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.

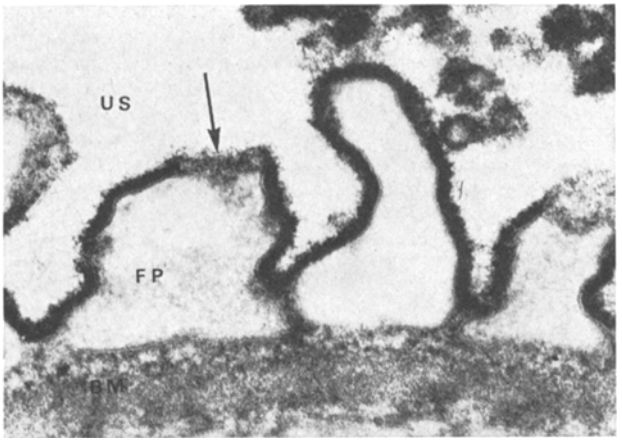

Fig. 3. Densely stained cell coat attached to plasma membrane. Basement membrane less intensely stained. The electron density of the basement membrane approximates to that of obliquely cut cell coat (arrowed). (Ruthenium red. × 145,000).
US, urinary space; FP, foot process; BM, basement membrane; SM, 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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Cytochemical demonstration of 'marker' enzyme activity is thus another favorable evidence of nervous tissue culture; together with the maturation of Nissl pattern[11], the formation of myelin sheaths de novo[12,13], the establishment of functional synapses[14], the fidelity existing in fine structure[15], and the achievement of enzyme activity to the adult level[16]; presenting the conditions close to the physiological, biochemical and basic morphological characteristics of the tissue as observed in vivo.

There is increased interest among researchers in the use of nervous tissue cultures in pharmacological and pathological fields. Application of the cytochemical approach to nervous tissue in vitro undoubtedly can provide extensive and refined information concerning structural and functional relationships within the nervous system. One example of such an application involved a study of the response of cultured nerve cells to anoxic conditions[17].

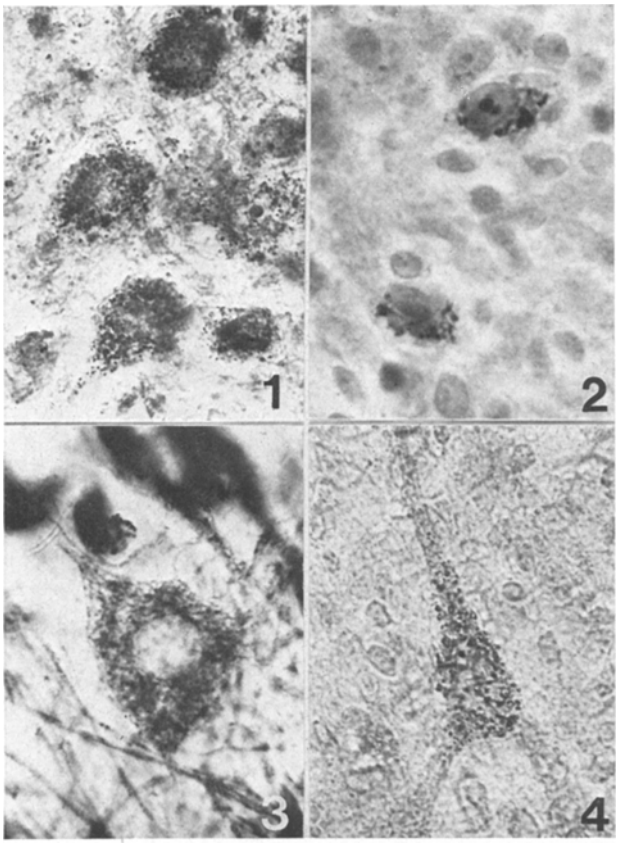

Fig. 1. New-born mouse cerebellar neurons, 24 days in vitro. Acid-phosphatase reaction for lysosomes. × 600.

Fig. 2. New-born mouse cerebellar neurons, 24 days in vitro. Thiamine pyrophosphatase reaction for Golgi apparatus. × 600.

Fig. 3. New-born kitten cerebellar neurons, 30 days in vitro. NADP-tetrazolium reductase reaction for mitochondria. × 1000.

Fig. 4. New-born mouse cerebellar neuron, 28 days in vitro. Acetylcholinesterase reaction for endoplasmic reticulum. × 600.

Zusammenfassung. Die Enzymaktivität des Nervengewebes in Kulturen wird untersucht um funktionelle und morphologische Befunde zu vergleichen. Es werden sogenannte «Marker» Enzyme beschrieben sowie deren histochemische und färberische Darstellung.

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The Adrenergic Innervation of the Efferent Arterioles and the vasa recta in the Mammalian Kidney

Studies of the adrenergic innervation of the intrarenal vessels has yielded conflicting results as far as the efferent arterioles and vasa recta are concerned.

McKenna and Angelakos[1] reported that in the dog the vasa recta received an adrenergic nerve supply but that the efferent arterioles did not. In the rabbit Nilsson[2] was unable to demonstrate adrenergic fibres that supplied either the vasa recta or the efferent arterioles. These different findings may reflect either species differences or limitations of the techniques used. The differences have not been resolved by studies with the electron microscope.

We have therefore studied the adrenergic innervation of the kidney in a number of species, using a histochemical method to demonstrate catecholamines.

Materials and methods. Rats, gerbils, guinea-pigs and rabbits were used. They were killed by an i.p. or i.v. injection of sodium pentobarbitone. A kidney was removed 5–10 min after death of the animal and a portion frozen in solid carbon dioxide and sectioned at 16–30 μ in a cryostat. The sections were mounted on glass slides, dried at room temperature, treated by the method of El-Badawi and Schenk[3], and exposed to formaldehyde

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