Stratified Laminae Fenestratae (Alveolus Fenestratus Endothelialis) in the Glomerular Capillaries of the Mouse Kidney

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Summary. The fine structure of the stratified lamina fenestrata of the mouse glomerulus was described in detail in both transmission and scanning electron microscopy. We propose to name the extremely developed structure of the stratified lamina fenestrata as the alveolus endothelialis. It consists of numerous small irregular spaces partitioned by thin cytoplasmic processes. Individual spaces communicate with each other through the fenestra surrounded by the cytoplasmic partitions. This structure occurs not only on the basal lamina but also on the perikaryal portion of the endothelium. The constant appearance of the alveolus fenestratus without association of any pathological or degenerative changes suggests that this structure represents a certain general and fundamental feature of the fenestrated endothelial cell. Possible mechanisms for the genesis of this structure are briefly discussed.

It is generally believed that the lamina or areola fenestrata is the attenuated portion of the endothelium perforated by numerous round pores and underlined directly with a basal lamina (Ham and Cormack, 1979). This structure is assumed to represent a 'small pore system' proposed by physiologists in relation to the transendothelial transport mechanism (Landis and Pappenheimer, 1963). However, the occasional occurrence of the stratified lamina fenestrata has been reported in the glomerular capillary of both the rat and rabbit kidney (Wolff and Merker, 1966; Yoshinari and Fujita, 1982; Fujita and Miyoshi, 1985), and in the sinusoidal endothelium of the liver and adrenal cortex of monkeys and guinea pigs (Nozaki and Miyoshi, 1984; Ohata et al., 1984). Furthermore, the lamina fenestrata has been described as occurring even on the thickened perikaryal portions of the glomerular endothelium of the kidney, designated as pored domes (Yoshinari and Fujita, 1982). If this structure is the constant feature of the endothelium, a question is raised on the current view about the functional significance of the fenestra only from the aspect of transendothelial transport. However, no information has been available concerning the frequency of the occurrence and the extent of the stratified lamina fenestrata; therefore little attention has so far been paid to such a stratified lamina fenestrata.

During the study of the fine structure of the normal mouse kidney, we often encountered such stratified laminae fenestratae, some of which were extensively developed. In this report, we describe structural details of the stratified lamina fenestrata
in the transmission as well as scanning electron microscopy, and the constant appearance of this structure is stressed. The functional significance of the structure is also briefly discussed.

MATERIALS AND METHODS

Young male albino dd-mice weighing 20–25 g and showing no pathological symptoms were used in this study. The animals were anesthetized with Nembutal (35 mg/kg) and perfused for 10 min with 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, via the ascending aorta. The kidneys were dissected out, minced into small pieces and immersed in the same fixative for an additional 2 hrs at 4°C. The tissue blocks were postfixed with 1% OsO₄ in the same buffer for 2 hrs. After en-bloc staining with 1% uranyl acetate, the tissue blocks were dehydrated in a graded series of ethanol and embedded in Epon 812. Random ultrathin sections and a short series of serial ultrathin sections were made and observed in a Hitachi H-700 electron microscope after staining with uranyl acetate and lead citrate. For the scanning electron microscopy, the tissue blocks, after fixation with 2.5% glutaraldehyde, were treated with the tannin-osmium impregnation method by Murakami (1974). After dehydration in a graded series of ethanol, the tissue blocks were frozen by liquid nitrogen, and cracked with a chisel and hammer (Tokunaga et al., 1974). The cracked specimens were im-

Fig. 1. A transmission electron micrograph of the mouse glomerulus showing several alveoli fenestrati (*). Note the excellent preservation of this material and the absence of any pathological or degenerative changes. * endothelial cells, P podocytes. ×9,600
mersed in isoamyl acetate and then dried by the critical point process from CO₂. The dried specimens were evaporated with gold-palladium and observed in Hitachi HFS-2 scanning electron microscope with a field emission gun.

RESULTS

The structure of a typical, single-layered lamina or areola fenestrata of the glomerulus has been fully described by several authors using both transmission and scanning electron microscopy (Yamada, 1955; Fujita et al., 1976; Fujita and Miyoshi, 1985). The present findings were in good accordance with those already described.

In addition to the typical lamina fenestrata, focal stratified ones were frequently found in the capillary endothelium of this species. They occurred not only abutting on the basal lamina but frequently were seen capping thickened perikaryal portions (Fig. 1). Some of the stratified laminae fenestratae were enormously developed both in their extension (up to 3 μm) and height (up to 2 μm) and appeared in sections as an alveolar structure which consists of numerous small, irregular spaces, 100 to 500 nm in diameter, partitioned by thin cytoplasmic processes, 50 to 60 nm in thickness. The scanning electron microscopy and the three-dimensional observations using serial section analysis revealed that individual spaces communicated with each other through fenestrae, 60 to 100 nm in diameter, surrounded by cytoplasmic partitions (Fig. 2, 3). Round profiles demarcated by the plasma membrane representing transversely sectioned cytoplasmic partitions were frequently seen at the cross-point of the cytoplasmic partitions. We propose to name the extremely developed structure of the stratified lamina fenestrata as the “alveolus fenestratus endothelialis.” In contrast to this structure in a section, an en-face view of the single layered lamina fenestrata is shown in Figure 4, in which the rounded or oval profiles of fenestrae demarcated by the plasma membrane are embedded in the endothelial cytoplasm. Therefore, it is easy to discriminate these two different structures. The luminal surface of the alveolus

![Fig. 2. Higher magnification electron-micrograph of the alveolus fenestratus. The structure is extensively developed not only on the basal lamina (*) but also on the perikaryal portion of the endothelial cell. L capillary lumen, N nucleus of endothelial cell, P podocyte. ×19,000](image-url)
Fig. 3. A scanning electron micrograph showing a luminal view and cut-face of an alveolus fenestratus of the mouse glomerulus. Marks (*) indicate the basal lamina region interposed between endothelium and podocyte (P). N perikaryal portion of endothelial cell. ×16,500

Fig. 4. A transmission electron micrograph of a thicker section showing the alveolus fenestratus (A) in comparison with an en-face (grazing) view (**) and a regular (transversely cut) view (arrows) of the single-layered lamina fenestrata. ×23,000
fenestratus was disclosed by scanning electron microscopy to be covered by a thin lamina fenestrata (Fig. 3). The thickened cytoplasm capped by the alveolus fenestratus contained numerous free ribosomes and some mitochondria, but no particular abundance of vesicles was noticed. No Q-shaped invaginations of the plasma membrane into the thickened cytoplasm were seen at the bottom of the alveolus fenestratus. No degenerative changes such as an increase in density of the cytoplasmic matrix, the appearance of numerous lysosomes and myelin figures or the disintegration of the membranous organelles were noticed in the endothelial cytoplasm adjacent to the alveolus fenestratus.

DISCUSSION

The structural details of the alveolus fenestratus endothelialis of the normal mouse kidney in the present study is basically the same as the simple two or three fold stratified laminae fenestratae in normal rats, guinea pigs and rabbits already reported by others (WOLFF and MERKER, 1966; YOSHINARI and FUJITA, 1982; NOZAKI and MIYOSHI, 1984; OHATA et al., 1984; FUJITA and MIYOSHI, 1985), although, the frequency and development of the structure are much more extensive in mice. This constant appearance, regardless of species difference, suggests that the alveolus fenestratus is not an artifact but represents some general and fundamental feature of the fenestrated endothelial cell.

With regard to the mechanism for the genesis of the alveolus fenestratus, several explanations are possible. The first is that this structure represents the transendothelial channel in permeability and that the acceleration of transendothelial permeability by some cause requires the involvement of the thickened portion of the endothelial cell, resulting in the formation of the alveolus fenestratus by the extensive fusion of intracellular vesicles. However, no particular abundance of vesicles was seen in the endothelial cytoplasm in continuity with the alveolus fenestratus. They frequently formed to rest not only on the basal lamina but also on the perikaryal portion of the endothelium. These two phenomena are incompatible with this first explanation.

The second and most probable explanation is that the alveolus fenestratus represents the rapid proliferative response of the endothelial cell to some unknown stimuli. Previous studies have noted the regional abundance of microvilli on the luminal surface of the endothelium, and the occasional fusing of the top of the microvilli has been described (FUJITA et al., 1976; FUJITA and MIYOSHI, 1985). These findings suggest that active and repeated budding and fusion of the microvilli from the endothelial surface may result in the formation of this structure. The abundance of free ribosomes in the endothelial cytoplasm favors this line of thinking. An alternative interpretation is that the structure represents the degenerative and desquamative process of the endothelium. However, this notion is unlikely because no histological changes suggesting degeneration were found in or near the alveolus fenestratus. More attention should be paid to the appearance of the alveolus fenestratus, and further studies on this structure of the mouse kidney in its pre- and postnatal development stages and in various pathological conditions will surely give us more information about the nature of the alveolus fenestratus.
REFERENCES


