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STAGES OF DEVELOPMENT OF RENAL GLOMERULI IN THE NEWBORN RAT KIDNEY

M. NOBAKHT*, M.S., M.T. TAKI*, Ph.D., M. REZAZADEH*,Ph.D., AND S. SHARIAT TORBAGHAN**, M.D.

*From the *Department of Anatomy, Tarbiat Modarres University, and the **Cancer Institute of Imam Khomeini Hospital, Tehran, Islamic Republic of Iran.*

ABSTRACT

Glomerular development of the kidney was studied in newborn rats by electron microscopy. Four different stages of glomerular development were defined: vesicle formation, S-shaped body stage, capillary loop formation, and glomerular maturation.

In the first stage, the mesenchymal cells form a spheroid mass. This is followed by the S-shaped body stage in which clefts appear in the mass. Afterwards, capillary loop formation, junctional migration, podocyte differentiation, and interdigitation of epithelial processes occur. Finally, the cytoplasm of endothelial cells becomes thinner. The urinary space is visible in this stage. The fusion of epithelial and endothelial basement membranes results in formation of the layers of the GBM. The increase in the number of podocyte processes and endothelial cell fenestrations are important events in the maturation phase.

Key Words: Development, Glomerular stages, Basement membrane

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INTRODUCTION

The development of the renal glomerulus has been studied by transmission electron and scanning electron microscopy.[5,17,19,20,27,28] Some quantitative information has been obtained from light microscopical studies.[3,12]

Morphologic studies have been carried out on the glomerulus,[19] and Saxen and Lehtonen have decribed the aggregation of mesenchymal cells in the blastema and their differentiation into coiled tubules.[3]

The aim of the present study was the differentiation of the glomerulus. We studied the kidneys of 1,3,5,7 and 9 day old newborn rats at ultrastructural levels to sequentially evaluate the changes that occur during the process of glomerular differentiation.[19,28]

MATERIAL AND METHODS

Sprague-Dawley rats were used. Kidneys were removed at 1,3,5,7 and 9 days of age. The rats were divided into five groups with five animals per group.

Left and right kidneys were used for light and electron microscopy respectively. The right kidney was placed in buffered formalin, washed, dehydrated, infiltrated, and embedded in paraffin. Tissue sections were cut 4 microns thick and stained with hematoxylin and eosin, periodic acid-schiff (PAS) and Jones' stain. Thick sections were viewed and photographed on DX Konica film (ISO 400) with a Ziess photomicroscope II.

The left kidneys were placed in 2.5% glutaraldehyde in 0.1 M phosphate buffersaline, pH 7.4, for 2 hours in 4°C. The tissue was then washed in buffer, postfixed four hours in 1% osmium tetroxide, rinsed again, dehydrated in graded acetone, and embedded in Epon 812 (Polysciences, Warrington, Pennsylvania). Semi-thin sections were cut at 0.5 micron thickness from each block and stained with 1% toluidine blue in 1% sodium borate.

Thin sections were prepared, placed on 200 mesh copper grids, and stained with uranyl acetate and lead citrate, before

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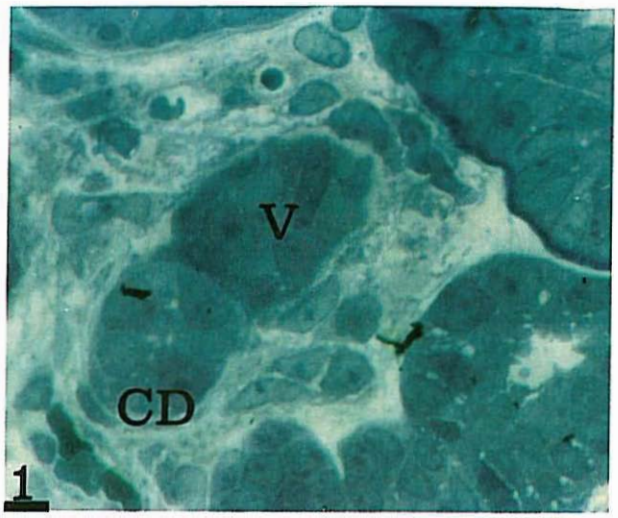

Figs. 1-6: Light micrographs of stages of glomerular development. Toluidine blue staining. Original magnification ×1000.

Fig. 1. Vesicle (earliest) stage: vesicle (V) clusters of cells are located near a collecting duct (CD). Note that vesicles are located just beneath the kidney capsule.

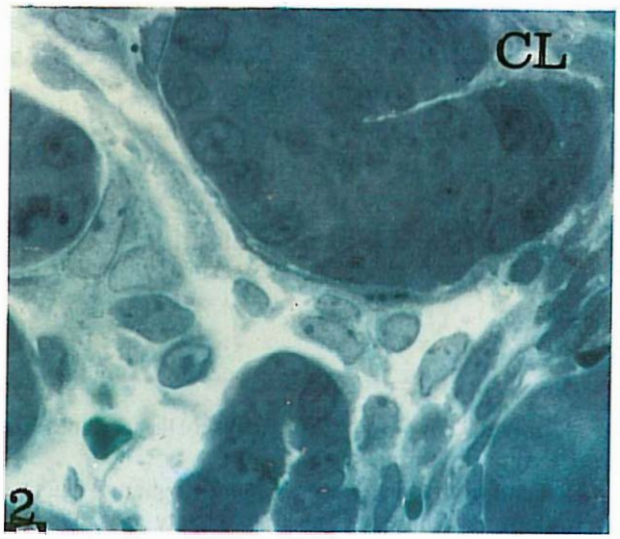

Fig. 2. S-shaped body stage (early): the first cleft (CL) gives the vesicles a comma-shaped appearance.

examination in a Ziess 10CR EM and Siemens 1A operated at 60 KV.[14]

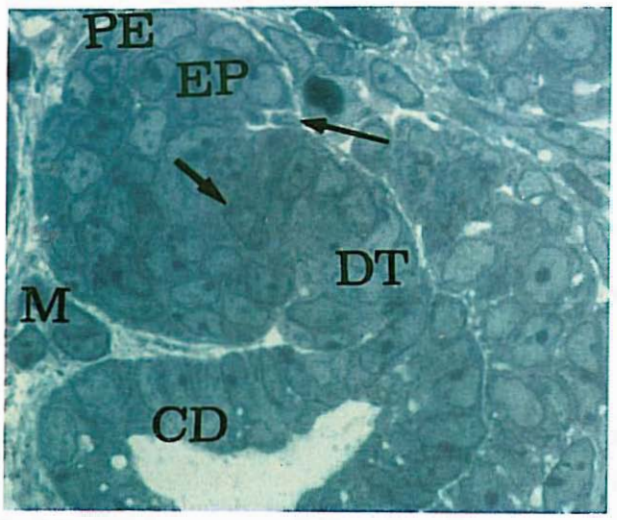

Fig. 3. S-shaped body stage (late): the second cleft appears (long arrow), and the vesicle has begun to differentiate into parietal epithelium (PE), vesicle epithelium (EP), distal tubule (DT), and collecting duct (CD) and become invaginated by mesenchymal cells (M). Note numerous mitotic figures (short arrow) indicating the extensive cell proliferation that takes place at this stage.

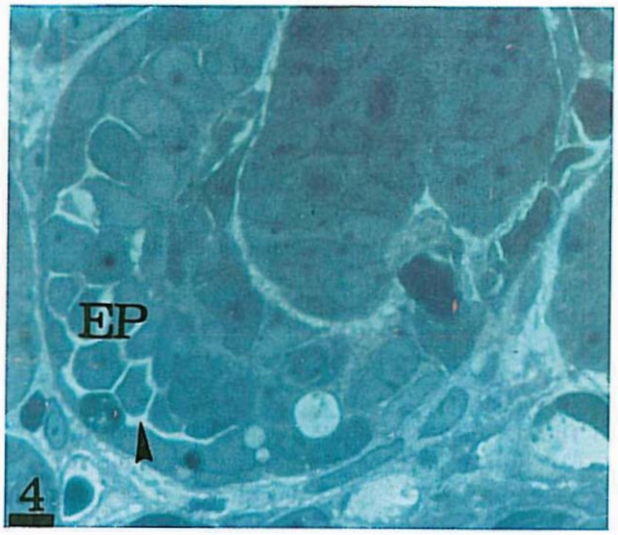

Fig. 4. Capillary loop development stage (early): visceral epithelial cells (EP) have proliferated and intercellular spaces can be seen; Bowman's space (arrow head).

RESULTS

The newborn rat is not fully developed until approximately 9 days after birth. Therefore, glomeruli were not differentiated at 1,3,5,7 and 9 days after birth, and different developmental stages were confronted; vesicle stage (stage I), S-shaped body stage (stage II), capillary loop stage (stage III) and the glomerular maturation stage (stage IV). Different stages of developing glomeruli can be found in the cortex of the same kidney. The immature glomeruli were located in the subcapsular region while the mature glomeruli were seen in the cortico-medullary region. The principal stages of glomerular maturation are shown in Figs. 1-6. Briefly, the main events in glomerular differentiation are as follows.

Vesicle stage (stage I)

During the vesicle stage, the mesenchymal cells

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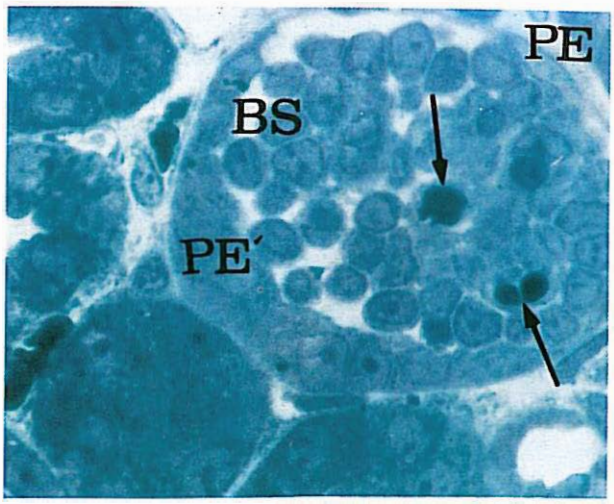

Fig. 5. Capillary loop development (BS) is more prominent and the parietal epithelium (PE) has become flattened at the top of the glomerulus but is still cuboidal below (PE). Several capillary loops can be recognized (long arrow).

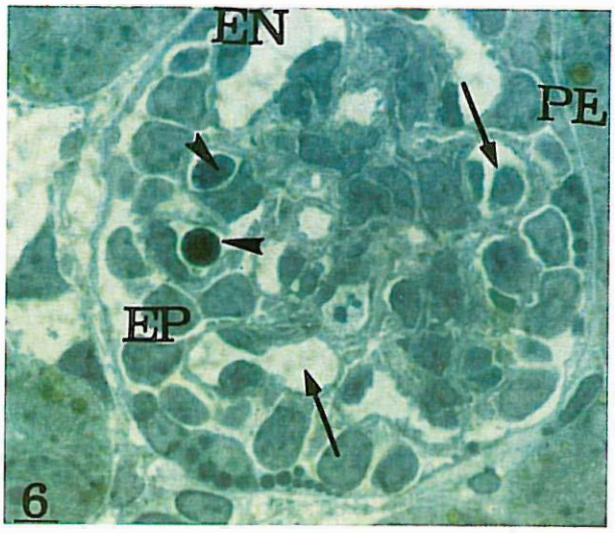

Fig. 6. Glomerular maturation stage: multiple capillary (long arrow) lumina containing red blood cells (arrow head) are evident. The parietal epithelium (PE), visceral epithelium (EP), and endothelium (EN) have become more flattened but have not yet reached their mature configuration.

Fig. 7. Electron micrograph of an early vesicle body comparable to the light micrograph shown in Fig. 1. Original magnification ×2500.

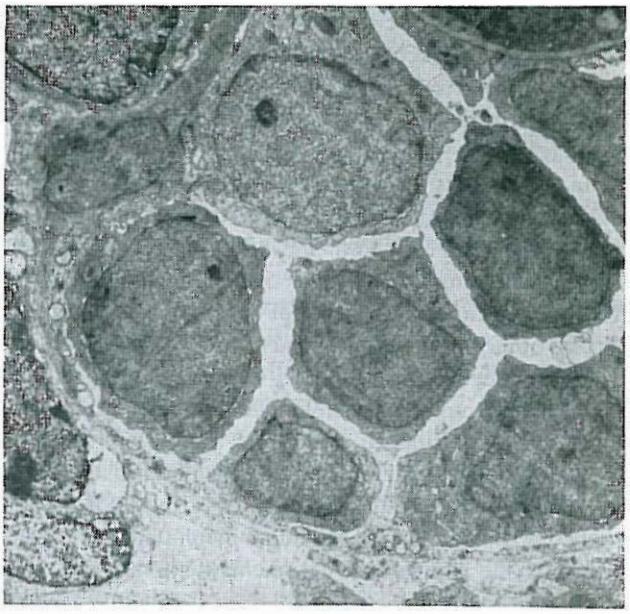

Fig. 8. Late S-shaped body stage; the parietal epithelium (PE) is thick and Bowman's space (BS) is beginning to form. The visceral epithelium (EP) appears to be several cells thick. Original magnification×4000.

condensate to form a spheroid mass. The mass consists of a cluster of columnar epithelial cells which form the lining of the vesicle lumen. As predicted, most of the glomeruli of 1 day old rats were in developmental stage I (Fig. 7).

S-shaped body stage (stage II)

Initially, a cleft appears which changes the vesicle to a comma-shaped body. Another cleft soon appears in the upper third of the mass. Thus, an S-shaped body consisting of three major parts develops. The upper portion situated close to the collecting tubules forms the distal tubules, and the proximal tubules develop from the middle part. The lower cleft is invaded by cells which will form the mesangium and endothelium. The earliest endothelial cells are cuboidal. The mesangium cells, like the endothelial cells, arise from the developing mesenchyme that invades the vesicle. Initially, before endothelial fenestrae develop and lumina are open, these cells are difficult to distinguish from the endothelium. The end of the tubule, the lowest limb of the coiled body, forms a relatively thin bilaminar disc, with its concavity

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toward the rest of the tubule. This disc, which forms the glomerulus, organizes into two layers. The outer layer of flattening epithelial cells below forms the capsule of the glomerulus and remains thin. The inner layer consists of columnar epithelial cells situated between the mesangial\ endothelial cleft and the lumen (Bowman's capsule) and is believed to form the podocytes (Fig. 8). Basement membrane material is laid down first in the cavity of the bilaminar disc and then within the expanding inner layer. On the other hand, the earliest precursors of the glomerular basement membrane are recognizable during the S-shaped body stage and consist of a loose and amorphous layer closely applied to the epithelial cell base and a corresponding layer closely applied to the endothelium.

In the kidney of 1 day old rats most of the glomeruli were in developmental stages I and II.

Capillary loop stage (stage III)

At the beginning of the capillary loop stage, the height of the visceral epithelium decreases. These cells are joined at their apices (to the parietal epithelial cells) by tight junctions. Subsequently, these junctions progressively migrate from the cell apex towards the basement membrane. Later, foot processes are formed by interdigitation of the epithelial cells.

In the capillary loop stage, endothelial cells proliferate and fenestrae appear, the cytoplasm becomes thinner, and the cells rearrange to form the lining capillary lumina. Later, after maturation of the endothelium, they can be identified along the periphery of the presumed filtrating surface of the glomerular capillary.

At the beginning, these two layers are separated by extracellular matrix. Mesenchymal cells, present previously, are gradually eliminated during the capillary loop stage with further differentiation. The layers become progressively thicker and more electron dense, and with fusion of the epithelial and endothelial basement membranes, the three layers of the glomerular basement membrane become indistinguishable (Figs. 9-10).

In kidneys of 3 day old rats, most of the glomeruli were in developmental stages II and III.

Stage of glomerular maturation (stage IV)

In this stage, endothelial fenestrae and foot processes increase in number. The number of interdigitations and frequency of slits between cells also increase (Figs. 11-12). The narrow intercellular (urinary) spaces, present in stages II and III, usually widen in stage IV. Therefore, the final differentiation of glomerular components with filtration slits and attenuated endothelium occurs in this stage.

As a result of fusion of endothelial and epithelial basement membranes, we have a three layer glomerular basement membrane (GBM) with increased thickness. The three layers are the lamina rara externa (LRE) adjacent to the visceral epithelial layer, the lamina densa (LD) in the middle, and the lamina rara interna (LRI) adjacent to the endothelial cells. In the kidneys of 5 day old, 7 day old and 9 day old rats, the number of stage IV glomeruli gradually increased.

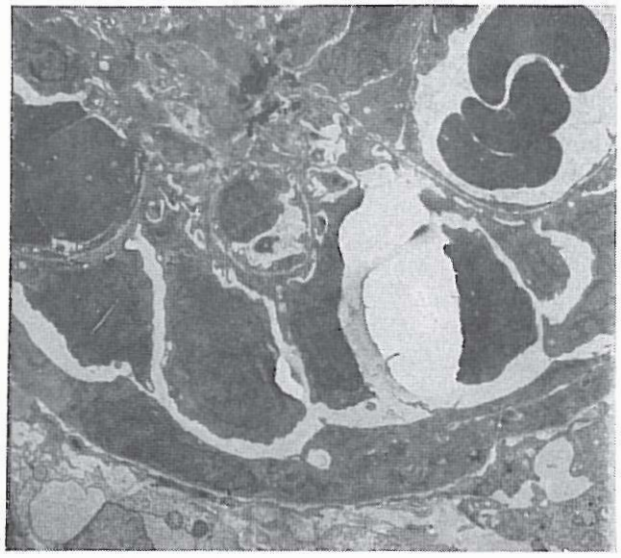

Fig. 9. Developing capillary loop stage: parietal epithelium (PE) is flattened and the visceral epithelium (EP) is differentiated. The endothelium (EN) has begun to flatten and several capillary loops are seen (long arrow). Original magnification ×2500.

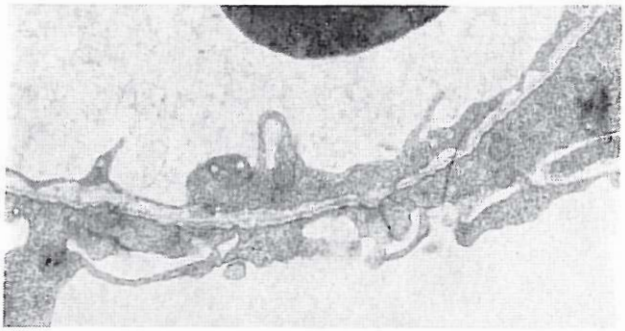

Fig. 10. Development of the glomerular basement membrane, capillary loop stage. The basement membrane matures into a structure containing a lamina rara interna (LRI), lamina densa (LD), and lamina rara externa (LRE). Here the lamina densa is narrower and the lamina rarae are wider than in the mature glomerulus. Note that endothelial fenestrae are not seen (short arrow). Foot processes are poorly differentiated
capillary lumen; EN: endothelial cell; N: nucleus. Original magnification ×5000.

DISCUSSION

Kidney development has been studied in the human

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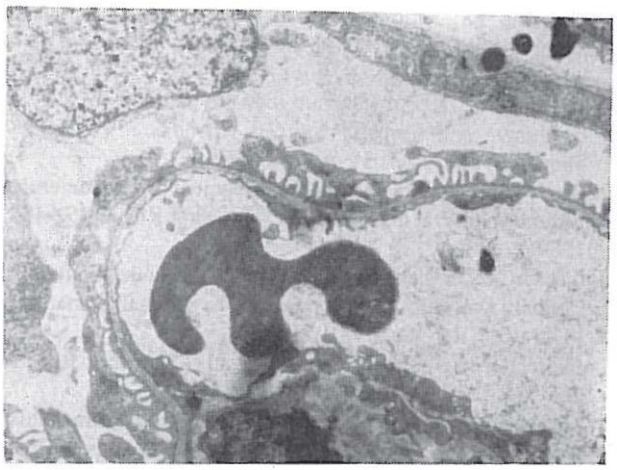

Fig. 11. Epithelial and endothelial differentiation; maturing glomerular stage. Epithelial differentiation (PO) has resulted in foot processes (FP) separated by filtration slits (FS). Note that endothelial fenestrae are visualized (short arrow). Original magnification ×6300.

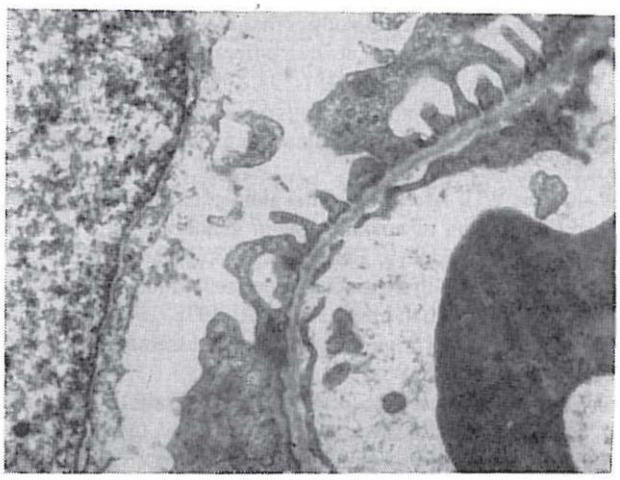

Fig. 12. Same as Fig. 11, original magnification × 16000. R: red blood cell; L: lumen; FS: filtration slits; FP: foot processes; PO: podocyte; BS: Bowman's space; arrow head: endothelial fenestrae.

being, the dog, the rabbit, the mouse, and the rat.[26]

Also, glomerular development has been studied in fetuses or in the newborn rodent kidney using light microscopy and transmission and scanning electron microscopy.[28]

Larsson has divided the developmental stages into vesicle, S-shaped body, capillary loop, maturing, and mature glomerulus. This form of staging was the basis of our investigation.

Zolani and Palkorits (1965) reported that the newborn glomeruli of the cortical zone are smaller than those of the cortico-medullary region.[26]

Similarly, we believe that a gradient in the degree of development exists so that more mature glomeruli are located toward the cortico-medullary junction while immature glomeruli are located toward the capsule. The glomerulus is organized into two layers, the visceral and the parietal epithelium. Formation of the vesicle lumen represents the presumptive Bowman's space.

At the beginning, the visceral epithelium has the morphology of pseudostratified epithelium without a defined apex or base.[1] In further development, the cells become columnar, and the nuclei become located against Bowman's space.[7,28] These cells separate at their apices but remain attached at their bases by modified occluding junctions.[25,27,28] These junctions may be modified in developing glomeruli for relocation along cell walls as structural differentiation and modification occur.[3]

After the process of migration, the epithelial cells cytoplasm appears to be continuous without foot processes or slits, on the outer aspect of the presumptive basement membrane.

During development, the cytoplasm of endothelial cells becomes thinner and endothelial fenestrae appear in increasing numbers. This is supported by many authors.[4,8,21,27]

During development of the visceral glomerular epithelium, foot process differentiation occurs after migration of junctions towards the base of the epithelium. This process correlates roughly with the later part of the capillary loop stage of development.[18,24,28]

We have classified glomeruli into four developmental stages. Okada and Morikawa (1988) reported that stages III and IV are functional in filtration, due to their well-differentiated GBM, but stages I and II are not functional because of poor GBM differentiation.[2,26]

Initially, before the slits open, the basement membrane is a loose layer with indistinct margins.[28] This basement membrane surrounds the capillaries within the glomerular anlage in the S-shaped body and is usually separate from the visceral epithelial cells. The basement membranes fuse and later form three layers.[9,10,11,22,23,27,29,30,31] Afterwards, the basement membrane increases in thickness and becomes a mature structure with a central dense layer or the lamina densa, the lamina rara interna adjoining the endothelium, and the lamina rara externa adjacent to the epithelium. This is supported by many reports.[6,15,19,27,30]

Osterby in 1965 reported that the epithelial and endothelial cell membranes both become part of a triple-layered membrane.[19] Thus, endothelial cells and epithelial cells both produce the GBM.[10,11,13]

In conclusion, the present findings indicate that the glomerular structure induces the growth and differentiation of the GBM in the postnatal rat kidney.

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