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].

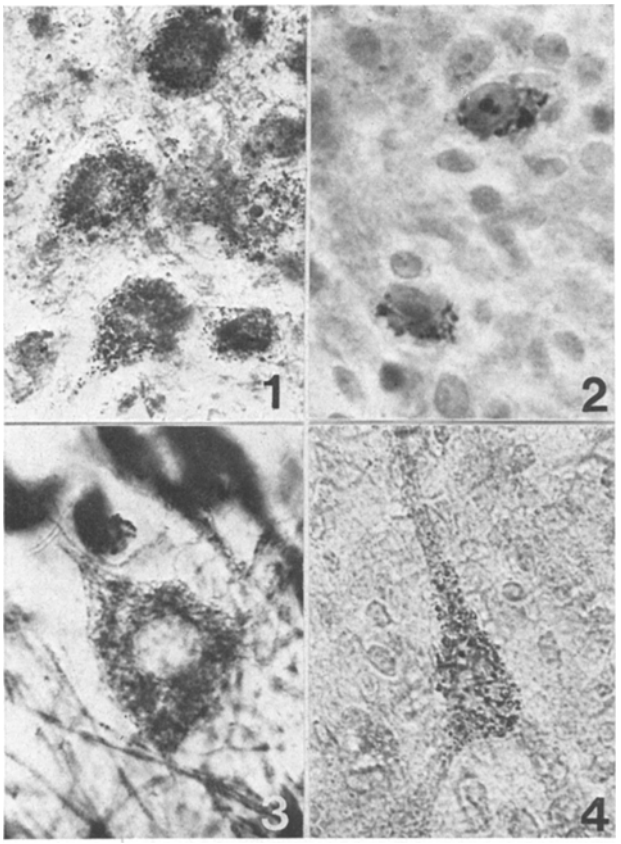

Fig. 1. New-born mouse cerebellar neurons, 24 days in vitro. Acid-phosphatase reaction for lysosomes. × 600.

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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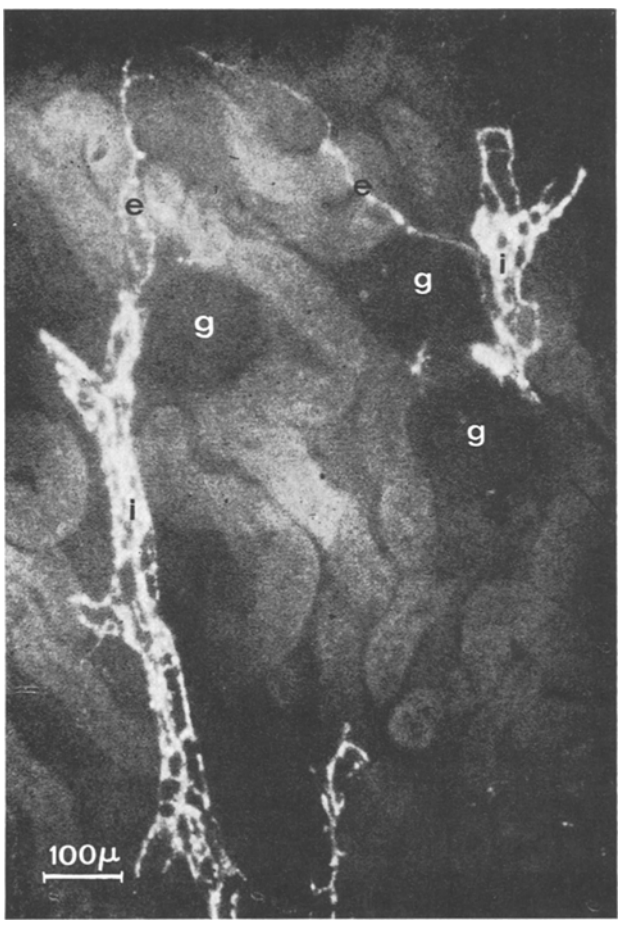
vapour for exactly 30 min. They were then dehydrated, cleared and mounted in non-fluorescent oil. Some sections were pre-incubated for 30 sec in a solution containing 2 μg/ml of noradrenaline bitartrate.

Results and discussion. The distribution of fluorescence in the kidney was similar in all the species studied. Fluorescent catecholamine-containing fibres were seen to accompany all the vessels of the arterial tree, including the efferent arterioles. In the outer part of the cortex adrenergic fibres accompanied the efferent arterioles of the subcapsular glomeruli (Figure). The fibres leaving the other cortical glomeruli were short and terminated in a few twigs passing between the tubules. These followed the efferent arterioles and there was no evidence of a nerve supply to the tubules. The long efferent arterioles leaving the juxtamedullary region were accompanied throughout their length by adrenergic fibres which extended to surround the vasa recta in the upper part of the vascular bundles.

In the rabbit and guinea-pig, but not in the rat or gerbil, some of the nerves around the vasa recta bundles appeared to accompany aglomerular vessels which left the connective tissue plexus. McKENNA and ANGELAKOS[1] reported a similar appeareance in the canine kidney. In this species as in rabbit, guinea-pig and man, descending vasa recta may arise from a vessel apparently without a glomerulus[4]. This has been shown to result from degeneration of a glomerulus so that the afferent and efferent arterioles become continuous. Such aglomerular vessels are not seen in the rat or gerbil kidney. The difference in the form of the vessels supplying the vasa recta may explain the reported differences in their innervation. Conversely the relatively sparse innervation of the juxtamedullary efferent arterioles in some species may reflect a less effective sphincter mechanism which might facilitate the shunting of blood from the afferent to the efferent arterioles and be a factor in the observed glomerular degeneration.



Gerbil Kidney. Sections of cortex showing distribution of adrenergic fibres. (i) Interlobular artery; (g) glomerulus; (e) efferent arteriole.

The results were not always reproducible, despite the fact that several sections from the same kidney were mounted on a single slide. Sometimes not all the sections showed the finer fluorescent fibres. In some kidneys no fluorescent fibres were demonstrated even around the large renal arteries unless the sections had been previously incubated in noradrenaline. Factors such as humidity of the formaldehyde or of the tissue may have influenced the results. McKENNA and ANGELAKOS[1] found that demonstration of the adrenergic fibres in the outer medulla required a greater humidity than was needed for those in the cortex. Catecholamines may have been released from the nerves at time of death. Electron microscope studies have shown that depleted vesicles in adrenergic fibres take up catecholamines. This may explain why FALK[5] and ourselves obtained better results if excision of the tissue was delayed for some 5–15 min after death. During this period amines released at the time of death could be taken up again. Incubation of the sections with noradrenaline would have a similar effect. It would appear that only a positive result is of value with these techniques.

These findings are consistent with GOSLING's[6] electron microscope demonstration that in the rabbit the nerves supplying the upper part of the vasa recta contain dense-cored vesicles typical of adrenergic nerves.

In the animals studied the distribution of the nerves containing monoamines is the same as that found using histochemical methods to demonstrate butyrylcholinesterase activity. It is not yet possible to say whether the efferent arterioles and vasa recta have a dual innervation with vasodilator and vasoconstrictor fibres or whether the cholinesterase is in adrenergic nerves (BURN and RAND[7,8]).

Résumé. En employant des techniques histochimiques, nous avons étudié l'innervation adrénergique du rein des mammifères et démontré que les fibres adrénergiques entourent les artérioles et la partie supérieure des faisceaux des vasa recta.

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