Our limited study of the distribution of a trace dose (375 ng/kg) of arsenate revealed the following: a) day 9 embryos contained detectable amounts of arsenate – approximately 3 pg/g tissue; b) the pattern of arsenate distribution and the fractional decrease in tissue arsenate between day 9 and day 12 were the same as for the teratogenic dose. Thus, arsenate is partitioned in identical fashion for doses of teratogen differing by more than 2100 times. Constancy in the pattern of arsenate distribution over a wide concentration range implies that very little teratogen is bound to macromolecules or other tissue components, contrary to expectations for heavy metal ion teratogens.

The biochemical basis of arsenate-induced teratogenesis may depend on the chemical similarity of arsenate and phosphate. For example, arsenate competes with inorganic phosphate in a variety of metabolic reactions[5] and could interfere with one or more biochemical steps at a critical stage of organogenesis. Alternatively, arsenate might be reduced to arsenite, as Ginsburg has demonstrated in renal clearance studies[4], and further metabolized to an organo-arsenical with teratogenic properties. Regardless of the biochemical mechanism of arsenate-induced teratogenesis, our data show that the teratogen does enter embryonic tissues during organogenesis. These findings, therefore, offer support to Ferm's suggestion that arsenate exercises a specific effect on embryonic cephalic mesenchyme[6].

5 L. M. Klevay, Pharmac. Ther., A, *1*, 189 (1976).
6 V. H. Ferm, Adv. Teratol. *5*, 51 (1972).

Histochemical demonstration of adrenergic nerve fibres in the renal capsule of rats

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Summary. Adrenergic innervation in rat renal capsule was demonstrated using the histochemical fluorescence method with glyoxylic acid.

The fluorescence histochemical method of Falck and Hillarp[2] has revealed adrenergic innervation in the renal blood vessels[3–5] and tubules[6]; however, localization of monoamines in the renal capsule has not been reported. A new histochemical technique using glyoxylic acid instead of formaldehyde has been developed[7,8] and good results are seen with the method using aqueous solution and heating in the stretch preparation of peripheral thin tissues[9]. We attempted to demonstrate adrenergic innervation in the renal capsule using this glyoxylic acid method.

Male Wistar rats weighing 150–200 g were sacrificed under pentobarbital anesthesia. The kidneys with the capsules were rapidly excised and immersed in ice-cold 2% glyoxylic acid solution. Glyoxylic acid monohydrate was dissolved in 0.1 M phosphate buffer adjusted to pH 7.0 with NaOH. 10 min later, the capsules were removed and stretched on clean glass slides. The specimens were dried with a hair-dryer for 20 min, heated at 100 °C for 5–10 min and then mounted with the entellan-xylene mixture. For microscopic analyses, a Zeiss epifluorescence microscope was used with a high pressure mercury lamp as a light source. The excitation filter used was a BG12 and the barrier filter was Zeiss '47' or '50'.

The outer surface of the kidney is covered with a delicate fibrous capsule composed of collagenous and elastic fibres and a few smooth muscle cells. Outside the tunica fibrosa is a fatty layer called the tunica adiposa. Fluorescent nerve fibres with varicosities were found to innervate the hilus arteries of the kidney and their ramifications to enter the renal capsule. The renal capsular arteries were accompanied by 2 main bundles in which ran 2 or more intensely fluorescent fibres and both bundles were connected by a few fine varicose terminals (figure 1). There were 2 types

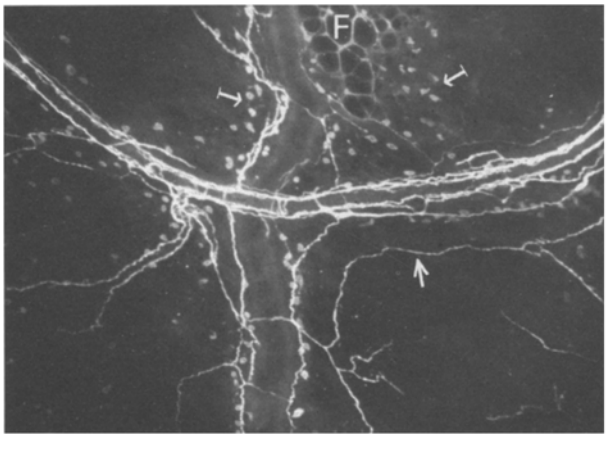

Fig. 1. The renal capsule of the rat. The adrenergic nerve fibres run along the blood vessels, on to the fat cells (F) and freely on the renal capsule (↑). Yellow fluorescent mast cells (↑) were seen around the blood vessels and the fat cells. ×30.

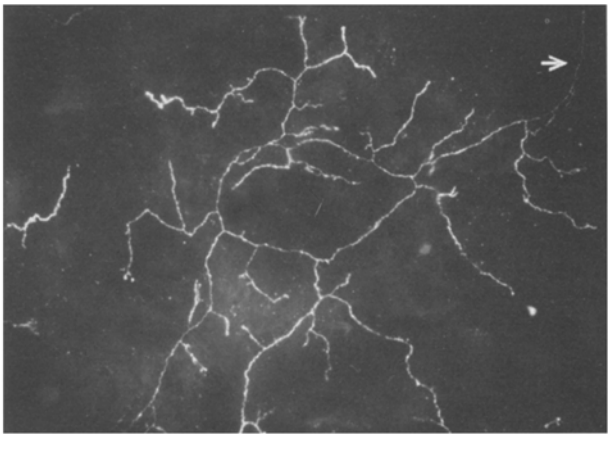

Fig. 2. The tunica fibrosa of the rat kidney. A system of branching terminals arising from one preterminal axon (↑) is visible in the tunica fibrosa. ×30.

of fibre in the perivascular fluorescent fibre bundles, one being intensely fluorescent winding fibres with protruding varicosities, and the other thinner fibres with elongated varicosities, the so-called varicose terminals. The intervaricose segments were more clearly seen in the stretch preparation treated with glyoxylic acid as compared with that treated with formaldehyde vapor. 2 fine varicose fibres were visible along the small branches of the arterioles. Occasionally, only one single varicose fibre could be traced to the side branches of arterioles having diameters of 8 µm. The adrenergic nerve supply was less in the vein than in the artery.

In the tunica fibrosa, a system of branching terminals arising from one preterminal axon was visible (figure 2). The preterminal axon was smooth and weakly fluorescent as compared with the branching terminals with abundant intensely fluorescent varicosities. The branching systems originated from the perivascular plexus and extended into tunica fibrosa. These branches appeared to innervate the smooth muscle cells which intermingled with connective tissues and elastic fibres. Other adrenergic terminals unassociated with the blood vessels were found around the fat cells in the tunica adiposa. Yellow fluorescent mast cells with non-fluorescent nuclei were evident around the blood vessels and the fat cells. Distribution of the adrenergic innervation and mast cells was rich in the medial half of the renal capsule, particularly around the hilus renalis.

To our knowledge this is the first demonstration of the distribution of adrenergic fibres and serotonin-containing mast cells in the renal capsule of rats. These findings suggest that in the renal capsule, as well as many other peripheral tissues, these adrenergic fibres assist in regulating blood flow and fat metabolism. Herbert et al.[10] proposed that the renal capsule provides the major force opposing expansion of the outer cortex when intrarenal pressure was increased. Our findings of a direct adrenergic innervation in the tunica fibrosa may provide a morphological basis for adrenergic regulation of the elasticity of the renal capsule.

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2 B. Falck, N.-Å. Hillarp, G. Thieme and A. Torp, J. Histochem. Cytochem. *10*, 348 (1962).
3 O. Nilsson, Lab. Invest. *14*, 1392 (1965).
4 O. McKenna and E. Angelakos, Circulation Res. *22*, 345 (1968).
5 J. Muller and L. Barajas, J. Ultrastruct. Res. *41*, 533 (1972).
6 N. Ohgushi, K. Mohri, M. Sato, K. Ohsumi and K. Tsunekawa, Experientia *26*, 401 (1970).
7 S. Axelsson, A. Björklund, B. Falck, O. Lindvall, L. Å. Svensson, Acta physiol. scand. *87*, 57 (1973).
8 O. Lindvall and A. Björklund, Histochemistry *39*, 97 (1974).
9 J. B. Furness and M. Costa, Histochemistry *41*, 335 (1975).
10 L. H. Herbert, K. A. Stuart and J. A. Stemper, Kidney Int. *7*, 45 (1975).

Cytoarchitecture of monoamine-containing cells in the frog's gustatory epithelium[1]

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Summary. With the availability of 5,6-dihydroxytryptamine, the overall morphology and the characteristic distribution of monoamine-containing cells in the frog's gustatory epithelium came to be clearly elucidated by fluorescence histochemistry.

It has recently been reported that a serotonin-like monoamine is present in a certain cell type of the frog's gustatory epithelium[3]. However, the overall morphology of the monoamine-containing cells (MC cells) has not been elucidated as yet. An analogue of serotonin, that is 5,6-dihydroxytryptamine (5,6-DHT), is selectively taken up in the MC cells and enhances temporally their fluorescence intensity during the first few h following the drug injection[4]. By making use of this drug, the MC cells of the frog's gustatory epithelium were observed by fluorescence histochemistry under the condition of the complete accumulation of the drug in the cells.

Materials and methods. These are described in detail elsewhere[4]. Frogs (Rana catesbiana) weighing 150–200 g were used throughout the present work. At various intervals after intraperitoneal injection of 5,6-DHT (20 mg/kg), tongues were excised and cut into small blocks. After being dried in a freeze-drying unit, they were processed according to the Falck-Hillarp technique[5] for the localization of monoamine. The sections of 8 µm thickness were mounted with liquid paraffin and photographed by fluorescence microscopy. Then, the cover-slips were carefully detached by dipping in xylene, and the mounting medium was removed by further soaking in fresh xylene. The sections were transferred into a descending series of alcohol and finally into water. They were mounted on albuminized glass slides and stained with 0.1% toluidine blue solution on a hot-plate (70 °C) for 2 min. After being washed briefly, they were dehydrated in alcohol and mounted in balsam. The papillae that had been examined by fluorescence microscopy were also photographed by light microscopy.

Results. A specific yellow fluorescence of normal fungiform papillae always appears at the basal region of the gustatory epithelium. In the transverse section, the dot-like fluorescent structures stand in a line along the basal lamina (figure 1). In horizontal sections made just above the basal lamina, round or oval fluorescent rings are observed at the periphery of gustatory disc (figure 2, arrows). From there the fluorescent structures stretch toward the centre and their inner ends are considerably dilated (figure 2).

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2 The authors gratefully acknowledge the support and helpful advice of Dr T. Kanaseki, Professor of Anatomy, Faculty of Medicine, Kyushu University, Fukuoka 812 (Japan).
3 K. Hirata and O. Nada, Cell Tiss. Res. *159*, 101 (1975).
4 O. Nada and K. Hirata, Histochemistry *45*, 121 (1975).
5 B. Falck, N.-Å. Hillarp, G. Thieme and A. Torp, J. Histochem. Cytochem. *10*, 348 (1962).