC). The endothelial membrane is smooth on both the luminal and abluminal surfaces. The nuclei (N) are on the vitreal side of the vessel, and the nuclear margins are smooth. A process from a pericyte (arrow) follows the contours of the endothelial cell. A thin basement membrane surrounds the vessel. (x6250.)

mounted on uncoated copper grids, stained with uranyl acetate and lead citrate, and examined in a JEM 100B electron microscope. The other fixed eye was embedded in glycol methacrylate (JB-4; Polysciences, Inc., Warrington, Pa.). Sections (3 to 4 μm) were stained with toluidine blue or periodic acid–Schiff stain (PAS) for examination by light microscopy. One eye from days 1, 6, 12, and 18 was fixed, opened, incubated overnight in 1% α-amylase (Sigma Chemical Co., St. Louis, Mo.) in 0.1M phosphate buffer, pH 7 at 37° C, and processed for transmission electron microscopy (TEM) or methacrylate embedding as previously described. The other eye was treated with ruthenium red (RR) (BDH Chemicals, Ltd., Poole, England) according to Luft24 and processed for TEM as previously described. Additional eyes from these same days were fixed, incubated in 1% osmium tetroxide in 0.1M sodium cacodylate containing 0.05M K3Fe(CN)6 (Allied Chemical Corp., Morristown, N.J.) overnight at 4° C, and processed for TEM as previously described.

Results

Rate of involution. The surface area of the posterior lens was calculated from an area defined anteriorly by the lens equator and posteriorly by the posterior lens pole. Growth of the posterior lens surface area was in a linear pattern increasing twofold between days 1 and 9 and more than threefold by day 21 (Fig. 1, A). Measurements of the changes in topographical area of the vessels indicated a decrease through the postnatal period. The raw data demonstrated a rapid decline in vessel area, with almost total obliteration by day 21.

The normalized data demonstrated that the vessels actually involuted at a slower rate than was seen in the raw data (Fig. 1, B) and appeared to do it in almost a biphasic pattern. Between days 15 and 18 involution appeared to slow, followed by a rapid drop to day 21, but still about 18% of the vessels remained (Fig. 1, B).

Morphological changes

Day 1. In the 1-day-old rat SEM revealed two groups of vessels that radiated from the optic disc. An outer peripheral group reached the lens at its equator, and an inner group extended to the posterior pole of the lens. No vessels from the vitreous joined the TVL between these points. On the posterior surface of the lens the TVL formed a dense network of interlacing vessels that covered a large proportion of the lens surface. The larger vessels appeared tortuous and were connected by many short vessels (Fig. 2, A).

TEM demonstrated in the 1-day-old rat that the vessels were mostly capillaries or pericytic venules of the continuous type. In both the endothelial cells and pericytes, the luminal and abluminal cell margins were smooth and there were a moderate number of organelles. The nuclei had smooth contours and were usually seen at various positions on the vitreal side of the vessels. Many of the pericytes had thin extensions that reached over and were closely applied to the
Fig. 4. A, Scanning electron micrograph of vessels from a 4-day-old rat. Two large vessels are seen connected by a small regressing vessel (arrow). The small vessel at one end is reduced to a thin strand. A vitreal cell (V) is associated with the vessel remnant. (×900.) B, Scanning electron micrograph of a vessel from a 6-day-old rat. Two vitreal cells (V) are seen associated with the surface of vessel. The vitreal cell surfaces are ruffled, and small pseudopodia (arrow) appear to be extending to the vessel wall. The bulge (asterisk) in the vessel wall appears to belong to a cell within the basement membrane, which probably is a pericyte. (×1400.)
Early regression. SEM revealed that the vessels were less tortuous and farther apart, with few small interconnecting vessels. Earlier in this period the vessels were approximately the same diameter in width (Fig. 2, B), but later (as seen in Fig. 2, C) some of them had uniformly smaller diameters. Within the first postnatal week, many of the short interconnecting vessels were reduced to threadlike remnants (Fig. 4, A) and then disappeared.

TEM demonstrated that some of the endothelial cells had an increase in membrane processes on both the luminal and abluminal surfaces. The nuclei with folded margins were seen on both the vitreal and lens sides of the vessels. Many of the endothelial cells appeared separated from the lens capsule. In some vessels pericytes or vitreal cells separated the endothelium from the lens capsule. The basement membrane, frequently multilaminated, often showed discontinuous contact with the lens capsule (Fig. 5).

Late regression. SEM demonstrated that the vessels were thin and spread apart. Some of the larger vessels had areas that appeared collapsed, whereas others were reduced to

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Fig. 6. Transmission electron micrograph of a vessel from a 15-day-old rat. The lumen (L) is flattened and contains a dense flocculent material. The endothelial cell cytoplasm appears dense, the cell margins are irregular, and most organelles are difficult to recognize. The pericyte appears similar to the endothelium. The basement membrane (BM) is thick and contains many cell processes. (×13,000.) Inset, Higher magnification of the cytoplasm from the pericyte demonstrating the electron-dense particles that remain in the cytoplasm after the other organelles become less apparent. These particles are also seen in the cytoplasm of the endothelial cells. (×35,000.)

thin, threadlike strands (Fig. 2, D). TEM revealed some vessels to be flattened, but intracellular or extracellular gaps were not observed. In many of the older endothelial cells the mitochondria were dark and closely associated with strands of interlacing rough endoplasmic reticulum (RER). The cytoplasm appeared dense and contained many small, electron-opaque particles believed to be ribosomes (Fig. 6). The particles did not stain with K_3Fe(CN)_6 and amylase did not disrupt them. RR reached the vessels from the vitreous, covered the outer surfaces, and was seen in the abluminal portions of the junctions, but not in the lumens or luminal portion of the junctions (Fig. 5).

In the end stages of regression, TEM revealed that many vessels were collapsed and the lumens were filled with a dense flocculent material. The endothelial and pericytic cytoplasms were condensed, and organelles were not easily recognized. The endothelial cells, however, still appeared intact with no apparent discontinuities in the membrane. The pericytes were separated from the endothelial cells by a thick basement membrane. The basement membrane was wide and filled with cell fragments and cell processes of en-
Lather and Kuwabara


Fig. 7. Transmission electron micrograph of a vessel from a 15-day-old rat. A filamentous structure (asterisk) similar in character to the basement membrane surrounding the vessel seen in Fig. 6, is adjacent to the lens capsule (LC). Several spaces are seen within this structure. (×17,000.)

dothelial cells and pericytes (Fig. 6). Finally, the cells disappeared, leaving a basement membrane–like remnant (Fig. 7). These remnants were rarely seen before postnatal days 8 to 10, becoming more common in the 15- to 20-day-old rats.

Vitreal cells. Vitreal cells were seen to accompany the vessels through the period studied and were seen adjacent to the outer surface of the vessels, free within what had been the vitreous, and on the lens capsule. The accompanying vitreal cells varied in appearance. SEM demonstrated that the outer surfaces of some of the vitreal cells frequently were ruffled and had several small pseudopodia, which extended onto the outer surfaces of the vessels (Fig. 4, B). Other vitreal cells had smooth contours and few pseudopodia (Fig. 4, A). TEM demonstrated that most vitreal cells had irregular cell membranes, clear vacuoles, and a few small, membrane-bound dense bodies with homogeneously contents (Fig. 5). Later in regression a number of these cells contained large vacuoles with homogeneously dense contents. PAS staining was more intense in the older vitreal cells with large granules. Vacuoles with heterogeneous contents or recognizable cellular components were rarely observed at any stage investigated. Also seen near the vessels were cells with morphology similar to that of the pericytes. Although these cells were surrounded by a basement membrane, they were not closely associated with the endothelial cells.

Discussion

The TVL appears to have a definitive pattern of regression that enables these vessels to involute with minimal effect on the surrounding environment. Early in the postnatal period the larger vessels appeared to adapt to the rapidly expanding ocular tissue, whereas the smaller interconnecting vessels disappeared. Later the pericytes and endothelial cells became reduced in size but remained intact, thereby maintaining a separation between the luminal and vitreal compartments.

Changes in the relationship between the TVL and the enlarging ocular structures. We have shown that in the first week after birth the surface area of the lens increased more than threefold. Since the vessels on this rapidly enlarging surface appeared not to be increasing, they may have needed to adapt. Our SEM study demonstrated that the larger vessels in regression became thin and straight, possibly allowing the slack seen in the younger vessels to be taken up by the expanding lens surface. At the same time, these vessels also became further apart. The small interconnecting vessels, not having the resiliency of the larger ones, appeared stretched, became thin, and finally disappeared. Silver et al.16 have shown in the ZRD mouse, which is microphthalmic, a persistence of the HVS and TVL into the fifth and sixth postnatal week, although in the controls these vessels had regressed almost completely by the end of the third week. The rapidly growing lens, coupled with the increasing distance between the optic disc and
posterior pole, may have imposed stresses on these vessels that altered their morphology. TEM showed that by the end of the first postnatal week many of the endothelial cells were detached from the lens capsules. In the prenatal and young postnatal rat there was a close relationship between the endothelial cells and the lens capsule. This close association, which has been observed in several species, is believed to facilitate transport of substances between the lumen of the capillaries and the developing lens. The separation of the endothelium from the lens capsule may be related to the change in configuration seen in older vessels and may also indicate a change in their functional role.

**Ultrastructural changes in the TVL during regression.** Atrophy of the endothelial cells of the TVL occurred slowly without apparent cellular disruption or increases in lysosomes. Jack noted an increase in glycogen in regressing hyaloid vessels in rabbit but did not verify his morphological findings with histochemistry. In our study, older vessels, which showed an accumulation of electron-dense particles, did not appear more PAS-positive, amylase digestion did not seem to disrupt these particles, and $KFe(CN)_6$ did not enhance them. The electron-dense particles in regressing cells were more likely to be ribosomes. An increase in polysomes as well as an increase in RNA synthesis were observed in the endothelial cells and connective tissue of regressing rabbit corpus luteum. RNA and protein synthesis have been shown to be required for regression of tadpole tail in organ culture.

At the end of regression all that appeared to remain of the TVL was a filamentous remnant. The basement membrane, which was barely detectable around young vessels, became thick and multilaminated in older vessels and frequently contained cell processes and fragments as well as apparent spaces. Balazs had noted multilayered fenestrated sheath remnants after regression of the bovine HVS. Filamentous remnants similar to those reported by Balazs were seen with increasing frequency in the late postnatal period and may represent the nonmetabolized basement membrane.

Using RR as a marker for extracellular space, we found that it reached only part way through the endothelial junctions, indicating that a barrier existed even into regression between the two compartments. In monkey the HVS was found not to be permeable to fluorescein or peroxidase, although these studies did not follow the vessels into regression. Gieser et al. found little fluorescein leakage in a patient with persistent TVL. However, Easterbrook and Sloan reported leaks from cysts of the hyaloid system. Gaps were reported in the endothelial lining of regressing TVL in rabbit. Our study indicated that junctional areas between regressing endothelial cells may retain their integrity, but additional studies using tracers of various sizes will be needed to explore this further.

Free vitreal cells were associated with the vessels through the period studied. Although these cells were found near the vessels far into regression, they rarely had phagosomes containing recognizable cellular components. There appeared to be little change in the number of vitreal cells. Vitreal cells have been described as macrophage-like residents of the cortical vitreous gel, and the granules may contain degradative enzymes. Macrophages have been suggested to initiate closure of the TVL in rabbit by vascular occlusion and to be involved in regression of the pupillary membrane. Balazs indicated that in the developing bovine vitreous there was an impression of decreasing number of vitreal cells but that, in fact, the cell number was not increasing proportionally to the volume increase of the vitreous. Since de-
generating cells are known to exert a chemotactic effect on phagocytes,\textsuperscript{29}\textsuperscript{a} the number and character of the vitreal cells might be expected to change. However, regression of the TVL appeared to be a slow physiological involution in which the regressing vessels may not have stimulated the vitreal cells to become phagocytic or increase in number, or possibly these cells were unable to respond to the stimulus. It seems unlikely that the few vitreal cells present would be able to remove, in a short period of time, this large involuting vascular bed. The possibility of rapid turnover of macrophages or vitreal cells exists, but this was not evaluated in our study. Heterophagia did not appear to play a prominent role in the involution of the TVL of the rat.

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