Experimental Studies on the Formation of Glomerular Epithelial Cell Coat and Basement Membrane

Renal glomerular basement membrane is of considerable physiological interest because of its involvement in the initial ultrafiltration process of urine formation. This basement membrane is also of considerable pathological interest by reason of its involvement in a variety of kidney diseases.

Fundamental to an understanding of this membrane, in both normal and abnormal states, is information concerning its origin. It is the purpose of this report to describe experimental studies which indicate that a component of renal glomerular basement membrane is secreted by the visceral epithelial cells. This basement membrane component is produced at the same time and is secreted in the same manner as the cell coat, or 'fuzz', which covers the plasma membrane of the visceral epithelial cells.

0.012 *M* silver nitrate was administered orally to a series of young, male, Sprague-Dawley rats for up to 10 weeks. At weekly intervals animals were killed and renal glomeruli were examined in a Zeiss EM9A electron microscope. The specimens were fixed in phosphate buffered osmium tetroxide, embedded in Epon, sectioned at a nominal 600 A° and examined unstained, and stained with uranyl acetate and lead citrate.

A second series of rats were given silver nitrate for 10 weeks and then put back on ordinary tap drinking water. At weekly intervals thereafter animals were killed and the glomeruli were similarly examined. This experimental procedure is essentially similar to that of KURTZ and FELDMAN[1].

Electron-dense silver deposits were first observed simultaneously in the vacuoles or 'coated pits'[2], which characteristically open on to the plasma membrane of the foot processes both on the anchored portion into the basement membrane (Figure 1) and on the free portion into the urinary space (Figure 2). In subsequent specimens a progressive number of silver granules were observed in the lamina densa and a few granules were also apparently lying in the urinary space adjacent to the plasma membrane of the visceral epithelial cells. In the second series of rats the glomerular regions which were first cleared of electron-dense silver deposits were these vacuoles. These vacuoles, which open either into the urinary space or into the basement membrane are ultrastructurally indistinguishable and differ only in the locus of their discharge, one outwith the other inside the filtration slit membrane.

The contents of these vacuoles was not apparent in ordinary electron micrographs so similar glomerular tissue was stained by the ruthenium red procedure[3]. A densely-staining layer was observed on the outer aspect of the plasma membrane of the visceral epithelial cells including these open vacuoles: the basement membrane was also stained but slightly less intensely than the cell coat (Figure 3). In general these staining features indicate the presence of an acid substituted large polymer[3], probably a polysaccharide or glycoprotein.

The cell coat covering the visceral epithelial cells was first clearly demonstrated by RAMBOURG and LEBLOND[4] using thorium. Small amounts of thorium were also detected related to the anchored portion of the foot processes but not in the lamina densa. It has recently been shown[5], using colloidal iron techniques allied with neuraminidase digestion that the cell coat contains sialic acid and that small amounts of this material are present related to the anchored portion of the foot processes. There is general agreement that glomerular basement membrane contains, apart from a collagen-like protein, significant amounts of carbohydrate[6–8].

It has been shown that 2 distinct carbohydrate components are present[9–11], one of which is a glycoprotein

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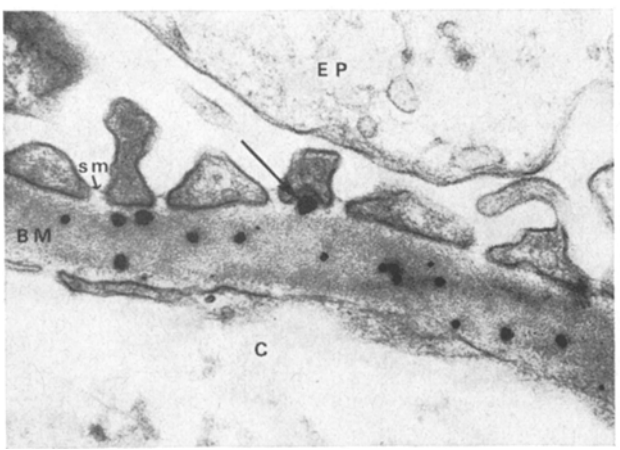

Fig. 1. Electron dense silver deposits in basement membrane. One such deposit (arrowed) is in a vacuole which is opening at the anchored portion of a foot process. (Uranyl acetate and lead citrate. × 57,000).

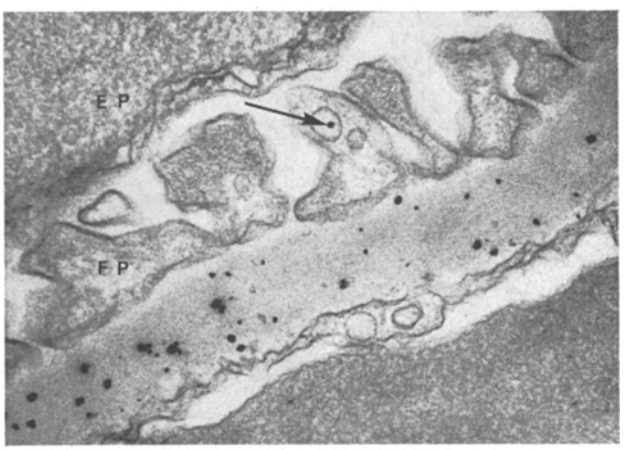

Fig. 2. Electron dense silver deposit in vacuole (arrowed) in foot process. Adjacent section showed that vacuole communicated with plasma membrane. (Uranyl acetate and lead citrate. × 51,000).

containing galactose, mannose, hexosamine, fucose and sialic acid. The available evidence makes it not unlikely that such a glycoprotein could be a component of both the epithelial cell coat and the basement membrane. Dilution of this mucosubstance with the collagen-like protein in the basement membrane could account for the basement membrane staining slightly less intensely than the cell coat in the ruthenium red procedure.

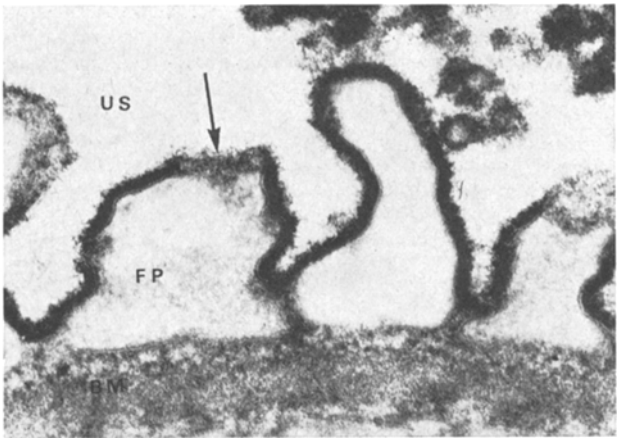

Fig. 3. Densely stained cell coat attached to plasma membrane. Basement membrane less intensely stained. The electron density of the basement membrane approximates to that of obliquely cut cell coat (arrowed). (Ruthenium red. × 145,000).
US, urinary space; FP, foot process; BM, basement membrane; SM, filtration slit membrane; EP, visceral epithelial cell; C, capillary.

In summary, on the basis of electron microscope studies on rat glomeruli labelled in vivo with silver and stained in vitro with ruthenium red, it has been shown that a component of renal glomerular basement membrane is secreted by visceral epithelial cells. This component is secreted in the same manner and at the same time as, and shares certain staining affinities with, the cell coat which covers the visceral epithelial cells.

Résumé. Les études faites au microscope électronique sur les reins de rats marqués in vivo à l'argent et colorés in vitro au rouge de ruthenium ont montré qu'une partie de la membrane de base glomérulaire rénale est constituée par les cellules épithéliale viscérales. Cette partie est sécrétée de la même manière et en même temps que la couche cellulaire recouvrant les cellules viscérales et sa coloration est semblable.

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Cytochemical Demonstration of 'Marker' Enzymes in Nerve Cells Cultured in vitro

The tissue culture method has contributed very much in the field of neurological sciences[1,2], however the main concern in previous works were restricted to morphological features of nervous tissue. It appears important and desirable to investigate the enzymatic activity of nervous tissue in vitro by cytochemical means in order to provide correlation of structural and functional characteristics. In this connection, it has previously been reported by NOVIKOFF[3,4] that certain cytoplasmic organelles can be visualized by cytochemical demonstration of selected 'marker' enzymes. In the present communication 'marker' enzymes in nerve cells grown in tissue culture were investigated with cytochemical staining methods.

Tissue culture explants were made from new-born mouse and kitten cerebellum. Cultures were grown either in Maximow's double-coverslip assemblies or in flying-coverslips in roller tubes. These procedures have been described in detail elsewhere[5,6]. After 2–5 weeks in vitro, the cultures on collagen-coated coverslips were fixed in cold formol-calcium and incubated for demonstration of the following 'marker' enzymes: (1) acid phosphatase for lysosomes[7], (2) thiamine pyrophosphatase for the Golgi apparatus[8], (3) NADP-tetrazolium reductase for mitochondria[9], and (4) acetyl cholinesterase for endoplasmic reticulum[10].

Nerve cells incubated in these cytochemical media showed well-developed lysosomes, Golgi apparatus, mitochondria and endoplasmic reticulum. Lysosomal acid phosphatase activity was demonstrated in neuronal perikarya in fine granules. The reaction was negative in nuclei (Figure 1).

Thiamine pyrophosphatase activity appeared in laminar structures in the perinuclear region of nerve cells where the Golgi apparatus is localized morphologically (Figure 2). NADP-tetrazolium reductase activity for mitochondria was seen as finely granular formazan deposits in neuronal cytoplasm (Figure 3). Acetyl cholinesterase reaction for endoplasmic reticulum appeared in the form of coarse granules localized in the neuronal cytoplasm. No reaction was seen in nuclear structures (Figure 4).

NADP-tetrazolium reductase was selected as the marker for mitochondria since neither succinate dehydrogenase nor mitochondrial-ATPase survives formol fixation[3]. The usefulness of another mitochondrial enzyme, NAD-tetrazolium reductase, is also limited, since this enzyme is present also in the endoplasmic reticulum of nerve cells; mitochondrial structures are therefore, masked by reaction-product deposited in endoplasmic reticulum. Strong reactions for the 'marker' enzymes in nerve cells indicate that enzymes of energy transformation, nervous transmission and hydrolytic reactions are fairly active even in the tissue culture environment.

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