with our anti-S antibodies. 2-Dimensional gels of myosin light chains, and peptide maps of myosin heavy chains from ^{14}C-labelled single fibres of red, pink and white muscle gave results identical to their equivalents obtained from myofibrillar preparations.

Thus, the light chains of pink and white muscle myosins from *Cyprinus carpio* are indistinguishable not only in apparent molecular weight[10] but also in isoelectric point. The heavy chains of these myosins, however, give slightly different peptide maps, suggesting that there are small differences in their amino acid sequences. An analogous situation, i.e. the association of light chains of the same type with different heavy chains, is found in mammalian skeletal muscle, in which the same 'fast' light chains are associated with the different heavy chains of IIA, IIB and neonatal myosins[17,19].

In conclusion, here we provide what we believe to be the first direct demonstration (by peptide mapping) that in the carp the pink layer of the lateral muscle has a distinct isoform of myosin. The small difference in primary structure between pink and white muscle myosins indicated by our results could account for the differences in histochemical amATPase and mATPase activities between these muscles (ATPase activity is a heavy chain property), but presumably have little or no effect on those parts of the molecule against which our anti-F and anti-FHC antibodies are directed.

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Substance P-like immunoreactive fibers in the frog taste organs

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Summary. In the frog tongue, substance P(SP)-like immunoreactive fibers were consistently present in each fungiform papilla, which contained the gustatory apparatus. Numerous SP-like immunoreactive fibers were usually distributed in the periphery of the gustatory disc and formed a varicose meshwork among the ciliated cells encircling the gustatory disc. SP-like immunoreactive fibers were rarely evident inside the gustatory disc. The role of SP-containing fibers in the frog taste organ was discussed.
Key words. Substance P; immunohistochemistry; taste organs; frogs.

Recent immunohistochemical studies demonstrated the presence of substance P(SP)-containing fibers in the taste buds in a few species of mammals[1-3], findings which suggested that SP may be involved in gustatory processes[1,3], as well as in other sensory processes, particularly those related to pain transmission[4]. However, the exact role of peripherally transported SP in the taste organ remains to be elucidated. Frog taste organs have structures different from those in other species[6] and are frequently used in physiology-related studies. There has apparently been no report on the presence of SP in the frog taste organs. In order to clarify the functional role of SP in the taste organ, it is important to elucidate the anatomical association between the SP-containing fibers and the taste apparatus in the frog tongue. The present study is concerned with the immunohistochemical demonstration of SP-containing fibers in the fungiform papillae of the frog tongue.

Materials and methods. The tongues of frogs (*Rana catesbiana*, weighing 240–400 g) were investigated.

Immunohistochemical procedures. The frogs were anesthetized with 5% urethane, and perfused with a cold solution of 0.2% picric acid and 2% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Small blocks of the tongues were immersed in the same fixative for 2 days, then placed in 0.1 M phosphate buffer (pH 7.4) containing 15% sucrose for 2 days, frozen with dry-ice in isopentane and cut into transverse sections (20 μm thick) in a cryostat. Free floating sections were immersed in 0.3% Triton X dissolved in phosphate buffer saline (PBS) for 1 week at 4°C. The sections were then stained with the following materials, in the order indicated: 1) rabbit anti-SP serum (IBL, Japan) at a dilution of 1:200 (diluted with PBS) for 24 h at 4°C; 2) goat anti-rabbit IgG (Cappel) (1:40) for 40 min at room temperature; and 3) peroxidase-antiperoxidase complex (1:40) for 40 min at room temperature. After the immunoreaction had been visualized with diaminobenzidine tetrahydrochloride, they were mounted on slides.

Control sections were processed in parallel, and in the same manner, except that they were incubated with primary antiserum preabsorbed (0.063 mg/ml) with synthetic SP (Peptide Institute Inc., Japan) or only PBS instead of primary antiserum.

For the immunoelectron-microscopic study, the method developed by Somogyi and Takagi[5] was used. In brief, the frogs were anesthetized with urethane and perfused with picric acid (0.2%)-paraformaldehyde(2%)-glutaraldehyde (0.15%) fixative. Small blocks of the tongues were immersed in picric acid (0.2%)-para-

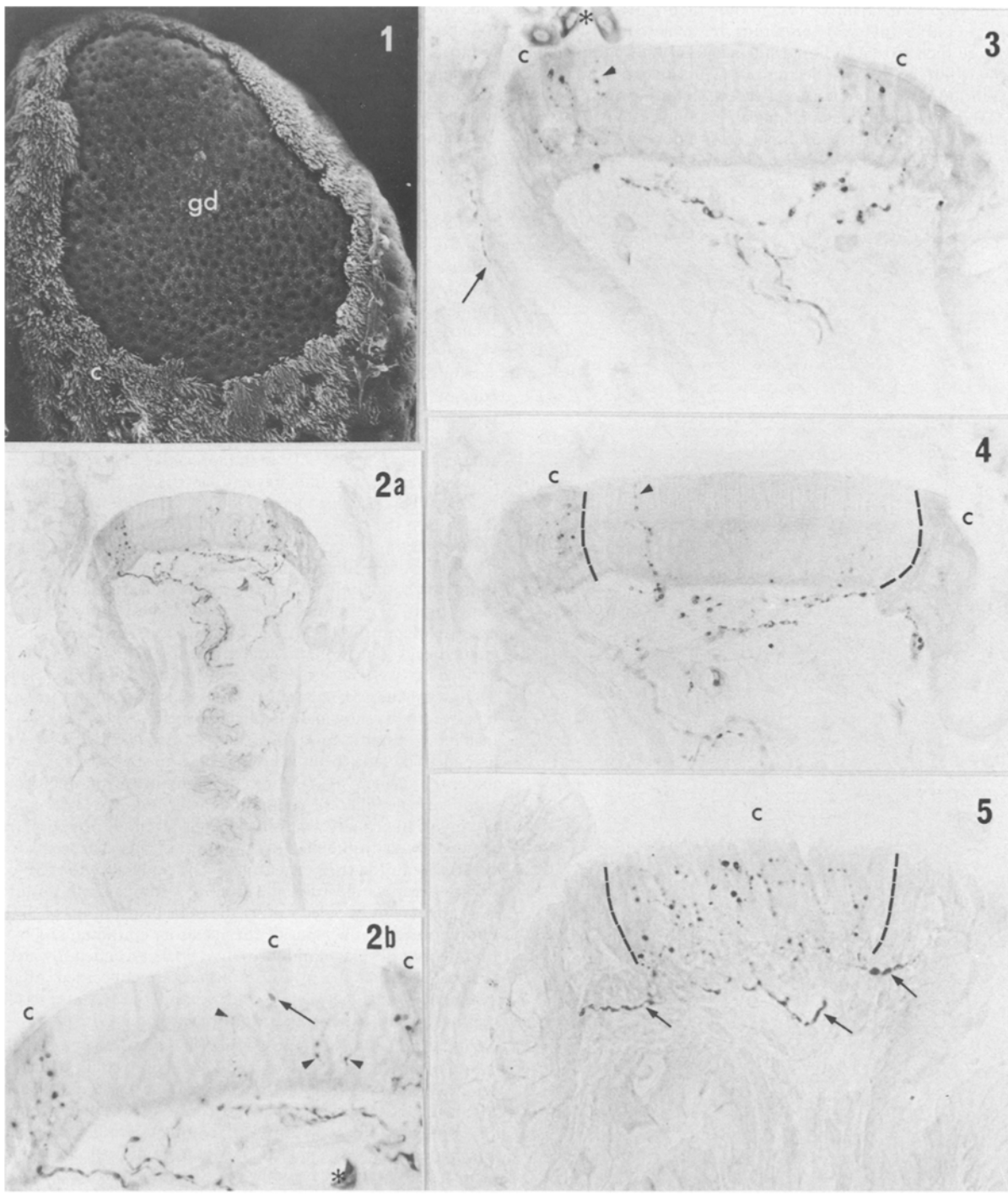

Figure 1. Scanning electron microscopic view of the frog fungiform papilla. The gustatory disc (gd) is obviously demarcated by cilia of ciliated cells(c); × 300.

Figures 2–5. Light-field photomicrographs showing SP-like immunoreactivity in the frog tongues. Fig. 2a. Transverse section through the middle of a fungiform papilla. SP-like immunoreactive fibers can be seen within the nerve bundle running through the core of the fungiform papilla. Note the lack of SP-like immunoreactive fibers in two adjacent filiform papillae; × 125. Fig. 2b. Details of the distribution of SP-like immunoreactive fibers in the epithelium of the fungiform papilla of fig. 2a. Note a SP-like immunoreactive fiber at the left margin just entering the ciliated cell group. An asterisk shows a non-specific SP-like immunoreactive cell in the capillary. c, ciliated cells; × 300. Fig. 3. Transverse section through the middle of a fungiform papilla. Note most of the SP-like immunoreactive fibers ascended between the ciliated cells(c). A fine SP- like immunoreactive fiber can be seen in the adjacent filiform papilla (arrow). An asterisk shows several non- specific SP-like immunoreactive cells, situated on top of the surface of the papilla; × 250. Fig. 4. Transverse section through the middle of fungiform papilla, showing a thin SP-like immunoreactive fiber ascending through the gustatory epithelium from the basal region to the surface. c, ciliated cells; × 250. Fig. 5. Transverse section through the edge of a fungiform papilla, showing a remarkable varicose meshwork of SP-like immunoreactive fibers among the ciliated cells. Note the varicose terminals of various sizes located just under the surface. Compare the meshwork with the profiles of SP-like immunoreactive fibers running under the epithelium (arrows). c, ciliated cells; × 300.

formaldehyde (2%) fixative for 2 days, then placed in 0.1 M phosphate buffer (pH 7.4) containing 15% sucrose for 3 days. They were then frozen in liquid nitrogen and thawed in 0.1 M phosphate buffer (pH 7.4). Sections of 60 µm thickness were cut on a Vibratome and immersed in the same buffer overnight at 4°C. After the immunohistochemical staining, using the same procedure as that described above, the sections were checked using a light microscope, postfixed in 1% OsO_4 in 0.1 M phosphate buffer (pH 7.4) for 1 h, dehydrated and embedded on slides in Epon 812. Ultrathin sections were contrasted with uranyl acetate followed by lead citrate and examined in a JEM 7A electron microscope.

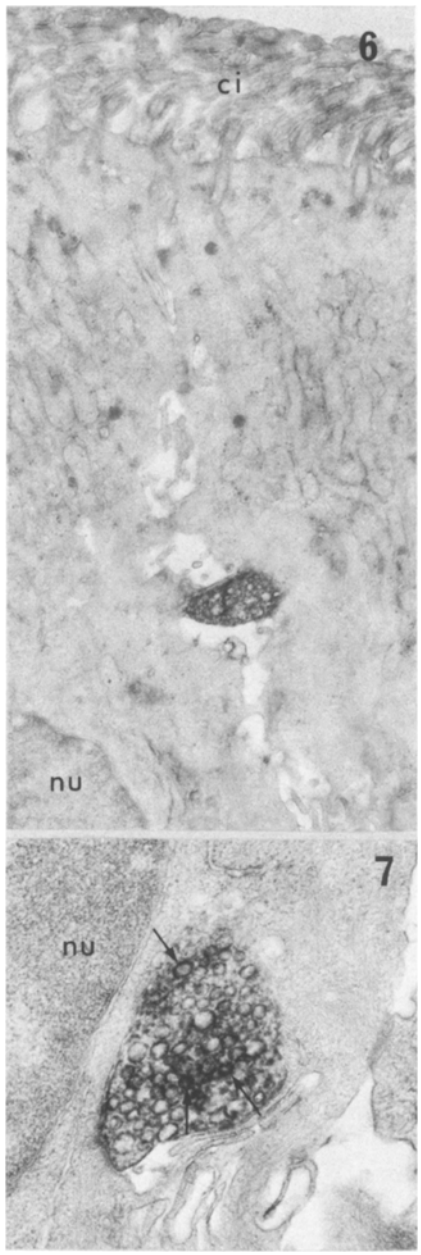

Figure 6. Electron micrograph showing a SP-like immunoreactive fiber located between two ciliated cells. Electron dense immunoreactive deposits are diffused throughout the fiber. ci, cilia of the ciliated cells; nu, nucleus of the ciliated cell; × 12,800.

Figure 7. Electron micrograph showing a SP-like immunoreactive fiber ensheathed by the cytoplasm of the ciliated cell. The fiber is filled with vesicles and electron dense immunoreactive products, particularly around the membrane of the vesicles (arrows). There is no evident synaptic contact between the fiber and the ciliated cell. nu, nucleus of the ciliated cell; × 24,000.

Scanning electron microscopy. To observe the surface of the fungiform papillae, the excised tongue, after pithing, was washed with 0.1 M phosphate buffer (pH 7.4) for the removal of excess mucous and cut into small blocks. After being fixed in a solution of 2% paraformaldehyde and 2% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 2 h, the tissues were postfixed in 1% OsO_4 in the same buffer for 2 h, were rinsed in distilled water and dehydrated in a graded series of ethanols. After critical point drying, the tissues were coated with gold and examined under a Hitachi-S430 scanning electron microscope.

Results and discussion. In the frog tongue there are two types of papillae, filiform and fungiform. The latter are the only ones related to taste organs[6]. The gustatory epithelium is of a disc-like shape, is located at the top of the fungiform papilla and is surrounded by ciliated cells. The ciliated cells are confined to the edge of the fungiform papilla and disappear on the sides of the papilla (fig. 1). SP-like immunoreactive fibers were consistently distributed in each fungiform papilla (figs 2a, 2b, 3, 4, 5), whereas only a few SP-like immunoreactive fibers were occasionally evident in the filiform papillae (fig. 3). SP-like immunoreactive fibers occupied part of the nerve bundle ascending from the base to the top of the fungiform papilla and branched just before entering the epithelium (fig. 2a). In most sections through the middle of the fungiform papilla they turned laterally and sent thin branches mainly into the periphery of the gustatory epithelium (fig. 3). The thin branches with obvious varicose profiles extended from the basal region to the free surface among the ciliated cells and formed varicose terminals just under the free surface but were never exposed outside the epithelium (figs 2b, 3). In some fungiform papillae, only a few thin SP-like immunoreactive fibers, showing less obvious varicose profiles appeared inside the gustatory disc (figs 2b, 3, 4, arrowheads). They often ascended directly from the basal region and terminated just under the surface (fig. 4). A recent immunoelectron-microscopic study[3] revealed that SP-containing fibers in the rat taste buds are not involved primarily in conveying gustatory information from the taste buds to the medulla oblongata. This also seems to be the case with the frog taste organ. The possibility that SP-containing fibers could transmit gustatory stimuli from gustatory cells appears to be ruled out by the poor distribution of SP-like immunoreactive fibers inside the gustatory epithlium and by the finding that SP-like immunoreactive fibers ascended toward the surface; as far as the frog taste organs are concerned, afferent synaptic contact between the nerve and the gustatory cell is confined to the base of the gustatory epithelium[6].

Some authors[1,7] have proposed that SP is involved in the maintenance of tast buds, as a trophic factor. Nagy et al.[2] denied this from the finding that the integrity of the taste bud was not affected, under the experimental conditions of neurotomy of SP-containing fibers or capsaicin treatment. On the other hand, the hypothesis was supported by an immunoelectron-microscopic study: the nerve endings of SP-containing fibers showed axon-like profiles rather than dendrite-like ones[3]. In the present study the distribution of SP-like immunoreactive fibers in the frog fungiform papillae was found to be similar to evidence obtained in the rat taste buds: dominant distribution of SP-like immunoreactive fibers in the perigemmal region[2]. Considering the results of autoradiographic studies; the renewal of taste bud cells occurred mostly at the peripheral region of the gustatory epithelium[8,9], this finding is at least compatible with the hypothesis.

On the other hand, in rare cases in which a few ciliated cells were situated in the middle part of the gustatory disc, the varicose profile of SP-like immunoreactive fibers was found in the ciliated cell group (fig. 2b, arrow). Thus, it would be expected that SP-like immunoreactive fibers are distributed in the ciliated cell group proper, rather than only in the peripheral region. In the

perpendicular section just through the edge of the papilla, the presence of varicose meshworks of SP-like immunoreactive fibers among ciliated cells was clearly shown (fig. 5). The meshwork with varicose profiles of various sizes was localized only in the ciliated cell group and covered the whole of it. Our immunoelectron-microscopic study confirmed the presence of nerve endings of SP-like immunoreactive fibers which made contact with the ciliated cells and contained numerous vesicles of various sizes (60–125 nm in diameter) (figs 6, 7). These findings strongly suggest that SP-containing fibers influence the ciliated cell proper. Little is known about the presence of ciliated cells in the taste organs of other animals. They may be supplementally involved in gustatory processes by propelling fluid or mucous films over the surface of the epithelium, because the structure of the cilia is typical of those commonly described as being motile[6] (fig. 6).

Interestingly, SP-containing fibers are distributed in other organs including motile cilia[10, 11]. The present study revealed that SP-like immunoreactive fibers in the frog tongue were distributed in each fungiform papilla, especially among the ciliated cells surrounding the gustatory epithelium.

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Calcium binding in chemically skinned fibers of rat myocardium during force development

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Summary. The amout of bound calcium and force were measured in chemically skinned rat myocardial fibers during isometric contraction at different concentrations of free Ca^{2+}. The data obtained suggest that calcium binding by cardiac myofibrils is cooperative and probably depends on mechanical tension.
Key words. Ca^{2+} binding; force; myofibrils; myocardial contraction.

It is well known that the contraction of cross-striated muscle is initiated by calcium ions binding to troponin. However, it is still not clear what is the exact quantitative relation between the degree of occupation of Ca^{2+} binding sites and muscle mechanical response, in particular the value of isometric tension. The results of a number of studies[2-5] suggest that the parameters of calcium binding by myofibrils are not constant, but depend on actomyosin interaction. If this is the case, then the characteristics of Ca^{2+} binding to troponin in the muscle, generating force, might be different from those for isolated troponin or myofibril suspensions. In some studies[6-9] calcium binding by skinned fibers of skeletal muscle was measured at different pCa values; however, the developed force was not determined. The only study of both Ca^{2+} binding and force at different pCa values was carried out by Fuchs and Fox using glycerinated skeletal muscle fibers[10]. There have been no similar studies on cardiac muscle. Therefore, the aim of the present study was to measure Ca^{2+} binding by chemically skinned fibers of rat myocardium during force development at different concentrations of free Ca^{2+}.

Materials and methods. The bundles of fibers, 0.3–0.4 mm in diameter, were isolated from left ventricular endocardial surface and incubated for 2 days at 0 °C in a calcium-free solution, with EGTA and 1% Triton X-100 to ensure that destruction and removal of the sarcolemma and the intracellular membrane structures would be as complete as possible. All the solutions used in the study contained 3 mM free Mg^{2+}, 5 mM MgATP, 15 mM phosphocreatine, 20 mM imidazole, 80 mM potassium propionate, 5 mM glucose, 100 μM Ca, 0.3 mM dithiothreitol, pH 7.0, and amounts of EGTA to adjust the pCa to the desired value between 7 and 4.5. The composition of the solutions was calculated by using the set of equations[11] with dissociation constant values given by Fabiato[12]. The solutions were made using deionized water. The contamination of the solution with admixed calcium was assessed by plasma emission spectrometry. The fiber bundles were fixed with tungsten clamps. One clamp was connected to the force transducer (FT.03, Grass Instrument Co.). The relaxed fibers were stretched to the point at which tension appeared, and additionally by 20% of this length. The preparations were incubated in 1 ml of test-solution with ^{45}Ca and ^{3}H-glucose (a marker for solvent space) for 5 min, then the labeled substances were extracted for 10 min in 1 ml of 'cold' solution with pCa 7. Then the procedure was repeated with another test-solution. Incubation and extraction were performed with intensive stirring of the solutions.

In preliminary experiments the time-course of elution of the bound ^{45}Ca from the fiber bundles into the extraction solution with pCa 7 was determined (fig. 1). It can be seen that after 10 min the label exchange between the preparation and the solution is completed. Therefore, it is clear that 10 min are sufficient for extraction of practically all the labeled molecules.

All the bundles were passed through 13 test-solutions with pCa from 7 to 4.5. The experiments were performed at room temperature (22–23 °C). The radioactivity of the incubation solutions and the extracts was determined using a 'Rackbeta' liquid scin-