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Ultrastructure of rat renal tubular basement membrane–meshwork structure demonstration by negative staining.

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Abstract

The tubular basement membrane (TBM) (i.e. tubular basal lamina) of rat kidney was shown to be a fine meshwork by electron microscopy after negative staining. Strands of the meshwork formed a regular three dimensional lattice work. The pores of the meshwork were polygonal. There were two main pore sizes: one approximately 30 Å in diameter, the other 42–60 Å. In view of our previous observation that glomerular and alveolar basement membranes were made up fine meshwork, it is quite possible that the basement membranes of other organs are also made up such fine meshwork.

KEYWORDS: renal tubular basement membrane, mesh work structure, pore, negative staining, electron micro scopy

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— BRIEF NOTE —

**ULTRASTRUCTURE OF RAT RENAL TUBULAR
BASEMENT MEMBRANE
— MESHWORK STRUCTURE DEMONSTRATION
BY NEGATIVE STAINING —**

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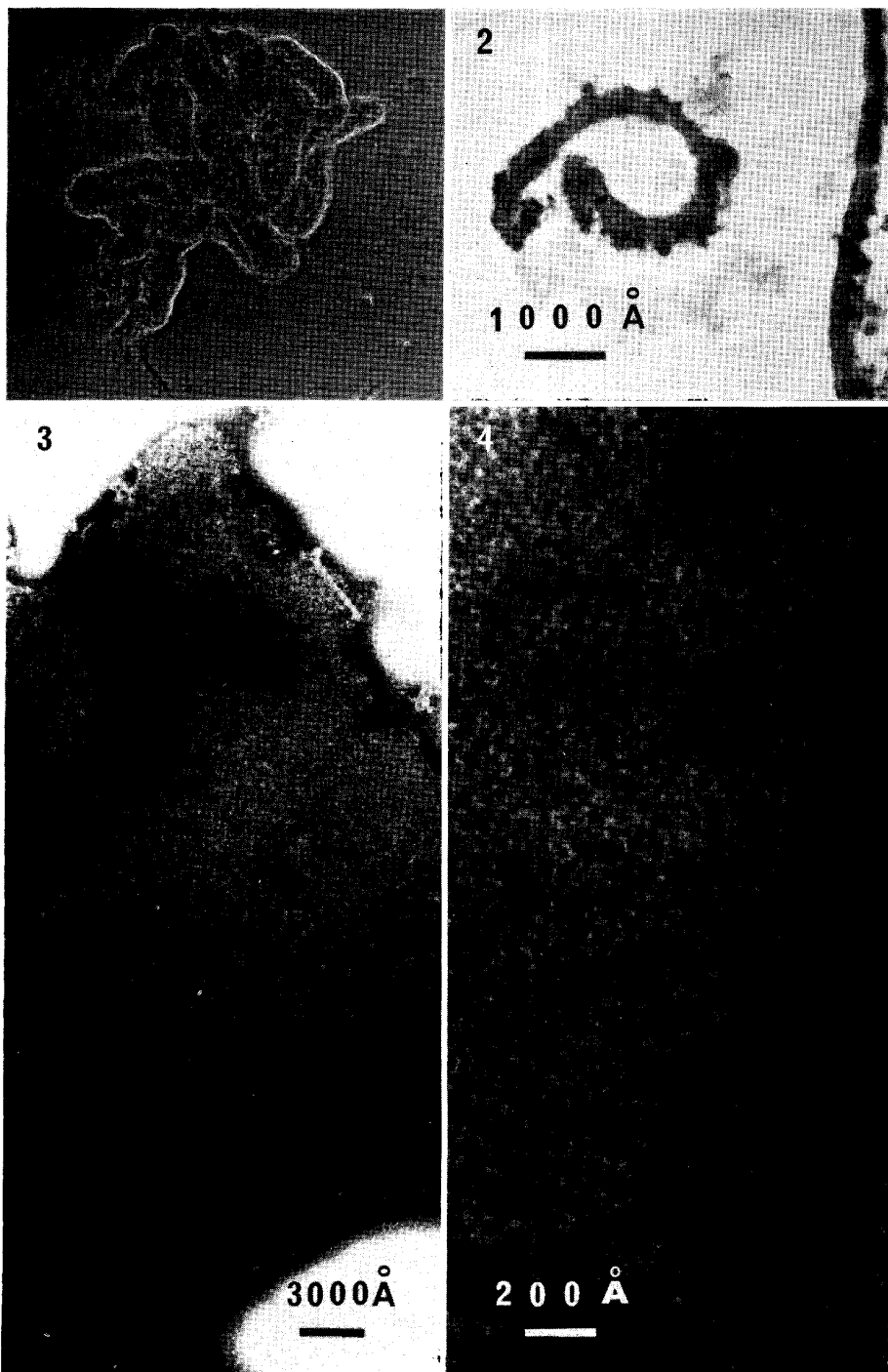
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Abstract. The tubular basement membrane (TBM) (*i. e.* tubular basal lamina) of rat kidney was shown to be a fine meshwork by electron microscopy after negative staining. Strands of the meshwork formed a regular three dimensional lattice work. The pores of the meshwork were polygonal. There were two main pore sizes: one approximately 30 Å in diameter, the other 42–60 Å. In view of our previous observation that glomerular and alveolar basement membranes were made up fine meshwork, it is quite possible that the basement membranes of other organs are also made up such fine meshwork.

Key words: renal tubular basement membrane, meshwork structure, pore, negative staining, electron microscopy

The tubular basement membrane (TBM) (*i. e.* tubular basal lamina) of the kidney is considered to play an important part in the reabsorption function of tubuli. Chemical analyses of the TBM have been performed by some workers (1–3), but little is known of the ultrastructure of the TBM. The TBM appears as a single PAS positive layer by light microscopy. With the transmission microscope, the TBM appears to be composed of fine osmiophilic granules and filaments. Electron microscopic observation by the freeze-etching method showed that the TBM was composed of very fine granules (4). These findings are almost the same as those for glomerular basement membrane. In previous reports (5–7), electron microscopy after negative staining showed that the glomerular basement membrane of human and bovine kidneys was composed of a fine meshwork. The present paper reports a study of the ultrastructure of rat TBM as seen by negative staining.

Approximately 100 Wister rats weighing 150 to 250 g were used. Isolation and purification of rat TBM were performed according to the method of Krisko



(3). In each step, the material was confirmed as being free of cells and glomerular fragments by examination with a phase microscope (Fig. 1). The final pellet containing TBM was fixed with glutaraldehyde and osmium tetroxide and observed with a transmission electron microscope to confirm that contamination was minimal (Fig. 2.). The pellet was then studied by electron microscopy using negative staining with 1.0% phosphotungstic acid pH 7.3.

In low magnification (Fig. 3), a fragment of TBM appeared to be as a piece of felt. The angular shape was characteristic of basement membrane and could be easily distinguished from other contaminants. Higher magnification of a basement membrane fragment (Fig. 4) showed a fine meshwork. The pores of the meshwork appeared pentagonal or hexagonal. The diameter of the pores and the width of the strands showed some variation among fragments. There were two main pore sizes. The smaller diameter was approximately 30 Å and the strands comprising the meshwork were approximately 20 Å wide. The larger diameter was 42–60 Å and the width of the strands was approximately 33 Å. Strands of the meshwork branched three-dimensionally resembling crystal lattice.

There have been no publications demonstrating the existence of pores in TBM. Rostgaard (8) observed TBM as a contaminants in his study of brush border. The magnification was not sufficient to demonstrate the meshwork. In our previous studies (5–7), we demonstrated the fine meshwork structure of the glomerular basement membrane previously only postulated by physiologists (9, 10). It is now clear that TBM, also, is made up of a fine meshwork and has a sieving function. The size of the meshwork showed some variation among samples. This may have been due to the existence of various segments of the tubular apparatus which have different functions. We have also seen a meshwork in alveolar basement membrane (11). In view of these results, it is quite probable that the basement membranes of other organs are also made up of fine meshwork.

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Fig. 1. Phase contrast micrograph of isolated rat tubuli.

Fig. 2. Ultrathin section of isolated tubular basement membrane from rat kidneys. Glutaraldehyde and osmium tetroxide fixation. $\times 11,000$

Fig. 3. Electron micrograph in low magnification of rat tubular basement membrane. Note the angular shape of the fragment. 1.0% phosphotungstic acid negative staining. $\times 30,000$

Fig. 4. Higher magnification of rat tubular basement membrane. Numerous small pores and strands are seen. 1.0% phosphotungstic acid negative staining. $\times 500,000$

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