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Development of the glomerular mesangium

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Abstract. The development of the glomerular mesangium was studied in fetal and newborn rat kidneys by using a widefield electron microscope which can cover a whole glomerulus within one low-power viewfield. A three-dimensional observation of the immature glomeruli was done by performing ultrathin serial sectionings of the specimen for electron microscopy. Scanning electron microscopic observation of the developing glomeruli was also performed. The developmental distribution of contractile protein (actin) in mesangial cells and the main intrinsic component of the extracellular matrix protein (type IV collagen) of the mesangium were examined by immunohistological techniques. The widefield electron micrograph revealed a precise relationship between the mesangium and other components of the glomerulus. The results confirmed that the capillary extends into the S-shaped body from the surrounding vascular system at the initiation of nephronogenesis. The mesangial cells are always continuous to the vascular pericyte-smooth muscle cell system during the whole course of glomerular development and they participate in the subdivision of the capillary network during glomerulogenesis. Morphological findings and the changing distribution of intra- and extracellular proteins of the mesangium during development suggest that the mesangial cell differentiates from the primitive pericyte of the immature capillary.

Key words: Kidney development – Glumerulogenesis – Glomerular mesangium – Pericyte – Actin – Type IV collagen

Introduction

The glomerular mesangium was first described by Zimmermann [1] in 1933 as a branching framework of connective tissue supporting the glomerular capillaries. He considered the relationship between the mesangium and capillary loops as being similar to that between the mesentery and the small intestines, and postulated mesangial cells to be fibrocytes in the stalk area. Electron microscopic studies have revealed that the mesangial cell has characteristics similar to that of the vascular smooth muscle cell and the former is now designated as "a modified smooth muscle cell" [2]. However, the nature of the mesangium still remains somewhat obscure. The purpose of this study is to elucidate further the nature of the mesangial cell and its scaffold by analyzing the developmental process of glomerular formation.

Materials and methods

Twelve kidneys obtained from 15- to 20-day embryos, six kidneys from newborn and two kidneys from adult Wistar rats were used in the present investigation.

Electron microscopy. The cortical tissue fo the kidneys were cut into small blocks, fixed in 2.5% glutaraldehyde and postfixed in 1% osmium tetroxide. They were dehydrated according to standard procedures and embedded in epoxy resin (Epok 812). Ultrathin sections were mounted on 7-mm-diameter extra-large grid (Akashi Co. Ltd) and stained with uranyl acetate-lead citrate. The specimens were observed by a widefield electron microscope (Akashi LEM 2000), which allowed the examination of a whole glomerulus at any stage of maturity in one electron-micrographic viewfield.

Ultrathin serial sectioning study. A series of 240 ultrathin serial sections of a specimen from the subcapsular nephronogenic zone of a newborn rat was prepared and every 10th section was observed by the LEM 2000 widefield electron microscope.

The three-dimensional relationship of glomerular components was examined by electron micrographs of different aspects of the same glomerulus.

Scanning electron microscopy. Small pieces of the cortex of kidneys were processed for scanning electron microscopy with application of the frozen resin cracking method [3]. The specimens were examined in a Hitachi S430 scanning electron microscope.

Distribution of actin. Rabbit antiserum for the contractile protein "actin" from chicken gizzard was purchased from Transformation Research, Inc. Framingham, MA, USA). Distribution of actin in glomeruli of newborn and adult rat kidneys was examined by the ABC-immunoperoxidase method [4].

Distribution of type IV collagen. Anti-serum for type IV collagen was prepared from rabbit lens-capsule subsequent to immunization of sheep, according to a method described previously by a co-worker [5]. The distribution of this main component of the extracellular matrix protein in the glomerulus was examined by standard immunofluorescence techniques.

Results and discussion

It is generally accepted that the formation of the glomerulus corresponds with the appearance of capillary components within the cleft of the S-shaped body in the subcapsular zone of a developing kidney [6]. However, there are two main controversial hypotheses regarding the initiation of glomerulogenesis. One is the in situ theory, which states that capillaries are formed by primitive mesenchymal cells located in the S-shaped body and that the renal arterial supply joins the glomerulus after the formation of a vascular network [7–9]. The other theory states that a glomerular network is formed by the entrance of a capillary branch from a renal arterial vessel into the S-shaped body at the beginning of nephronogenesis [10–12]. Our observations of the vertically sectioned S-shaped body from the markedly congested kidney of a newborn rat show that the degree of congestion in the capillary of the S-shaped body is always parallel with that of the surrounding vessels in the interstitium. Our injection experiments of colloidal carbon particles via the heart of newborn rats revealed similar results: the capillaries of the S-shaped body filled immediately with carbon particles [13]. These observations demonstrate that the blood supply exists from the very beginning of glomerulogenesis.

Electron microscopic examination of serial sections confirms the continuity of the blood supply from arterial vessels to the capillaries of the S-shaped body (Fig. 1).

The electron micrographs reveal that the cleft of the S-shaped body is occupied by a capillary surrounded by primitive pericytes and lined with endothelial cells. The pericytes are known to be the precursors of vascular smooth muscle cells [14]. A developing glomerulus in the next stage of development reveals the continuous relationship between vascular pericytes and primitive mesangial cells, which proliferate mainly in the core portion of the developing glomerulus (Fig. 2). This relationship suggests that the mesangial cells originate from the pericytes.

The primitive glomerular capillary first forms large cavity-like dilations, giving rise to the initial lobulation of the glomerulus. In the rat, the initial lobulations are usually three or four in number. Then, a capillary network begins to form within the lobules, establishing the basic structural pattern of the glomerulus. The primitive mesangial cells continue to increase in number in the core portion of the glomerulus and so do, to a lesser extent, the extraglomerular vascular pericytes. Glomerular epithelial cells are also increasing in number and the basement membrane along the epithelia shows increasing concavity corresponding to the increase in surface area of the developing glomerulus. Although the most developed glomerulus of the newborn rat, situated deep in the cortex, remains somewhat immature in structure, the basic pattern is similar to that of the adult glomerulus (Fig. 3). At this stage, the mesangial cells are continuous with arteriolar smooth muscle cells through lacis cells of the vascular pole, which are considered to be differentiated from primitive pericytes.

Cross-sectional scanning electron micrographs performed at various stages of glomerular development reveal that the mesangial cells that proliferate into cavity-like lobules originate in the core portion of the glomerulus, as the glomerular capillary network is being formed (Fig. 4).

Electron microscopic observations also reveal the way in which mesangial cells participate in vascular subdivision. The core portion of the glomerulus expands due to the increase in the number of mesangial cells. The glomerular capillary surface area also increases, resulting in the formation of concavities. The mesangial cells extend their cytoplasm into these concavities of the capillary lumen, displacing the overlying endothelial cells and anchoring themselves into the basement membrane (Fig. 5). The paramesangial area is thus established. Endothelial cells also participate in the formation of the glomerular capillary network by proliferating and anchoring their cytoplasm into the other side of the capillary wall.

The distribution of intracellular contractile protein, actin, in the glomerulus differs with the developmental stage. Light micrographs of renal

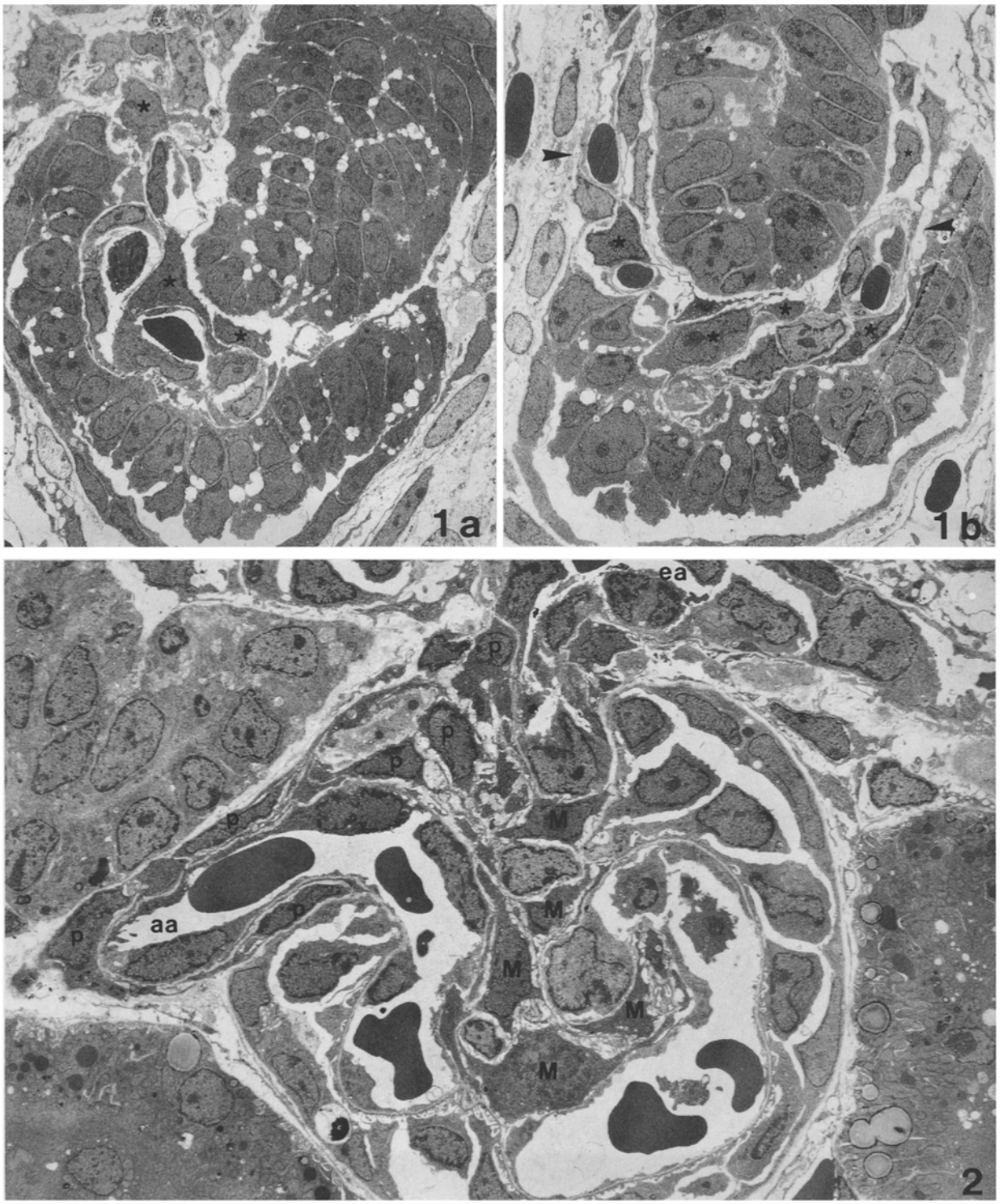


Fig. 1a, b. Different views of an S-shaped body. Capillary structure in the S-shaped body is continuous with the blood vessel outside the S-shaped body (*arrowheads* in **b**). *Asterisks* indicate primitive pericytes. EM, ×1,100

Fig. 2. A glomerulus in a later stage of the S-shaped body development. Note the continuity between the extraglomerular vascular pericytes (*P*) and the intraglomerular mesangial cells (*M*); *aa*, afferent arteriole; *ea*, efferent arteriole. EM, ×2,000

tissue from the newborn rat prepared by the ABC-immunoperoxidase method for detection of actin distribution reveal no staining even in the vascular wall of the most superficial S-shaped bodies. In the deeper layers, positive staining can be seen in the extraglomerular arteriolar wall. The primitive immature glomerulus shows very weakly positive staining in the mesangium. In the adult rat kidney, the reaction product is strongly positive in the arteriole and weakly positive in the JG-lacis area and in the mesangium (Fig. 6). These findings suggest that contractile protein in the mesangial cell is not as abundant as previously assumed [15, 16].

The distribution of extracellular matrix protein, type IV collagen, in the mesangium of the developing glomerulus differs from that of the adult glomerulus. In the newborn rat kidney, the staining indicating the presence of type IV collagen is almost negative in the S-shaped body, and weakly positive in the mesangial area of the primitive glomerulus in the subsequent cavity stage. In the adult glomerulus, type IV collagen is distributed mainly in the mesangium and less so in the glomerular basement membrane (Fig. 7).

These immunohistologic findings suggest that the mesangial cell differentiates from its precursor cell, the pericyte, whereas the extraglomerular vascular pericytes develop into vascular smooth muscle cells.

It is therefore appropriate to surmise that: (1) the glomerulus is formed by the extension of a ca-

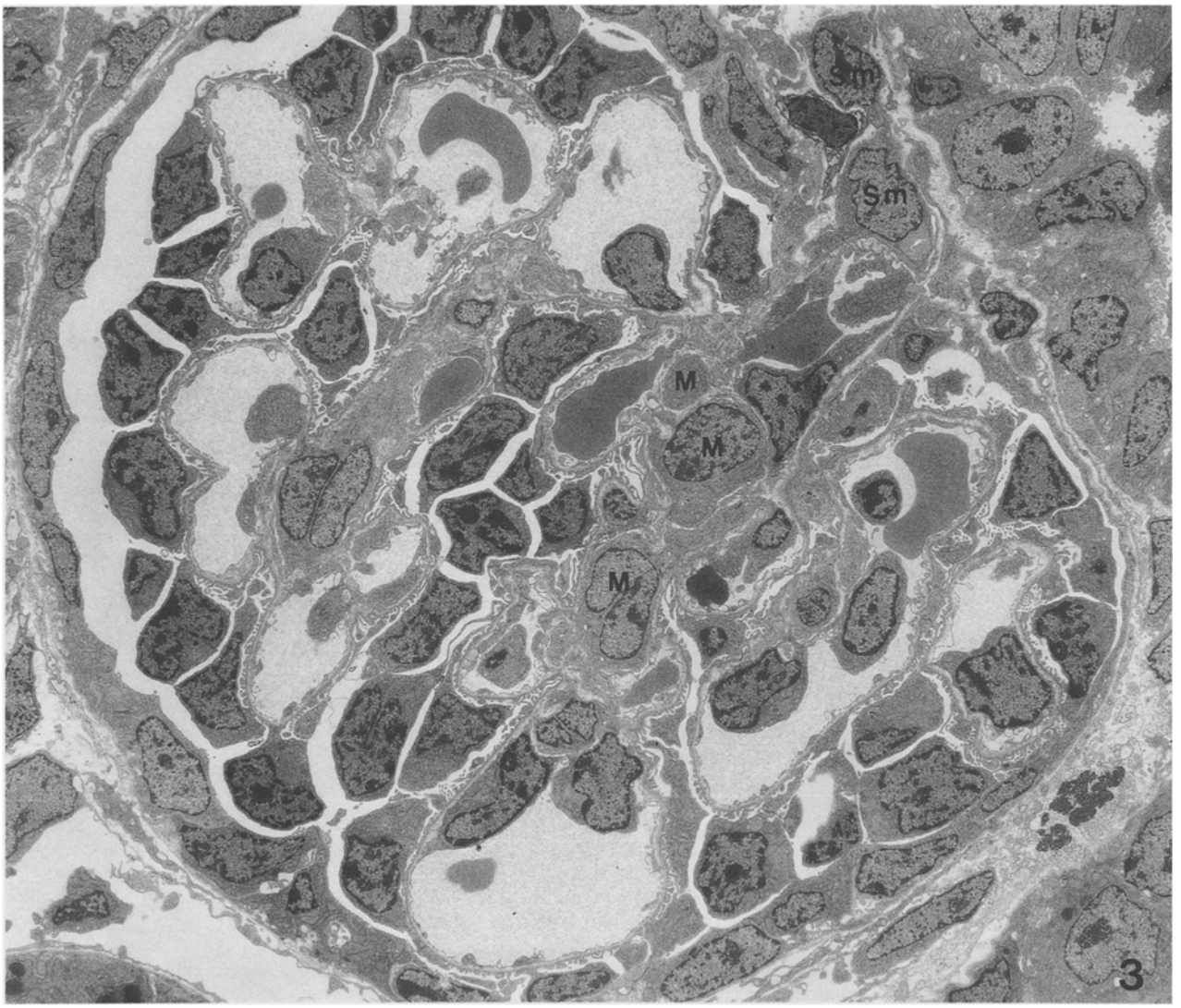


Fig. 3. In the newborn, the most developed glomeruli are found in the juxtamedullary region. Mesangial cells (*M*) are continuous with arteriolar smooth muscle cells (*Sm*). EM, ×2,500

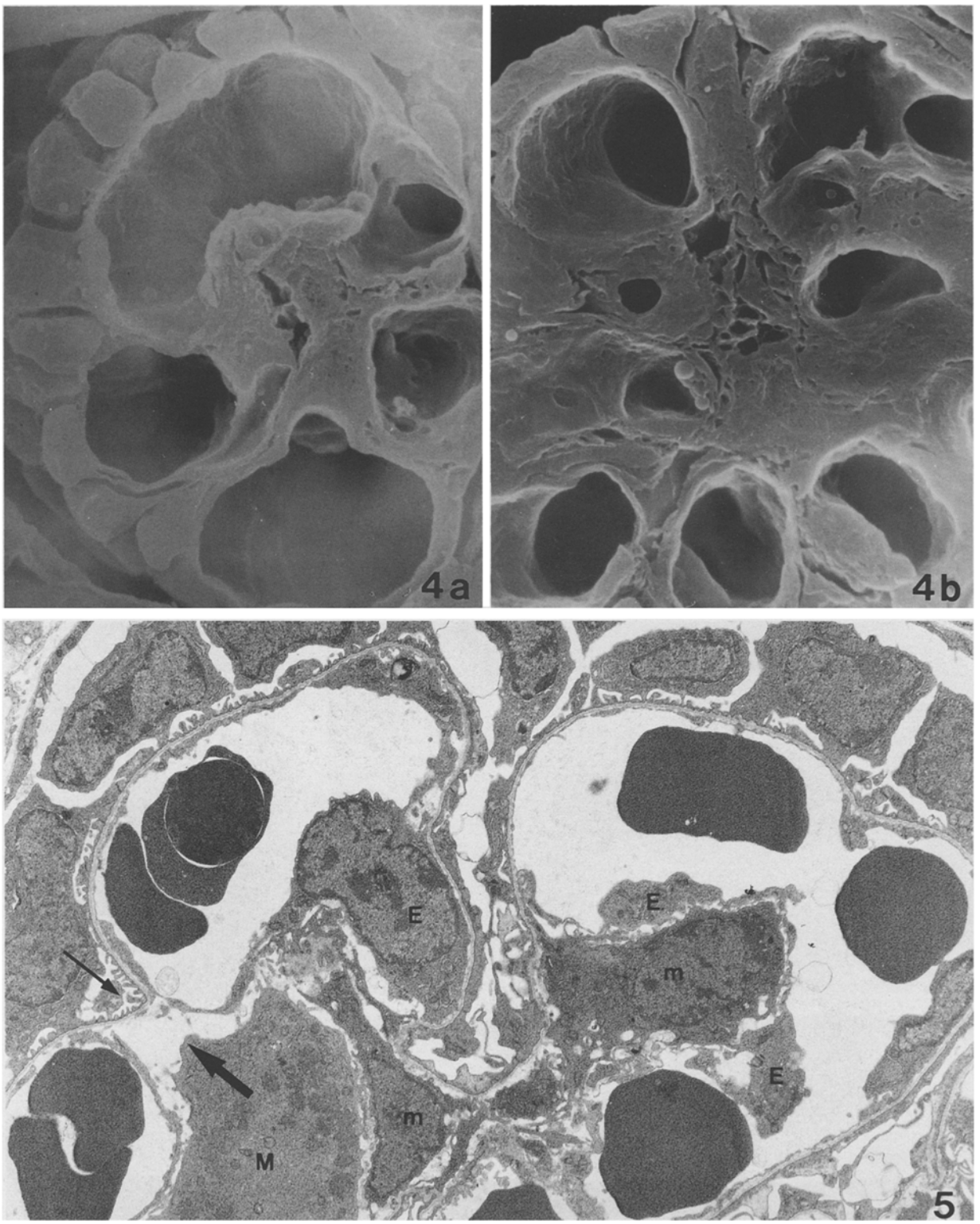
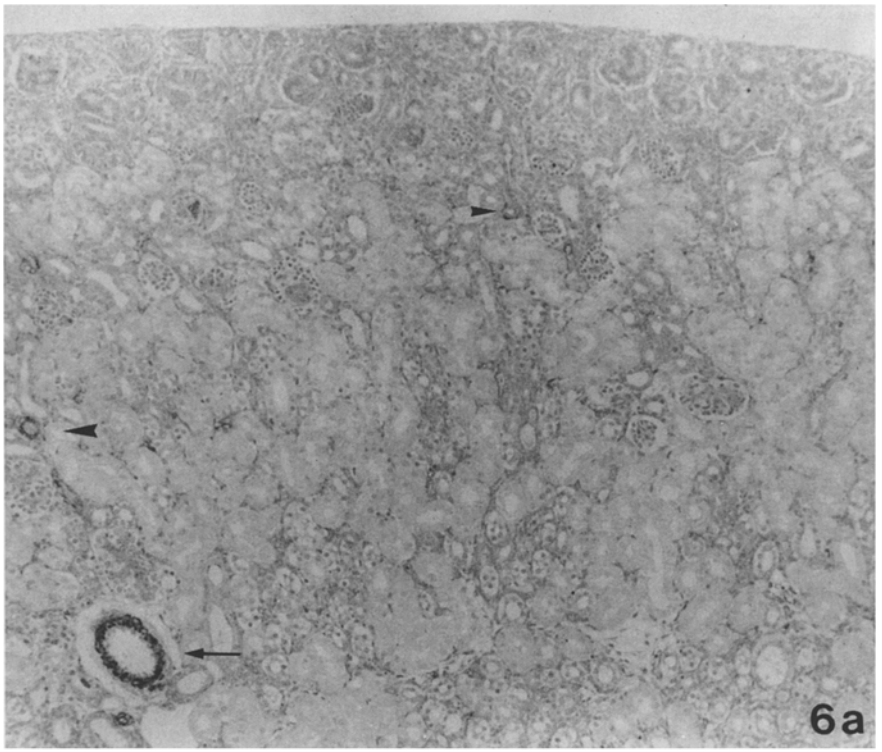


Fig. 4a, b. Scanning electron micrographs of developing glomeruli. **a** Primitive glomerular capillary with large cavity-like dilations forming the initial lobulation; SEM, ×3,000. **b** More developed glomerulus forming capillary network within the lobules; SEM, ×2,800

Fig. 5. Proliferating primitive mesangial cell (*M*) undergoing mitosis. The cytoplasm (*big arrow*) extends into the concavity of the capillary loop (*small arrow*); *m*, other mesangial cell; *E*, endothelial cell. EM, ×3,600



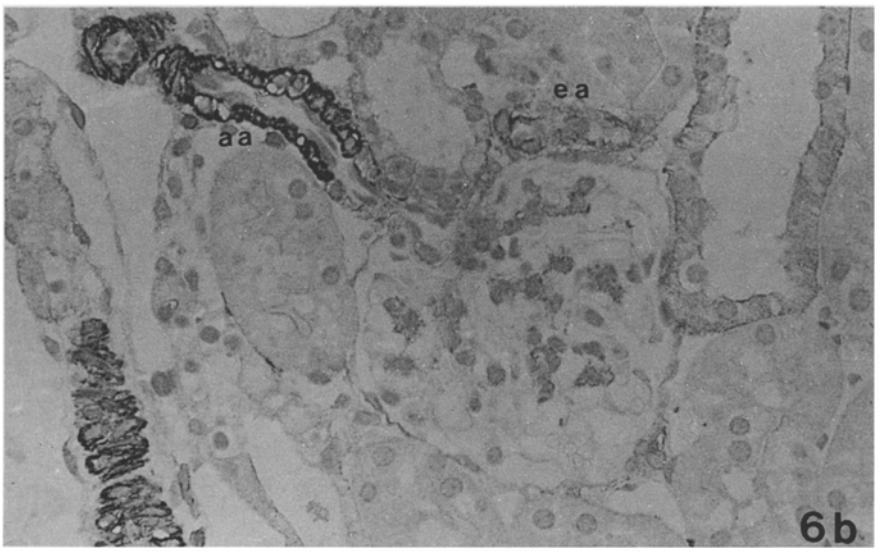


Fig. 6a, b. The distribution of actin (ABC-immunoperoxidase method). **a** Newborn rat kidney at low magnification. No staining is seen in the S-shaped body and in the vascular wall outside. Very weakly positive staining in the small arteries (*arrowheads*) and strongly positive in the large arteries situated in the juxtamedullary region (*arrow*). LM, × 120. **b** Adult rat glomerulus. The reaction product is strongly positive in the afferent arteriole (*aa*), weakly positive in the efferent arteriole (*ea*), and very faint in the JG-lacis area and the mesangium. LM. × 560

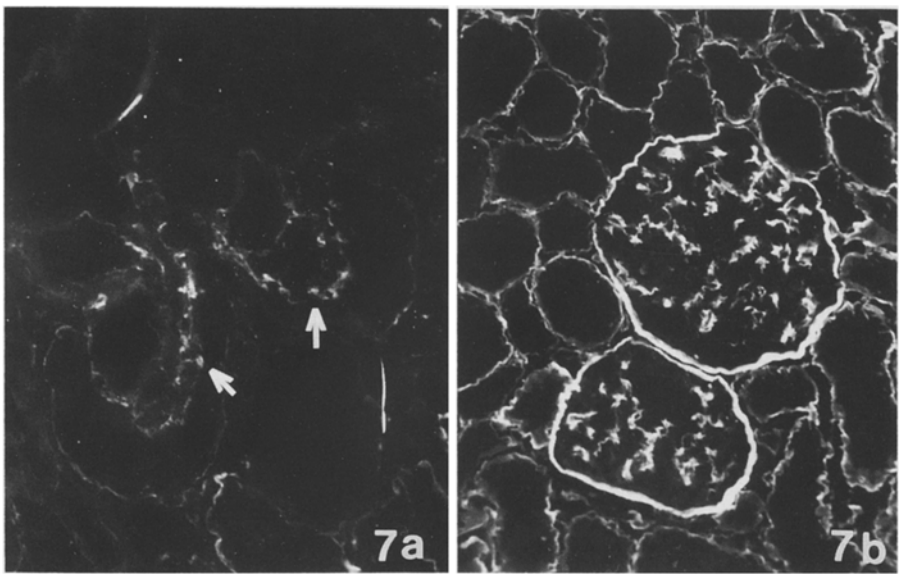


Fig. 7a, b. Immunofluorescence micrographs showing the distribution of type IV collagen. **a** Primitive glomeruli of the cavity stage in newborn rat kidney. Very weakly positive fluorescence in the mesangial area (*arrow*). FM, × 400. **b** Mature glomeruli in the adult rat kidney. Fluorescence is strongly positive in the mesangium and less positive in the glomerular basement membrane. FM, × 400

pillary into the S-shaped body at the initiation of nephronogenesis; (2) there is a continuity between mesangial cells and the vascular pericyte-smooth muscle cells during the entire course of glomerular development; (3) the mesangial cells participate in the subdivision of the capillary network during glomerulogenesis; (4) the mesangial cells differentiate from the primitive pericytes of the immature capillary.

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