

## Fenestrated Glomerular Capillaries Are Unique

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The article by Ichimura *et al.*<sup>1</sup> in this issue of *JASN* reports during embryologic development in the rat and under regenerative conditions that glomerular endothelia form PV-1–positive fenestrae bridged by a diaphragm, as fenestrated capillaries elsewhere in the body, including peritubular capillaries. Conversely, this study also provides conclusive evidence that under orthologic conditions in the adult, glomerular capillaries do not equal fenestrated capillaries but represent a unique type of a porous capillary. A diaphragm does not bridge the pores of adult glomerular capillaries, unfortunately also called fenestrae by most in the field. This is revealed in side-by-side transmission electron micrographs in developing glomeruli that still have both types of endothelia. These structural observations are in agreement with positive PV-1 staining of developing capillaries and the absence of PV-1 staining in mature capillaries, confirming previous studies.<sup>2</sup> These results are also in agreement with many previous studies using transmission electron microscopy, including freeze-fracture studies,<sup>3</sup> showing the adult glomerular endothelium is equipped with nondiaphragmed fenestrae, or what I call pores.

Nevertheless, the question of whether the fenestrae in the glomerular endothelium have diaphragms has been a persistent controversy over several decades. Transmission electron micrographs of fenestrae along glomerular capillaries with and without diaphragms are well described. Diaphragms at one time were considered fragile structures that tended to get lost during vigorous fixation. A clarifying observation made by Elger *et al.*<sup>4</sup> demonstrated the intraglomerular segment of the efferent arteriole and its direct tributaries (capillaries that may well be located deep within the tuft) in the rat indeed have fenestrae with diaphragms, whereas the vast majority of glomerular capillaries

do not; however, this observation has never been generally appreciated.

The article Ichimura *et al.*<sup>1</sup> confirms that endothelial fenestrae/pores of glomerular capillaries in the adult rat are not bridged by diaphragms. Simultaneously, this study shows the types of fenestrae, with or without a diaphragm, are closely related to each other. During development, the endothelium is first established with diaphragmed fenestrae changing into the mature type without a diaphragm during and after the capillary loop stage. This holds true for regenerative processes in rats recovering from Thy-1 nephritis; frequently under such recovery conditions, the first capillaries with diaphragmed fenestrated endothelia are subsequently replaced by the nondiaphragmed type.

This sequence suggests that there might be a switch, some additional signal that induces the transition from the diaphragmed to the nondiaphragmed type. Eremina and Quaggin<sup>5</sup> reported that high dosages of vascular endothelial growth factor (VEGF) are necessary to establish the mature endothelium of glomerular capillaries. This notion has been confirmed by the appearance of nondiaphragmed endothelial portions in peritubular capillaries in response to VEGF overexpression in tubules (own unpublished observations<sup>6</sup>). Such a dosage dependence might also explain the occurrence of diaphragm-bridged fenestrae in direct tributaries to the glomerular efferent arteriole,<sup>4</sup> because these capillaries exhibit an additional thin layer of mesangial matrix between the GBM and the endothelium. This layer increases the distance from the podocyte to the endothelium, probably exposing the endothelium to lower levels of a suggested VEGF gradient starting at the podocyte.

The role of the glomerular endothelium in the filtration barrier is still insufficiently understood.<sup>7</sup> That there are no diaphragms bridging the fenestrae of the glomerular endothelium in adults in no way contradicts the observation that endothelial pores contain dense assemblies of glycoproteins that fill the pores like “sieve plugs”<sup>8</sup>; therefore, the pores should not be imagined as being fully patent. As summarized in a recent review,<sup>9</sup> it is safe to conclude that endothelial glycocalyx, together with the more loosely associated endothelial surface layer, plays a crucial role in preventing the bulk entry of albumin into the glomerular filter.

So we have to ask, “What is the gain for the glomerular filter in switching from diaphragmed to nondiaphragmed fenestrae during development?” Compared with a continuous, nonfenestrated endothelium, a typical fenestrated endothelium with diaphragms can readily be expected to have a much higher hydraulic conductivity for water and solute while maintaining a barrier for macromolecules and thus needs a transcytosis system for macromolecular (albumin) transport. Isn't that what we would like to have for the glomerular filter: High hydraulic conductivity and tightness for macromolecules? It is obvious that the non-

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diaphragmed fenestrae/pores in glomerular capillaries are leaky for albumin and other macromolecules, at least to a certain extent, corroborating that the glomerular endothelium does not have a transcytosis system<sup>3</sup> (own unpublished observations in freeze-fracture replicas). So what is the gain to changing to open fenestrae? In my view, the only plausible answer is a further and probably significant increase in hydraulic conductivity. It seems in adults the dominant goal in evolution is to achieve high filtration rates by accepting a decreased tightness for macromolecules. This is a surprising conclusion considering the many problems that obviously arise from leakiness to albumin.

## DISCLOSURES

None.

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See related article, "Glomerular Endothelial Cells Form Diaphragms during Development and Pathologic Conditions," on pages 1463–1471.

# Hemoglobin in the Kidney: Breaking with Traditional Dogma

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Hemoglobins (Hb) in both prokaryotes and eukaryotes belong to an ancient family of heme-associated, iron-containing globin proteins. Mammalian Hb are tetrameric metalloproteins (approximately 68 kD) that are present in millimolar concentrations within red blood cells, where they function as O<sub>2</sub>-carrier proteins, facilitate CO<sub>2</sub> transport in deoxygenated blood, and react with nitric oxide. Tetramers are composed of identical heterodimers that consist of  $\alpha$  and  $\beta$  chains encoded by gene families located on separate chromosomes. The expression of various  $\alpha$  and  $\beta$  genes in erythroid cells is tightly coordinated during development and in adulthood, HbA ( $\alpha_2\beta_2$  tetramer) being the most abundant adult Hb.

Although Hb have been traditionally thought to be exclusively expressed in cells of erythroid lineage, reports now suggest expression in nonerythroid cells, such as activated macrophages, respiratory epithelial cells, and Clara cells.<sup>1,2</sup> As part of a genome-wide microarray and proteomics screen to identify differentially regulated genes in chronically hypoxic kidneys, Nishi *et al.*<sup>3</sup> unexpectedly found increased expression of  $\alpha$  and  $\beta$  globin in kidney homogenates and investigated the cellular source of renal Hb in this issue of *JASN*. Using manual and laser capture microdissection of nephron segments, as well as sieving and *in situ* hybridization techniques, Nishi *et al.* demonstrate that renal Hb are specifically expressed in mesangial cells, where they may exist in dimeric form. Although the biologic functions of nonerythroid Hb are poorly understood, Nishi *et al.* propose that mesangial Hb may function as a scavenger for reactive oxygen species and thus protect cells from oxidative stress. This notion is supported by overexpression studies in cultured mesangial cells demonstrating that intracellular generation of reactive oxygen species is decreased in cells transfected with  $\alpha$ - and  $\beta$ -Hb and that increased Hb expression is associated with improved cell viability, albeit modest, as measured by lactate dehydrogenase release.<sup>3</sup>

Although Nishi *et al.* establish that both  $\alpha$ - and  $\beta$ -Hb are expressed in mesangial cells, several questions regarding expression levels and biochemical structure remain unanswered. For a comprehensive understanding of the biologic functions of mesangial Hb, it will be important to determine the ratio of  $\alpha$ - and  $\beta$ -polypeptide chains in mesangial cells under physiologic conditions, establish whether mesangial Hb are predominantly expressed as individual polypeptide subunits or whether they form heterotetramers, and clarify whether the heme prosthetic group is efficiently incorporated.

The heme group contains a porphyrin ring, which pro-

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