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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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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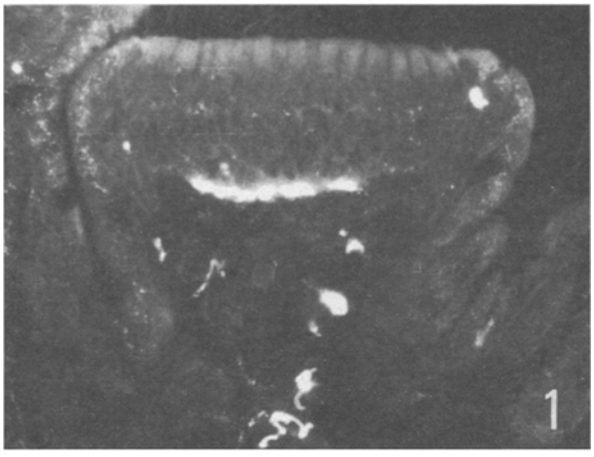
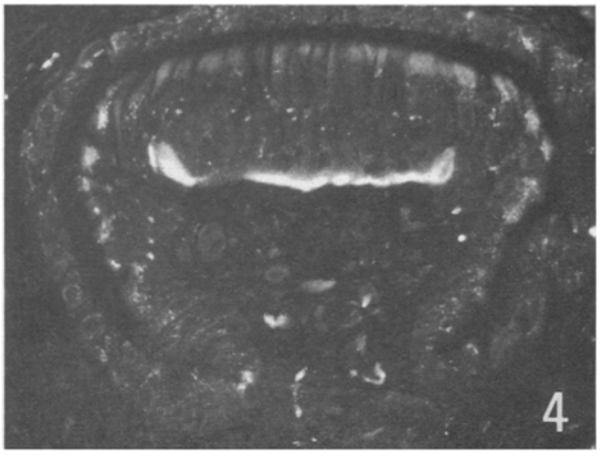
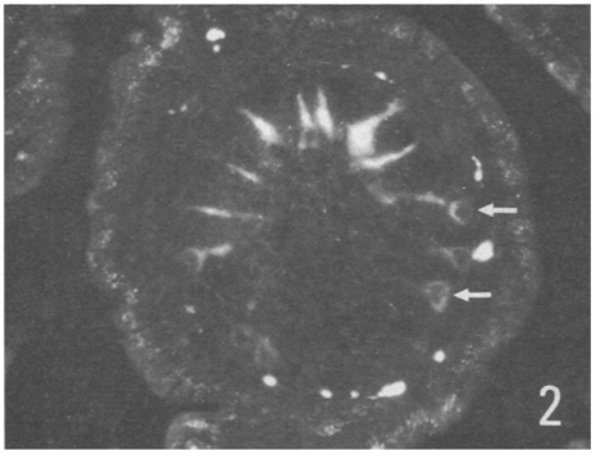
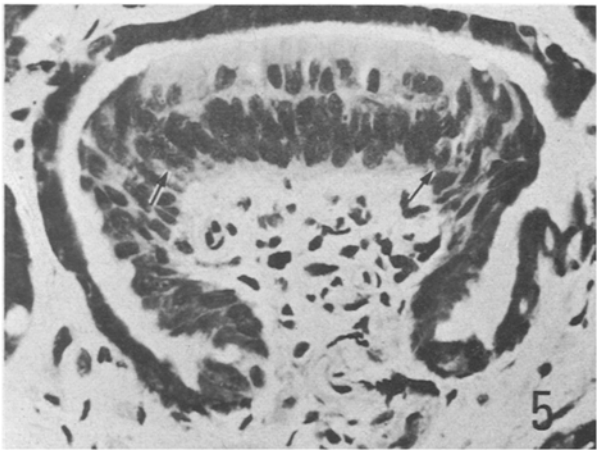
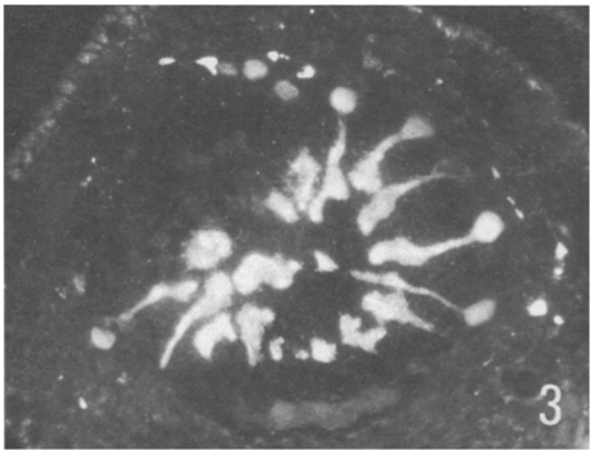
As reported previously, a single injection of 5,6-DHT could enhance the fluorescence intensity of MC cells for the first few h (figures 3 and 4). In horizontal section, the overall profiles of the cells were easily detected (figure 3). Their expanded inner ends were irregularly contoured and showed evidence of ramification. They progressively tapered down along their length toward the periphery where they were connected with round or oval fluorescent structures (figure 3). These structures were always observed at the periphery and were fairly uniform in size. It can be assumed that these portions indicate the sites of MC cell nuclei. Figures 4 and 5 show the fluorescence and light micrographs, respectively, of the same papilla. The oval fluorescent structures are found at the left and right margins of the base of the gustatory epithelium in figure 4. When figure 4 was superimposed on figure 5, these fluorescent structures in the former coincided with the nuclei indicated by arrows in the latter. Thus, it could be clearly demonstrated that the round or oval fluorescent structures correspond to the sites of nuclei. It is likely that they were produced by a great accumulation of fluorophore in the perinuclear regions, while the ringform fluorescent structures observed in the normal papilla can be attributable to the low accumulation of endogenous monoamine in the perinuclear cytoplasm. The perikarya of MC cells are always localized at the periphery of the











Figs. 1–4. Fluorescence micrographs of the frog's fungiform papilla. Fig. 1. Transverse section of a normal papilla. The specific monoamine fluorescence is localized along the base of the gustatory epithelium. ×300.

Fig. 2. Horizontal section through the base of the gustatory epithelium from a normal animal. Notice the fluorescent rings (arrows) at the periphery of the gustatory disc. ×300.

Fig. 3. Horizontal section through the base of the gustatory epithelium from 5,6-DHT-treated animal. The overall profiles of several MC cells are revealed by the drug treatment. ×300.

Fig. 4. Transverse section of the papilla from 5,6-DHT-treated animal. Notice the oval fluorescent structures at the left and right margins of the gustatory epithelium. ×300.

Fig. 5. Light micrograph of the same papilla as in figure 4 after toluidine blue staining. Notice that the oval fluorescent structures of figure 4 are observed on the sites of nuclei (arrows). ×300.

disc and their processes extend from there toward the centre. As a whole, their arrangement in the frog's gustatory disc shows a characteristic pattern resembling the spokes of a wheel.

Discussion. The present study has revealed a characteristic cytoarchitecture of MC cells in the frog's gustatory epithelium under the condition that the cells are probably filled with the fluorophore derived from 5,6-DHT. Recently, the ultrastructural study suggested the presence of Merkel cells in the frog's taste organ[6]. MC cells do not have apical processes extending toward the free surface. Serotonin-like monoamine-containing cells had been also observed in the taste organ of other species of vertebrates. In the rabbit's taste bud, the MC cell[7] was confirmed to be identical with the type III cell[8] which had been regarded as a gustatory receptor[9]. It extends from the basal lamina to the free surface and makes afferent synaptic contacts with intragemmal nerves. In the taste bud of the sheat-fish (Amiurus nebulosus), the basal cell contains an aminergic transmitter, serotonin[10]. Reutter[10] suggested that the basal cell modifies the impulse which is transmitted from sensory cells to afferent nerves. Further study is needed to clarify the role of MC cells in the frog's taste organ.

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The calcium-magnesium-deficient rat: A study on the distribution of calcium in the spinal cord using the electron probe microanalyser[1]

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Summary. Using the scanning electron probe X-ray microanalysis technique, calcium distribution in the spinal cord of the calcium-magnesium-deficient rat was studied. Calcium accumulations were observed within and around the perikaryon of certain unspecified motoneurons in the spinal cord.

In experimental rats fed a calcium-magnesium (Ca-Mg)-deficient diet for a period of several weeks, the concentration of spinal cord calcium showed an occasional rise despite a reduced blood calcium and magnesium level[3]. Furthermore, histochemical studies on the neuronal perikaryon of the spinal motoneuron showed a decrease in succinate dehydrogenase (SDH) activity with the perikaryon appearing swollen, and an atrophy and decrease in SDH type 2 muscle fiber was noted in gastrocnemius muscle tissue specimens in these Ca-Mg-deficient animals[3,4].

It became of interest to know the location of the calcium deposit within the spinal cord. The purpose of this study is to report the distribution of calcium in the spinal cord of the Ca-Mg-deficient rat by use of the electron probe microanalyzer, and to discuss a possible relationship between the calcium accumulations in the spinal cord and the appearance of a neuromuscular disorder in the experimental animals.

A Ca-Mg-deficit was induced in juvenile male albino rats by a synthetic diet containing 0.01% Ca and 0.003% Mg. The control rats were fed a normal diet containing 2.0% Ca and 0.34% Mg. Both groups were sacrificed after 6 weeks of this diet schedule, and the spinal cords were obtained for the experimental material. Atomic absorption spectrophotometry was used to determine the concentration of calcium in entire spinal cords with the following readings[3]:

	μg/g wet weight
Ca-Mg-deficient	286.6 ± 90.2
Controls	91.0 ± 10.7

Each value is the mean ± SE of the mean of 14.

The specimens were immersed in 4% formaldehyde containing 1% sodium oxalate, dehydrated gradually with alcohol, then cleared in xylene and embedded in paraplast using the method of Iwata et al.[5]. 10 micron sections were picked up from the solution onto a quartz disc and dried at 45 °C. The paraplast was removed and sections were coated with a thin layer of vacuum-evaporated carbon. The scanning electron probe microanalyzer (JXA-500A) was operated at 25 KV accelerating voltage and at about $1–10 \times 10^{-11}$ A absorbed current. The calcium LαX-ray integration time was 250 sec. Secondary electron images were recorded before recording the X-ray images. Polaroid photographs were then made of the display on the cathode ray tube.

The characteristic calcium LαX-ray images of the spinal cord specimens of the Ca-Mg-deficient and control animals and the secondary electron images appear in the figure. The image generated by the LαX-ray of calcium in the Ca-Mg-deficient animal is seen in figure B. Observation of the secondary electron image in figure D showed

Ratios of calcium-accumulated motoneurons in the anterior horn of the spinal cord of rats fed Ca-Mg-deficient or control diets

	Total motoneurons	Ca-accumulated motoneurons	Ca-accumulated motoneurons/total motoneurons
Control[6]	52	3	5.8%
Ca-Mg-deficient[6]	53	19	35.8%*

Values in parenthesis refer to number of animals studied significant level; *$p < 0.001$.

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