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SEM Observation of Mesangial Cells in the Rat Kidney by NaOH Maceration Combined with Freeze Cracking

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Summary. **A scanning electron microscopic study of glomerular mesangial cells was conducted in the rat kidney after freeze cracking and subsequent removal of the glomerular basement membrane and mesangial matrix with NaOH maceration. Mesangial cells extend long branching processes in accord with previous light microscopic observation by Zimmermann, and further reveal numerous short microvilli and fine grooves on their surfaces. Mesangial cells interdigitate with each other by the microvilli, forming an intercellular labyrinth. Mesangial processes are closely attached to the basal surface of the capillary endothelium. Sometimes the processes encircle the capillary for more than half of its circumference, suggesting that they might effectively regulate its caliber. Mesangial cells frequently protrude small cytoplasmic blebs into the capillary lumen, entering into direct contact with circulating blood. Goormaghtigh's cells resemble the intraglomerular mesangial cells, extending branching processes covered with microvilli and revealing labyrinthine intercellular spaces formed by the microvilli. The intercellular labyrinth among the mesangial cells and that among Goormaghtigh's cells connect with each other at the hilus, providing a channel leading from the periphery of the glomerulus through the hilus to the interstitial space outside the glomerulus.**

INTRODUCTION

Mesangial cells were first identified light-microscopically by Zimmermann[1,2] as connective tissue cells in the renal glomerulus. He described the cells as extending long branching processes in the whole mesangium, and as anastomosing with each other by the processes. However, later light microscopists[3] could not confirm the shape of the cells reported by Zimmermann. In early studies by transmission electron microscopy (TEM), Yamada[4] recognized intercapillary cells, or mesangial cells enclosed in a basement membrane-like matrix, while most investigators denied their existence.[5-7] Subsequently, Farquhar and Palade[8] clearly differentiated mesangial cells by TEM, and described them as displaying numerous profiles of cytoplasmic processes. However, it has been difficult to realize their three-dimensional structure from studies of tissue sections. Actually, many current textbooks of histology depict the cells as rather round forms devoid of processes, and as loosely dispersed in the mesangial matrix.[9-12]

According to Zimmermann, mesangial cells sometimes encircled glomerular capillaries with their processes, resembling pericytes. However, previous TEM studies showed that only a small portion of the circumference of each capillary was in contact with the mesangial cell. On the other hand, it has been assumed that mesangial cells regulate the blood flow in the glomerulus, as the cells were found in TEM observations to gradually transform into vascular smooth muscle cells at the glomerular hilus.[4,13] Becker[14] immunohistochemically demonstrated that mesangial cells contained actomyosin, suggesting their contractility. More information is required concerning the relation of mesangial cells to capillaries in analysing the effect of their contraction.

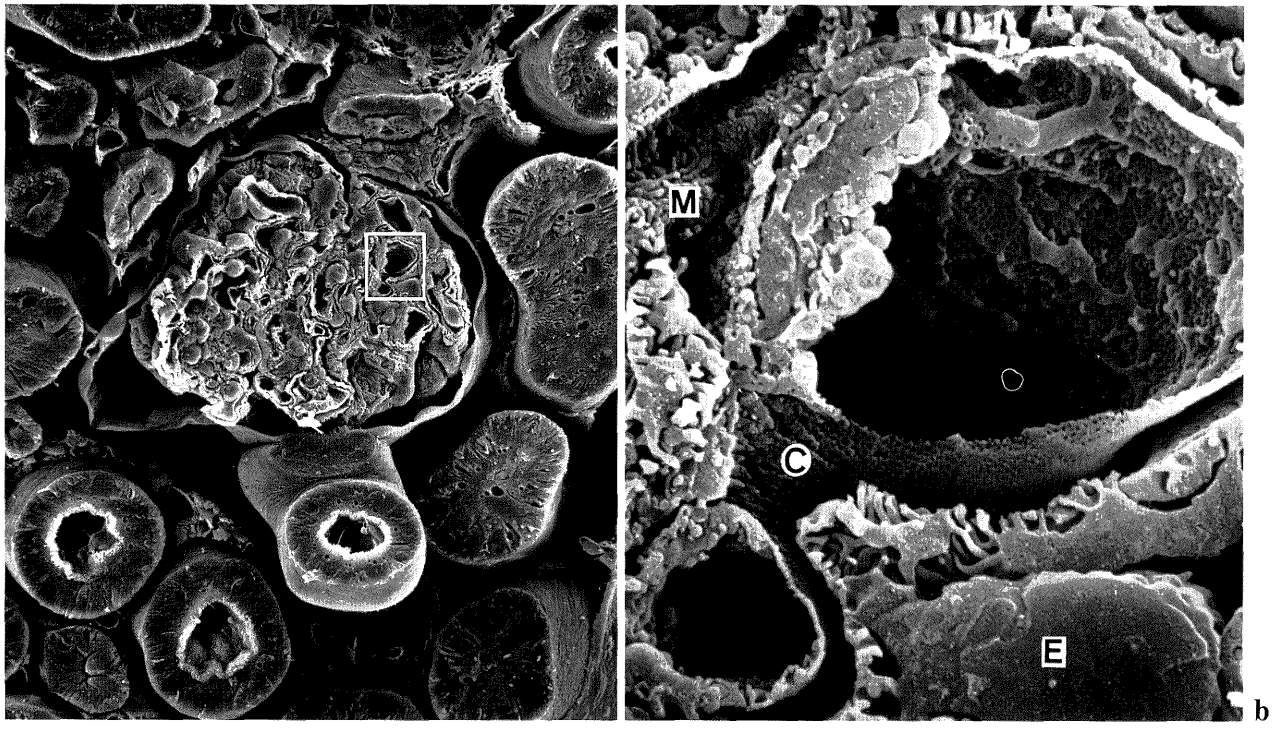
Zimmermann[2] showed that the processes of mesangial cells often penetrated the capillary lumen, where they formed "Intrakapillarhöckerchen", or small cytoplasmic mounds. Although his finding was corroborated by some TEM studies,[4,8,15] it does not seem to be widely accepted knowledge, nor neither has its physiological significance been analysed. Cook et al.[16] demonstrated that the intraluminal processes became larger and more numerous after ischemic injuries, suggesting that such processes were arti-

facts. Scanning electron microscopy (SEM) will be useful in clarifying the nature and distribution of the intraluminal processes of mesangial cells.

Concerning the SEM of mesangial cells, we could find only one report by Jones,[17] who used enzyme digestion to remove the basement membrane-like matrix obscuring the cells. We previously demonstrated that maceration with NaOH has a potent effect in removing basement membranes of epithelia and vessels in various tissues.[18-20] The present study applies this method[21] to the clarification of the above-mentioned undetermined features of the mesangial cells.

MATERIALS AND METHODS

Adult male Wistar rats weighing 180-220 g were examined in this study. All animals were anesthetized with sodium pentobarbital and perfused through the ascending aorta with Lock's solution saturated with O_2, and subsequently, with 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.3. The renal cortex was excised and cut into elongated cuboids, measuring $1.5 \times 1.5 \times 3$ mm in size. The tissue pieces were immersed in the same fixative overnight, and rinsed in 0.1 M phosphate buffer (pH 7.3). The fixed specimens were transferred through 30% aqueous solution of dimethyl sulfoxide (DMSO) into 60% DMSO, and then cracked in liquid N_2. The cracked specimens were rinsed in 0.1 M phosphate buffer, and placed in 6N NaOH for 10 min at 60℃. After the NaOH maceration, the tissue was rinsed in 0.01 M phosphate buffer (pH 7.3) containing Tween 20 at 0.05%, and conductive-stained by the tannin-osmium method by Murakami.[22] The osmicated tissue blocks were dehydrated through a graded series of ethanol, transferred to isoamyl acetate and critical point-dried using liquid CO_2. The dried specimens were evaporation-coated with gold-palladium and examined in a Hitachi S-450 LB scanning electron microscope at an accelerating voltage of 10 kV.



a b

Fig. 1. **a.** SEM survey view of the rat renal cortex craked in liquid N_2 and then macerated with NaOH. A glomerulus and urinary tubules are clearly sectioned. **b.** Higher magnification of the square in Fig. 1a. The glomerular basement membrane and mesangial matrix are completely removed. A mesangial cell (*M*) reveals a free surface covered with microvilli. *C* basal surface of a capillary, *E* epithelium. a ×650, b ×9,500

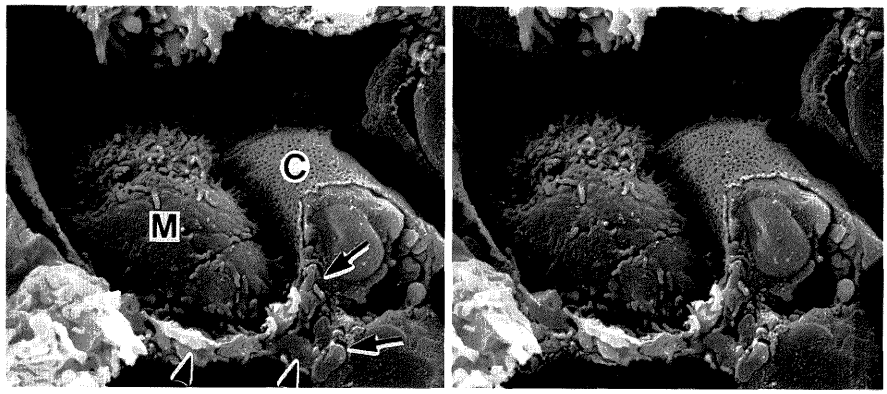


Fig. 2. A mesangial cell (*M*) extending cytoplasmic processes (*arrowheads*) (in stereo). *Arrows* indicate interdigitations between mesangial processes and capillary endothelium. *C* blood capillary. ×6,000

RESULTS

The renal cortex cracked in liquid N_2 and then macerated with NaOH appeared, at low magnification, similar to specimens processed by the conventional freeze-cracking method, displaying clearly-sectioned glomeruli and tubules (Fig. 1a). However, a closer view revealed that the former specimens exclusively contained cellular elements, the connective tissue fibers and matrix being completely removed. The glomeruli were devoid of a basement membrane and mesangial matrix. In places, the glomerular epithelium was detached from the specimen, exposing the smooth basal aspect of capillaries and free surfaces of mesangial cells covered with microvilli (Fig. 1b).

The mesangial cell possessed a round cell body, measuring 4-5 μm in diameter, and long branching processes, measuring 0.5-1.5 μm in thickness (Figs. 2, 3). It was difficult to pursue the entire length of the processes as they took complex courses among capillaries. Some processes were estimated to be 5 μm in length. The processes of mesangial cells were densely covered with short microvilli and fine grooves, while their cell bodies were rather smooth. The microvilli were mostly tapered and tilted in random directions. The grooves meandered in complex courses among the microvilli.

Mesangial processes deriving from the same cell or other cells interdigitated with each other by the microvilli, obscuring the outline of each cell (Figs. 3, 4b). The microvilli formed a labyrinthine channel in the subepithelial and intercellular spaces in the mesangium. The mesangial region was completely filled with mesangial cells and the labyrinth of their microvilli, leaving no acellular spaces.

Some long processes of mesangial cells coursed longitudinally to glomerular capillaries, extending numerous short branches toward them. The branches were intimately attached to the capillaries, and expanded membraneously along their basal surfaces (Fig. 3). Other processes were oriented circulary to the capillaries, embracing more than a half of their circumference (Fig. 3b).

Mesangial cells and endothelial cells were interdigitated with each other in the area of contact. The former extended small blebs, measuring 0.3-0.7 μm in diameter, while the latter showed corresponding indentations (Fig. 2). Some blebs of mesangial cells were penetrated through the endothelium, exposing their smooth surface into the lumen (Fig. 4a). The basal surface of the endothelium often displayed short microvilli in the area facing the mesangium (Fig. 4b, c).

Goormaghtigh's cells at the vascular pole resembled mesangial cells in the glomerulus, in that they extended branching processes covered with short microvilli (Fig. 5). These microvilli filled the intercellular spaces, forming labyrinthine channels similar to those seen among mesangial cells. The labyrinths of both types of cells were connected at the glomerular hilus, thus establishing an intercellular channel system leading from the mesangial region through the hilus to the interstitial space outside the glomerulus. At the proximal portions of the afferent and efferent arterioles of the glomerulus, Goormaghtigh's cells gradually gained in thickness and lost fine branches from the processes. Finally, they transformed into fusiform smooth muscle cells encircling the arterioles.

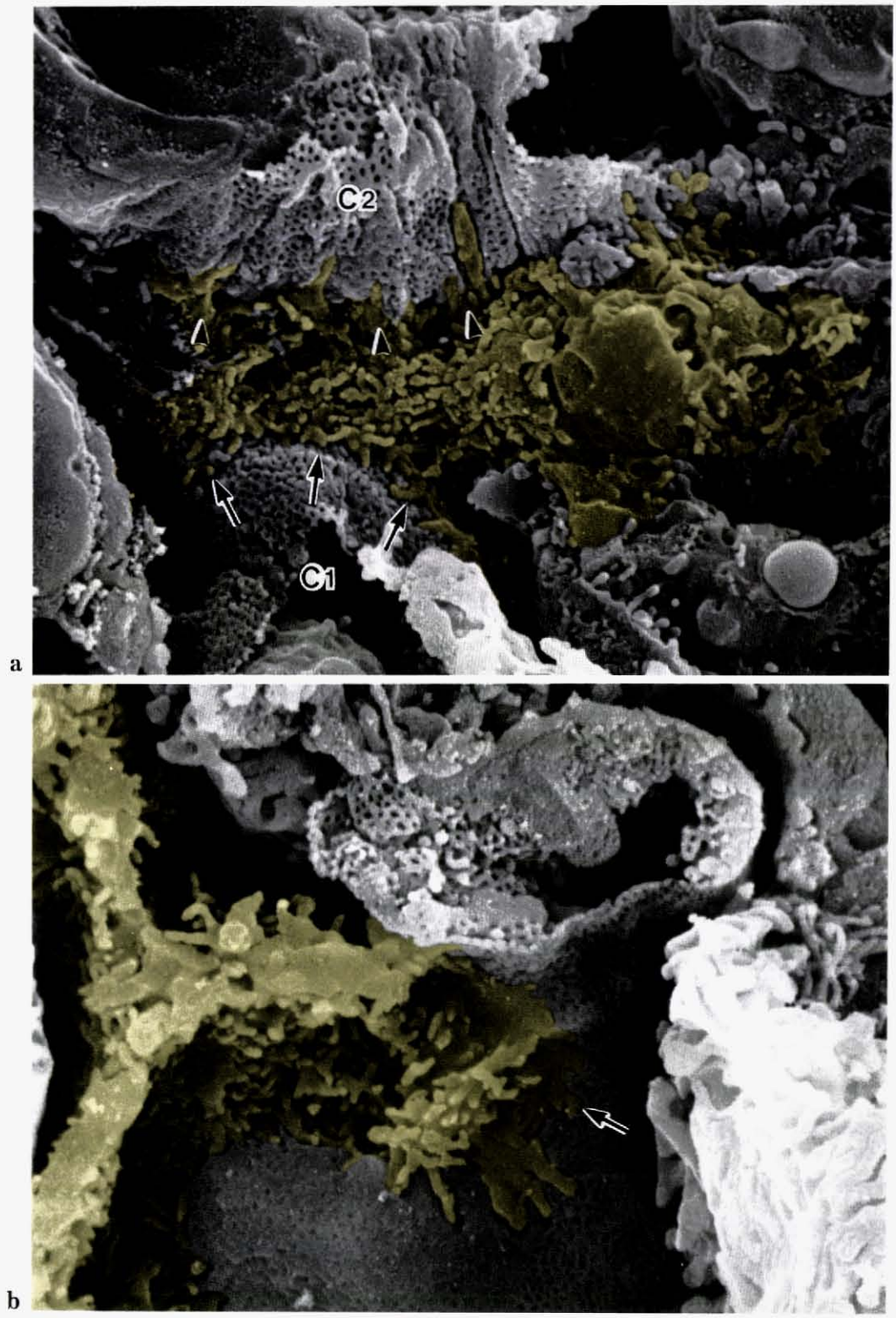


Fig. 3. **a.** A mesangial cell (colored in yellow) encircling a capillary (C_1) with its long process (*arrows*). The mesangial process extends short side branches (*arrowheads*) toward another capillary (C_2). **b.** Processes of mesangial cells (colored in yellow) expanding membraneously along the basal surface of a capillary (*arrow*). a and b ×15,000

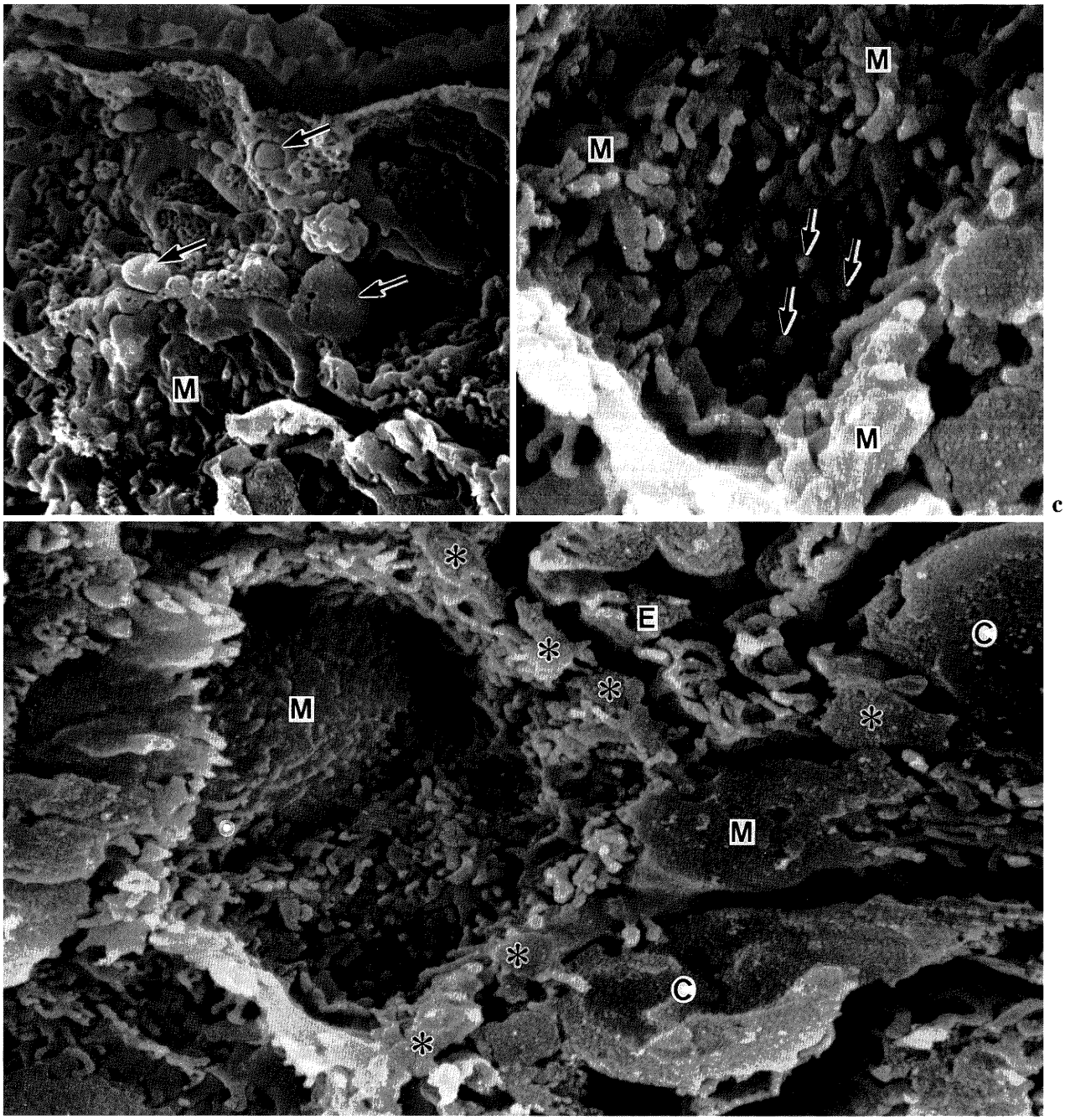


Fig. 4. **a.** Blebs of a mesangial cell (*M*) protruding into a capillary lumen (*arrows*). **b.** The mesangial region is filled with cell bodies of mesangial cells (*M*) and their processes (*asterisks*), the latter of which interdigitate with each other by microvilli. *C* capillary endothelium, *E* glomerular epithelium. **c.** A basal surface of a capillary endothelium revealing short microvilli (*arrows*) at the interface with mesangial cells (*M*). a ×12,000, b ×11,000, c ×19,000

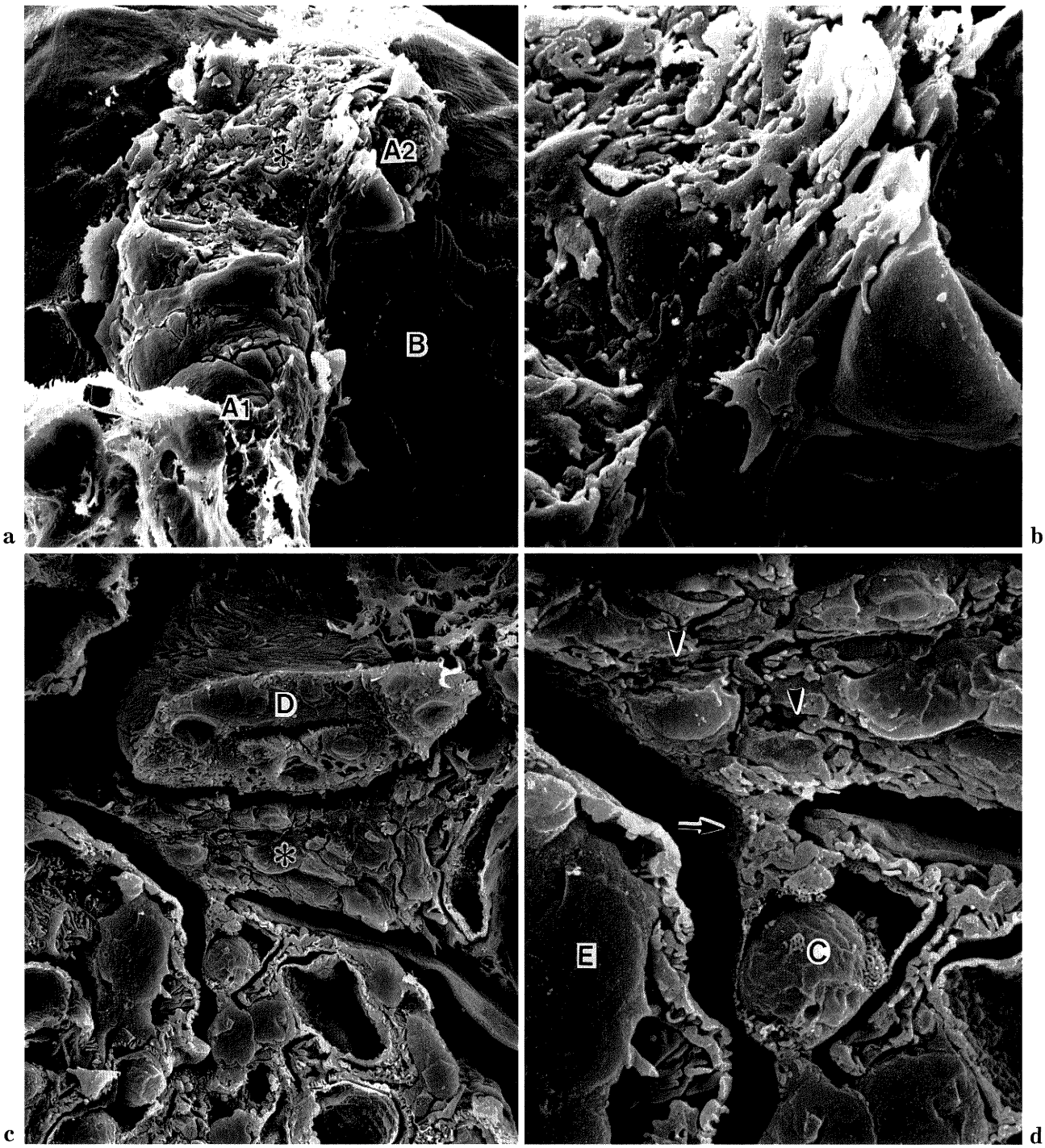


Fig. 5. Goormaghtigh's cells (*asterisks*). **a.** Smooth muscle cells embracing an afferent arteriole (A_1) gradually transform into Goormaghtigh's cells. A_2 lumen of an efferent arteriole, *B* basal surface of a Bowman's capsule. **b.** Higher magnification of the Goormaghtigh's cells in a. The cells interdigitate with each other by microvilli. **c.** Cross section of a vascular pole of a glomerulus. *D* distal tubule. **d.** Higher magnification of the central area in c. Labyrinthine channels (*arrowheads*) among Goormaghtigh's cells connect with those among intraglomerular mesangial cells at the hilus (*arrow*). *C* blood capillary , *E* glomerular epithelium. a ×1,400, b ×5,000, c ×2,500, d ×6,300

DISCUSSION

Jones[17] reported a SEM observation of the free surfaces of mesangial cells and the basal aspects of epithelial and endothelial cells in the renal glomerulus after removing the basement membrane and mesangial matrix by serial treatments with trypsin, pepsin and a type of bacterial protease. However, his method has not been pursued by other investigators, probably because it required 4 days of complicated digestion procedure and repeated centrifugation in order to collect the flocculent sediment containing digested glomeruli and tubuli. In the present study, on the other hand, maceration with NaOH disintegrated the intercellular matrices in the glomerulus within 10 min. In addition, the topographical relationships between cellular elements were found to remain intact after maceration procedure.

The present observations on the shapes of mesangial cells are in accord with those by Zimmermann,[1,2] with regard to their long branching processes. The microvilli of mesangial cells have been reported by previous TEM and SEM studies.[8,17,23,24] The present study further clarified that mesangial cells possess extremely numerous microvilli and that the cells interdigitate with each other by the microvilli to form an intercellular labyrinth. In this context, it is worthy to note that Zimmermann[1,2] depicted mesangial cells as being surrounded by a certain amount of "Interzellularsubstanz" stained deep blue with azan. The present study showed that the "Interzellularsubstanz" or the mesangial matrix was dispersed among the microvilli instead of forming a solid body.

Zimmermann[1,2] described mesangial processes as sometimes encircling a major portion of the capillary circumference. Previous TEM observations[8,13,25] could not recognize the circular processes, while the SEM study by Jones[17] showed that the mesangial cells close to the glomerular hilus tended to encircle the large hilar capillaries. The present SEM observations evidenced that the mesangial cells in the periphery of the glomerulus encircled capillaries.

It has long been assumed that mesangial cells might be contractile in function, thus regulating the glomerular blood flow.[13,14,26] Sakai and Kriz,[25] who observed fibrous links between mesangial cells and the glomerular basement membrane by TEM, conceived that mesangial cells might pull the basement membrane and regulate the capillary diameter. However, it seems reasonable to assume that the circular processes of mesangial cells recognized by Zimmermann[1,2] and us constrict the capillary lumen more directly and more effectively.

The intracapillary processes of mesangial cells have been reported by Zimmermann[2] and by numerous TEM investigators.[4,8,15] Cook et al.[16] suggested that such processes might result from an ischemic injury. However, the present SEM observation showed a considerable number of intracapillary processes in the specimens perfused with O_2-saturated solution, suggesting that they are normal, regular structures playing certain roles under physiological conditions. Some investigators assumed that they might be involved in monitoring capillary pressure or detecting circulating hormones such as angiotensin II or prostaglandins.[15]

Previous studies reported that colloidal particles or macromolecules entered the intercellular spaces between mesangial cells after intravenous administration.[27-29] The tracer particles were shown to move gradually from the periphery of the glomerulus through the mesangial stalk to the juxtaglomerular zone and finally to disappear from the glomerulus. The present SEM observation suggests that the continuous intercellular labyrinth formed by mesangial and Goormaghtigh's cells provides the pathway for the particles. The bush of mesangial microvilli may influence the passage of particles, entrapping larger ones. Previous tracer studies showed that small iron-dextran particles took 8 h to reach maximal concentrations in the juxtaglomerular matrix after administration,[29] whereas large carbon particles took weeks to reach maximal concentrations.[28]

Farquhar and Palade[8] showed by TEM that mesangial cells actively incorporated ferritin administered intravenously. In their study, ferritin molecules were found in round indentations and small vesicles in mesangial cells as well as in the intercellular spaces at 4 to 24 h after administration, being cleared from the mesangial area in 4 days. They demonstrated numerous large vesicles which varied in diameter from 0.2 to 0.7 μm, and several small vesicles with a diameter of about 0.1 μm, both containing ferritin. However, we are of the opinion that most tracer particles in the indentations and vesicles of mesangial cells are not in the process of uptake but on their way of passage between the cells. In fact, the present SEM observation demonstrated that mesangial cells were covered with complex fine grooves, which present images resembling the large intracelluar vesicles in sections. On the other hand, the small vesicles actually appear to incorporate ferritin by pinocytosis.

The present study showed that our NaOH maceration technique combined with freeze cracking is a

useful method for the SEM observation of mesangial cells. This method will help toward understanding physiological roles and pathological alterations of the cells.

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