Permeability Tracers and Serum Proteins

The knowledge of normal and abnormal vascular permeability and transport across the vessel wall is an essential prerequisite of the study of the pathomechanism of various vascular lesions[1-5]. The follow-up of such processes is partly by demonstration of the intramural deposition of a tracer substance or by light- or electron microscopic detection of the tracer's passage across the vessel wall[6-13]. But no unequivocal conclusions can be drawn until it is clarified whether or not the tracer present in the vessel wall is bound by serum proteins. If there is a durable linkage between the tracer and a given serum protein fraction, the former serves as an indicator of the fraction's mural transport, but if there is no such linkage, the presence of the tracer in the vessel wall signifies only the increase of vascular permeability.

The nature and duration of linkage between tracer substances and serum proteins have been examined in the present study.

Materials and methods. 18 male Wistar rats, weighing 110–150 g, were given the substances indicated i.v. 30 min prior to sacrifice.

Serum samples were examined by immunoelectrophoresis. Commercial antirhodent rabbit serum (Human) was used as immune serum. For the identification of iron, ferritin and peroxidase molecules in the precipitation lines, the Prussian blue and the peroxidase[10] reactions were used.

Group	Tracer	Dose
1	Suspension of colloidal iron (Ferrlecit; Natterman Co., Köln) Containing approximately 12.5 mg of iron/ml	1.5 ml/100 g body weight
2	Suspension of colloidal iron (Jectofer; Astra Co., Sweden) containing approximately 50 mg of iron/ml	0.5 ml/100 g
3	Ferritin, 2X cryst. B grade (Calbiochem Inc., USA) dissolved in physiologic saline	20 mg/100 g
4	Suspension of colloidal carbon (Pelican; Gunther Wagner Co., Hannover) containing approximately 100 mg of carbon/ml	0.2 ml/100 g
5	Evans blue (Gurr Ltd., England) dissolved in physiologic saline	20 mg/100 g
6	Horseradish peroxidase, B grade (Calbiochem Inc., USA) dissolved in physiologic saline	15 mg/100 g

Peroxidase was demonstrated in the β-globulin and IgG fractions; Evans blue was present in the albumin and α-globulin fractions; while the rest of the tracer substances examined were apparently not bound by any serum protein fraction. In vitro binding of Evans blue by albumin was demonstrated previously[14,15]. By electrophoresis on cellulose acetate, colloidal iron was shown to form a precipitate of varying charge and motility, migrating together with certain plasma components[12] but this does not mean the presence of a linkage.

It appears, therefore, that unless the binding between serum protein and tracer is not reversed during the former's passage across the vessel wall, peroxidase signifies the mural transport of β-globulin and IgG, and Evans blue that of albumin and α-globulin. The other tracer substances, being apparently not bound by serum proteins, serve only as indicators of increased vascular permeability.

Zusammenfassung. Es wird gezeigt, dass die Permeabilität der Gefässwand durch an Serumeiweissfraktionen gebundene Markierungssubstanzen geprüft werden kann.

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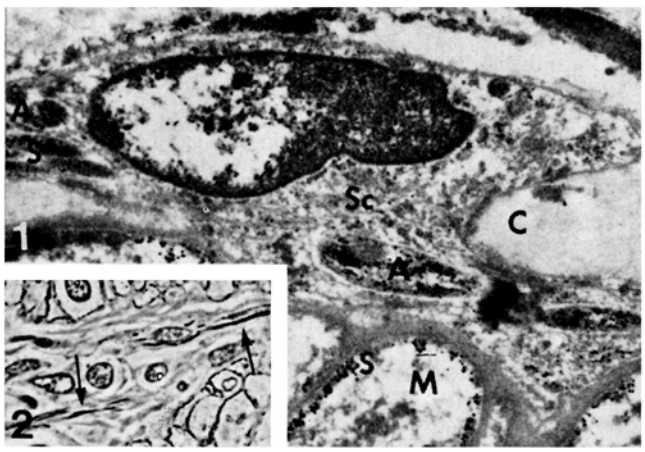
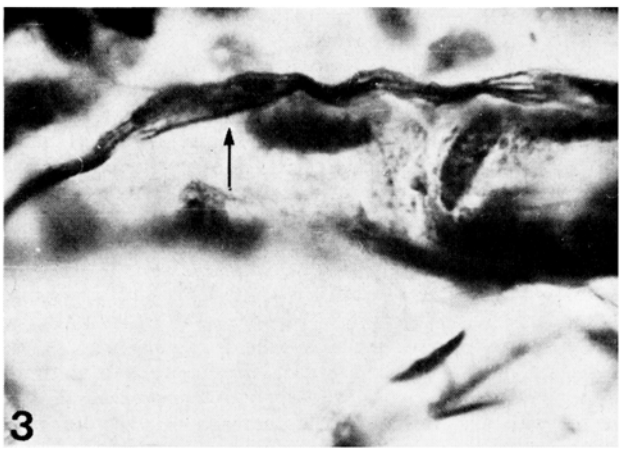
Direct Autonomic Nerve Fibers to the Renal Medulla in Man[1]

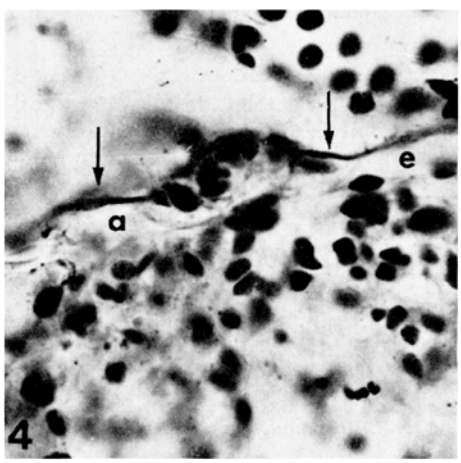
Blood flow to the renal medulla is regulated in part by the antagonistic action of the sympathetic and parasympathetic nerves on the medullary blood vessels[2]. Earlier studies[3-6] with the specific catecholamine fluorescence test showed that the arterioles supplying the renal pyramid – the juxtamedullary efferent arterioles and the proximal parts of the arterial vasa recta – receive sympathetic innervation from the periarterial plexuses of the afferent arterioles. These fibers traverse the vascular pole of the juxtamedullary glomeruli to reach the corresponding efferent arterioles.

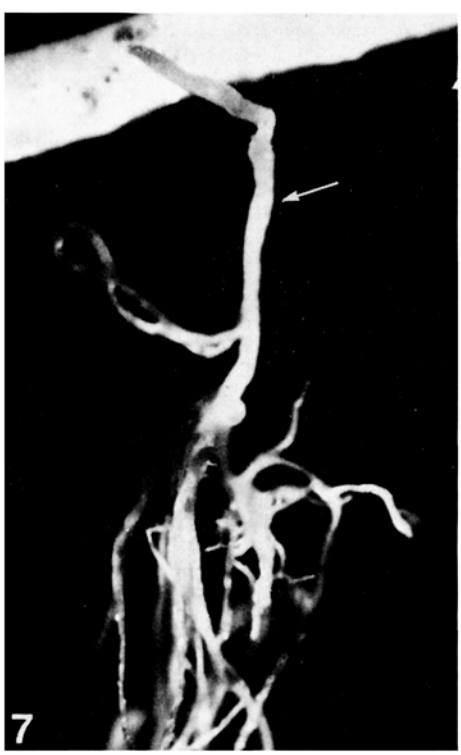
In the thin sections (4 μm) used for the fluorescence technique it is not possible to resolve the question whether some of the fibers innervating the medullary vessels originate directly from the plexuses around the arcuate arteries without passing across the juxtamedullary glomerular poles. If such direct nerve pathways exist, neural vasoregulation in the medulla may be achieved independently of any effects on the glomerular blood flow.

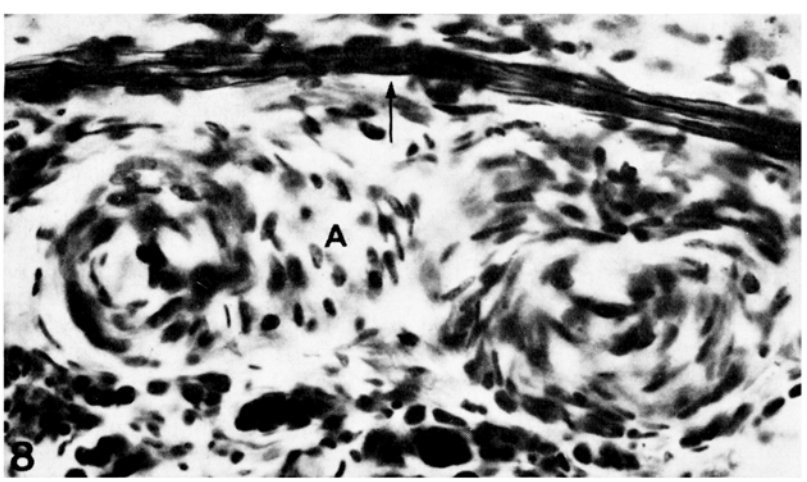
Materials and methods. Traditional silver impregnation (Bielschowsky, Gros and Schultze) was used to study











(Legend to Pictures 1–8 see next page) ➤

thick sections of human kidneys which appeared normal at autopsy, human kidneys extensively damaged by chronic glomerulonephritis, and kidneys of dogs and beavers. Blocks of tissue including the corticomedullary junction were fixed for 2–3 months in neutral formalin. Cryostat sections 20–30 µm thick were cut parallel to the vasa recta and impregnated with silver. Sections showing particularly well oriented vessels and nerve bundles were selected prior to gold toning and were embedded in Epon, sometimes after post fixation with osmium tetroxide. Sections from these blocks were examined with light or electron microscopy (Figures 1 and 2).

Results and discussion. The adventitia of all intrarenal arteries contains sympathetic nerve bundles which contribute fibers to the periarterial plexus (Figure 3). This pattern is similar along the afferent arterioles of both cortical and juxtamedullary glomeruli. A few fibers from these plexuses traverse the vascular pole of the glomerulus to the efferent arteriole (Figure 4). Nerve fibers from the cortical glomeruli do not extend beyond the first branches of the efferent arterioles, presumably because there are usually no more smooth muscle cells in the vascular wall beyond this site. The juxtamedullary efferent vessels, however, are innervated along their entire length and this innervation continues along the associated vasa recta deep into the outer medullary zone (Figure 5). This distribution of sympathetic nerve fibers in relation to the renal vasculature seems to pertain in mammals generally and suggests that neurally mediated vasomotion of the arterial vasa recta is not independent from that of the juxtamedullary afferent and efferent arterioles. In human kidneys, however, some bundles innervating the medulla are derived directly from the periarterial plexuses of the arcuate arteries without contributing to innervation of the afferent arterioles or of the vascular pole of the glomerulus (Figure 6). The size of these nerve bundles at their origin is similar to that of bundles running along the arcuate arteries and their distribution corresponds to the pattern of distribution of the arterial vasa recta. These nerve bundles can be regarded as aberrant or direct fibers since they do not follow the glomerular pathway.

Electron microscopic examination confirmed that even very small nerve bundles could be correctly identified in the preparations for light microscopy. Heavy deposition of silver in these preparations was associated only with axons of nerves, nucleoli, and the surfaces of smooth muscle cells (Figures 1 and 2).

Clearly, some nerve fibers reach the vasa recta across the vascular pole of the juxtamedullary glomeruli. Others seem to be derived directly from nerve bundles accompanying the arcuate arteries. In man, the direct fibers to the medulla may accompany the arteria recta vera which branch into vasa recta without becoming associated with a glomerulus and which are thought to develop when afferent and efferent arterioles become continuous across the vascular poles of damaged glomeruli. This explanation appears inadequate since in normal kidneys only a few small nerve bundles – usually 1 or 2 in a 20–30 µm section – can be observed passing across the vascular pole from each juxtamedullary afferent arteriole to the corresponding efferent arteriole. Direct nerve bundles are larger and contain more numerous nerve fibers than is the case at comparable distances along nerves accompanying efferent arterioles. Moreover, silver impregnations of the corticomedullary region in patients with chronic nephritis, in which progressive glomerular damage occurs and arteria recta vera develop in great density (Figure 7), do not show a density of the thick direct fibers greater than that found in normal kidneys. In general, the sympathetic peri-arterial nerve plexus is quite well preserved in chronic nephritis in spite of the degenerative processes occurring in the arterial walls (Figure 8). It is, therefore, unlikely that the direct fibers appear as a result of vascular remodelling following glomerular degeneration.

These direct fibers may have important functional significance since they may provide a means for neural control of the distribution of blood between cortex and medulla independently of any effect on the flow of blood to the glomeruli. Our investigation does not indicate whether the direct fibers are of sympathetic or parasympathetic origin, although previous investigations[7] confirm the presence of both types of fiber in the outer zone.

Résumé. Des fibres nerveuses passent directement du plexus nerveux des artères arcuates aux artérioles efférentes juxtamédullaires et aux vasa recta. Il est donc possible, que le contrôle neural de la distribution du sang entre le cortex et la medulla soit indépendant du passage du sang aux glomeruli.

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Fig. 1. Electron micrograph of a thin silver impregnated section adjacent to that illustrated in Figure 2 and showing some nerve fibers. Silver deposits (S) are confined to the axons of nerves (A) and the basement lamina of smooth muscle (M). Schwann cell (Sc) and collagen (C) are free of silver deposit. × 16,000 magnification.

Fig. 2. Nerves (arrows) accompanying an afferent arteriole in the juxtamedullary region of a dog's kidney. The nerves and the basement lamina of smooth muscle cells are heavily impregnated with silver. Phase contrast photomicrograph of a section 1 µm thick. × 860 magnification.

Fig. 3. Silver impregnation of autonomic nerve fibers (arrow) in the adventitia of an afferent arteriole in man. × 1200 magnification.

Fig. 4. Vascular pole of a glomerulus with nerve fibers (arrow) which traverse from afferent (a) to efferent (c) arteriole in man. Silver impregnation. × 1460 magnification.

Fig. 5. Nerve fibers (arrows) along the vasa recta in dog. Silver impregnation. × 310 magnification.

Fig. 6. Direct nerve bundle to medulla (arrows) from the periarterial plexus of an arcuate artery (a) in man. Silver impregnation. × 390 magnification.

Fig. 7. Vascular cast of an a glomerular (arrow) artery to medulla in chronic nephritis. × 140 magnification.

Fig. 8. An interlobular artery (A) in chronic nephritis. The accompanying nerve fibers (arrow) are well preserved. Silver impregnation. × 850 magnification.