Functional Anatomy of Frog's Taste Organs.

While frogs are extensively used in electrophysiological investigations on taste (RAPUZZI and CASELLA[1], SATO[2], NOMURA and SAKADA[3]) precise knowledge of the anatomy of their taste organ is still unsatisfactory. Comparison of the frog's taste organs with those of mammals are virtually absent and the available observations on frogs (UGA[4], UGA and HAMA[5]) provide a rather vague picture of the relationships between nerve fibres and taste cells and between taste cells themselves.

This report describes in preliminary form the data obtained with light microscope (LM), transmission electron microscope (TEM) and scanning electron microscope (SEM) with the intent to elucidate the fine morphology of the taste organ, the spatial arrangement between cellular and nervous components and their histochemical characteristics. Data on the turnover rate of the cells composing the taste organ are also included. A detailed study on the subject will be reported in a separate communication.

Materials and methods. Tissues from the tongue of adult frogs (*Rana pipiens*) were prepared for LM by fixing entire tongues in Bouin, embedding in paraffin and, after sectioning, staining with iron hematoxylin or Mallory. Impregnation of the tissue with Bodian method was also performed for the study of the nerves. Single fungiform papillae from tongues of the same animals were isolated and fixed for both LM and TEM in a mixture of 4% paraformaldehyde in phosphate buffer (pH7.2) followed by postfixation in 1% OsO_4 in the same buffer. Papillae were then dehydrated in alcohols and embedded in Araldite, oriented as desired, then sectioned to 1 µm thick sections and stained with toluidine blue for LM. For TEM, sections 600 A to 800 A thick were cut from similar material. One papilla was longitudinally sectioned into ultrathin sections with a complete and uninterrupted series to allow a complete three dimensional reconstruction of the taste organ at an ultrastructural level. Sections were picked up on formvar-coated, carbon-stabilized 75 mesh grids. The sections were subsequently stained with uranil acetate and lead citrate. An electron microscope, Philips EM 200, was used at 60 kV with magnifications on the negative ranging from ×1000 to ×50,000. Every section of the series was photographed and a three dimensional reconstruction of the papilla performed. Tongues to be used for SEM studies were thoroughly washed in frog's Ringer to remove the mucus and then fixed in 3% gluteraldehyde in phosphate buffer. Dehydration was carried out in ascending alcohols after which the pieces were coated in an evaporator with an estimated 200 A thick layer of gold or palladium. Observations were performed on a Cambridge SEM at magnifications ranging from ×16 to ×20,000. For the histochemical study of adrenergic elements the method of FALK and HILLARP[6] for the demonstration of catecholamines by fluorescence microscopy at LM level was used. At TEM level catecholamine containing vesicles were depleted by reserpine and then repleted by uptake of exogeneously-introduced catecholamines (AXELROD[7], HOKFELT[8]). Cholinergic nerves were demonstrated using the Karnowski's modification of KOELLE and FRIEDENWALD method (KOELLE and FRIEDENWALD[9], EL BADAWI et al.[10], KARNOWSKI[11]) for the demonstration of acetylcholinesterase activity at LM and TEM levels. Tritiated

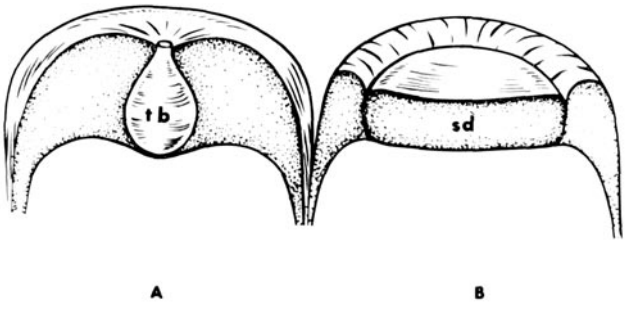

Fig. 1. a) Schematic outline of a fungiform papilla as observed in rat. Similar scheme holds for other mammals as well. The taste bud (tb) has a pear-like outline. One or more of these taste buds can be observed in these papillae. b) Schematic outline of frog's fungiform papilla. The bud-like structure of Figure a) is substituted by a disc-like (sd) structure which covers the entire dorsal surface of the papilla. While more than one taste bud can be observed in each mammalian papilla, only one sensory disc is found in each frog papilla.

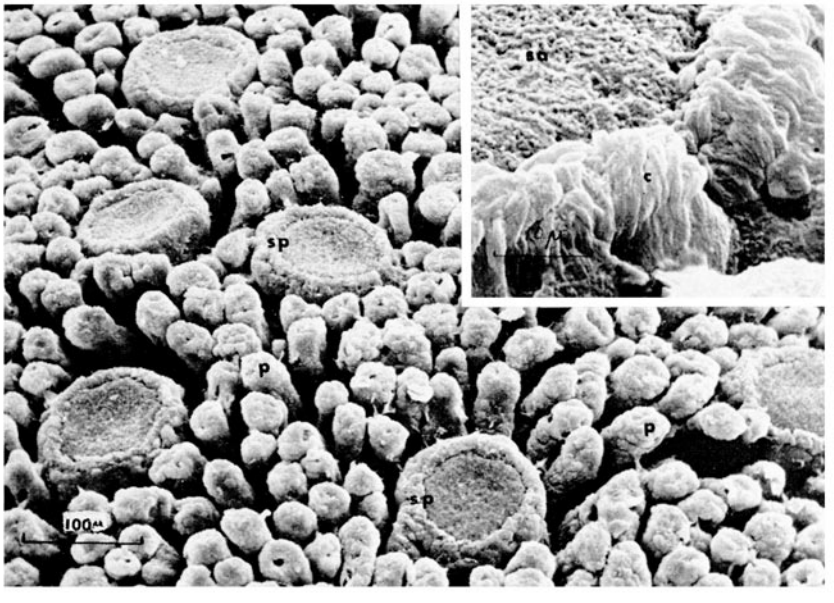

Fig. 2. Scanning electron micrograph of the frog's tongue. The surface is covered with a large number of filiform papillae (p). Many fungiform papillae (sp) are interspersed among them. The mark is 100 µm. The insert represents an enlarged portion of a fungiform papilla to demonstrate the sensory area (sa) and the crown of cilia (c) surrounding it. A carpet of microvilli covers the sensory area. The mark is 10 µm.

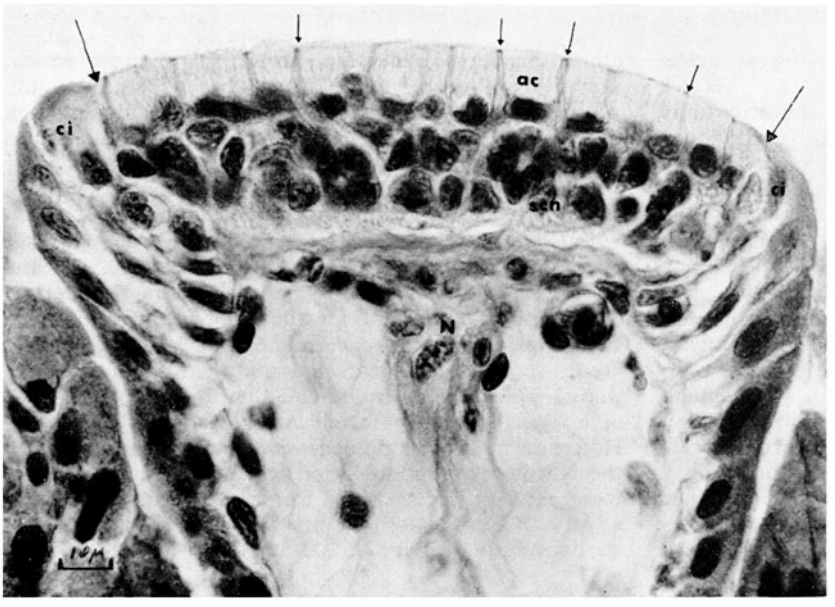

Fig. 3. Histological section 10 µm thick of the top of a fungiform papilla demonstrating the sensory disc (between the 2 large arrows) and the ciliated cells (ci) limiting its edges. The nerve bundle (N) reaches the sensory disc from underneath. Associate cells (ac) occupy most of the sensory surface while the dendrites of the sensory cells wedged in between the latter come in touch with the environment only through their tips (small arrows). Nuclei of sensory cells (scn) occupy the 2 inferior thirds of the disc.

thymidine was used to study the turnover rate of the taste organ cells and the labeled components were investigated by autoradiography (BEIDLER et al.[12]).

Results. Contrary to most other vertebrates, frog's taste organs are represented by an epithelial disc rather than a 'bud' (Figures 1a and 1b). The disc occupies almost the entire dorsal surface of the fungiform papilla, measures 100 µm in diameter and is covered by a layer of microvilli. Sensory papillae are illustrated in Figure 2 with the SEM, which shows in detail the disc surrounded by a crown of cilia. In order to investigate the internal structure of the organ, LM and TEM techniques have been used. Figure 3 represents a LM section longitudinal along the axis of the papilla. It shows the sensory disc area which measures 100 µm wide and 40 µm thick. The disc is clearly demarkated from the ciliated epithelial cells (ci) which surround it. The sensory disc is made up of 2 types of cells whose nuclei stain differently with the Mallory technique. The first cell type occupies the upper third portion of the disc and it will be referred to throughout the text as the 'associate cell'. The second cell type referred to as 'sensory cell', while extending from the free surface to the basal lamina of the disc with its processes, has the cell body (or nucleated part) located in the 2 inferior thirds of the epithelium.

The associate cells are arranged in a single row and make up most of the free surface of the sensory area which is covered with microvilli (Figures 3 and 4). Their cytoplasm is filled with secretory granules. The nuclei of these cells form a single, orderly row that never extends below the upper third portion of the sensory disc. Cytoplasmic processes of the associate cells, however, extend downwards to the level of the basal lamina of the sensory epithelium. These processes intermingle among the sensory cells and nerve fibers, with frequent contacts with both; such contacts however do not seem to be functionally specialized.

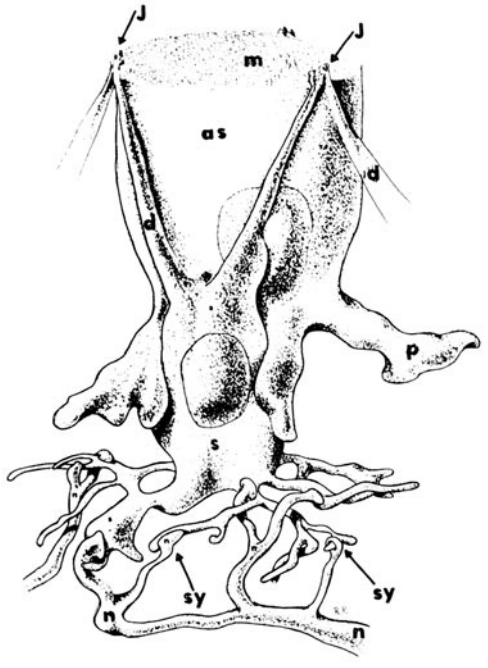

Fig. 4. Three-dimensional representation of the relationship between a sensory cell (s) and an associate one (as) with their nerve attachments. Dendrite-like processes (d) of the sensory cell partially engulf the associate cell body and join dendrites of neighboring sensory cells at tight junctional contacts (J) (arrows). Nerve fibers (n) contact the sensory cell by mean of synapses (sy) (arrows).

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The sensory cells strongly indicate their participation in the sensory process by their morphological characteristics and specialized contacts with the sensory nerves. These cells extend from the surface of the disc, that they reach with branching dendrite-like processes, to the basal lamina of the disc that they never surpass. At some distance above the nucleus, the cytoplasm of the sensory cells branches to three or more processes that engulf the upper part or nucleated portion of the associate cells and reach the free surface, however, leaving the microvillar surface free. This relation of the associate cell with the sensory one is diagramatically represented in Figure 4 which also shows the complexity of these synaptic contacts of the sensory cell cytoplasmic processes to the nerve fibers. Figure 5 shows a block of the sensory disc containing 5 sensory cells and 7 associate cells to demonstrate the interrelation between the 2 cell types as well as the mode of contact and the communication between sensory cells themselves. This communication is evident by the presence of tight junctional complexes of the gap type between the tips of dendritic processes. Such junctions are usually accepted as evidence of electrical coupling (BENNETT et al.[13]). At the proximal pole of the sensory cells the cytoplasm branches again in irregular, stumpy processes (Figure 4) which establish synaptic contacts with the incoming sensory fibres. In these branches the ultrastructural details of the cytoplasm are uniquely represented by mitochondria and dark-cored vesicles some 800 Å to 1000 Å in diameter. This highly specific organelles content is in contrast with the pattern shown by their perikarion, where no vesicles are observed and the common cytoplasmatic organelles such as ER, Golgi, free ribosomes, mitochondria and a dense matrix are usually observed. Along the processes of the sensory cells there are zones of specific contact with the sensory nerve fibres that show the morphological characteristics of synapses (Figure 6). The sensory cell processes with their vesicular content act as presynaptic profiles, while the sensory fibres are the post-synaptic element of the unit. The dark-core vesicles observed in the sensory cells have been proved to contain norepinephrine by means of LM fluorescence techniques and by means of TEM observations of material where depletion of the granular content was obtained with reserpine. Repletion of the same vesicles was subsequently obtained by means of uptake of exogeneously-introduced norepinephrine. The sensory nerve fibres forming the post-synaptic side of the contact were observed to contain clear vesicles 400 Å to 600 Å in diameter. While true synaptic contacts from the nerve fibre to the sensory cell were not so far observed, with typical accumulation of clear vesicles against a nerve membrane thickening, clumps of clear vesicles were indeed observed in the nerve lumen and further investigation is in progress to clarify their functional significance. The nerve profiles were proven to be cholinergic both at LM and TEM levels by means of the method of KOELLE and FRIEDENWALD[9].

Concerning the existence of a turnover mechanism in the taste cells of frog, there are data available in the literature (ROBBINS[14]) showing that the cells of the taste

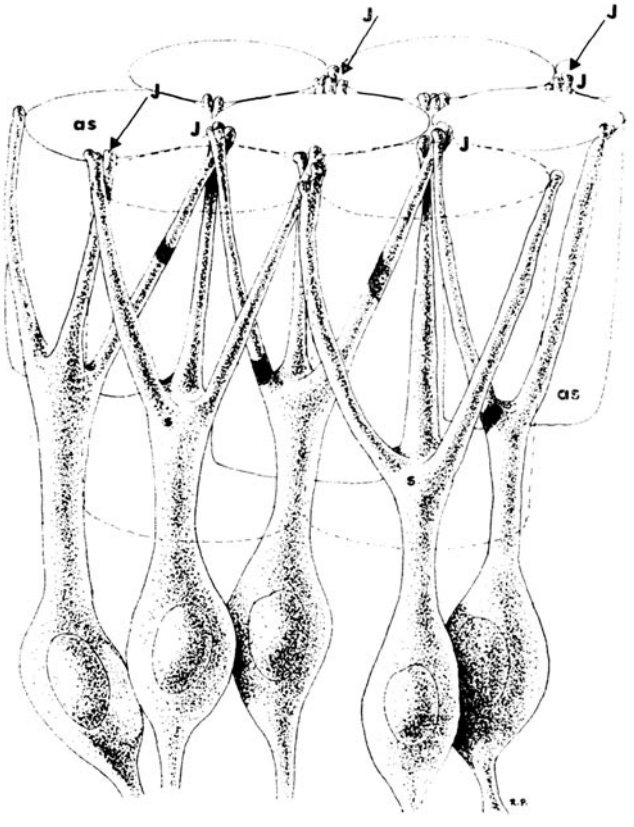

Fig. 5. A schematic three dimensional drawing which represents a block of the sensory disc made up of 7 associate cells (as) and 5 sensory ones (s), to illustrate the relationship between the 2 types of cells. Microvilli at the free surface of the associate cell are not drawn, so also the nerve. The tight junctional contacts (J) between the sensory cell dendrites are greatly emphasized (arrows).

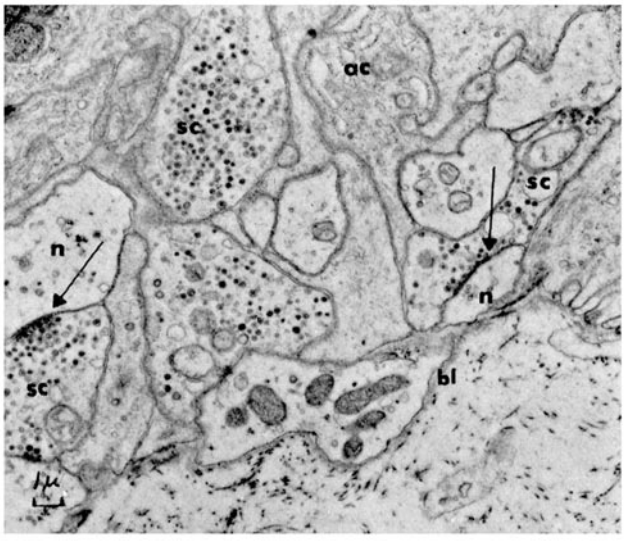

Fig. 6. TEM picture of the basal region of the sensory disc showing the basal lamina (bl) and profiles of sensory cells (sc) full of dark-cored vesicles and nerve fibres (n) with agranular (clear) vesicles. Arrows indicate synaptic contacts with clumping of vesicles between sensory cell profiles and a nerve fibre profile. The profile of an associate cell (ac) is also indicated.

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organ do indeed turnover. In our experiments so far we have observed that not only the cells of the taste organ turnover but that associate and sensory cells turnover at a different rate. The exact timing of the phenomenon for the 2 cells population will be furtherly elaborated in a subsequent report.

Conclusions. 1. Two morphologically-distinct types of cells exist in frog's taste organs, only one of which has direct functional involvement with the afferent nerve fibre.

2. The described sensory cells with their dendrite-like processes are typical of the frog and have not yet been described in any other animal. The authors believe that these processes are the receptive part of the sensory cell and that the tight junctional complexes observed between the distal parts of these dendrites should be furtherly investigated in regard to the possibility that it may subserve some form of interreceptor interaction.

3. The presence of typical synaptic contacts between nerve fibres and sensory cells shown by accumulation of dark core vesicles and mitochondria on the presynaptic membrane as well as membrane thickenings is taken as evidence for the existence of chemical transmission between the sensory cells and the sensory fibres. The data obtained through the outlined histochemical procedures, points to norepinephrine as the neurotransmitter involved in the sensory process of frog's taste organ[15].

Résumé. Dans les bourgeons gustatifs de *Rana pipiens* deux groupes de cellules ont été découverts. Le premier est directement en relation avec la réception de stimuli, tandis que le second ne comprend que des cellules d'association. Les cellules sensorielles offrent des contacts synaptiques avec les fibres sensorielles.

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Patterning of Resorption in the Cartilages from the Long Bones of Newts Treated with Parathyroid Extract[1]

Gross morphological studies of skeletal elements in *Rana pipiens* and *Triturus viridescens* treated with parathyroid extract have shown that there is an increase in the amount of matrix in the long bones which can be stained with methylene blue[2]. Widened and more densely staining matrix was observed at the ends of the tibiofibula, and the phalanges exhibited double bands of matrix between the articular cartilaginous cap and the ossified diaphyseal bone in the majority of animals after hormone treatment (Figure 1). When new bands of matrix appeared beneath the epiphyseal cap, they lay in a region topographically comparable to the zone of provisional calcification in mammalian long bone (= metaphysis). The types of cells which may be responsible for these changes in the bone matrix of hormone-treated amphibians are undetermined. Although SCHLUMBERGER and BERK[3] were unable to elicit a hormone-mediated increase of osteoclasts in bone of *R. pipiens*, other laboratories[4,5] have described elevated osteoclast numbers in several amphibian species as a direct or indirect (calcium-free lavage)[6] consequence of parathormone action. The exact nature of the 'metaphyseal' band of stainable matrix formed after hormone treatment is, however, unknown. The present study was, therefore, undertaken to investigate the origin of this tissue in the newt, *T. viridescens*, employing histochemical techniques.

Materials and methods. The majority of animals used in these studies were *T. viridescens viridescens*, but in a few cases, the closely related *T. viridescens dorsalis* were employed. All experiments were done with summer animals which were warm-acclimated in the laboratory at 18–22 °C for 1 week before use, in order to eliminate seasonal and temperature modifications of the response to parathormone. Newts were maintained in individual containers and environmental water was changed daily; calf liver was force fed once weekly. To avoid unfavorable changes due to conditions of captivity, no animals were used beyond 1 month after arrival in the laboratory.

The 104 *T. viridescens v.* used in this study weighed 1.0–4.5 g, and they were randomly placed into control and parathyroid extract-treated groups.

The experimental animals were injected i.p. with parathyroid extract (PTE) once daily for 7 days. Newts weighing over 3.0 g received 15 U; those with body

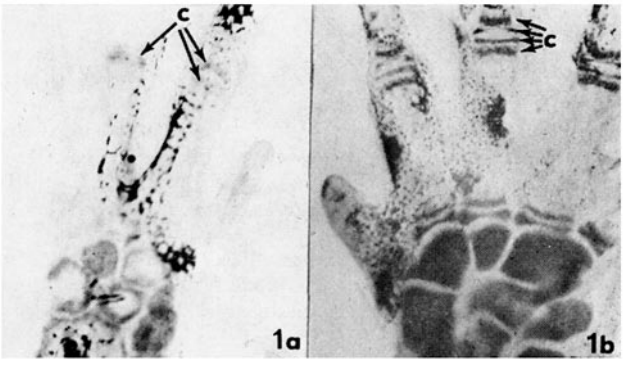

Fig. 1. Micrographs of appendages from control (1a) and PTE-treated newts (1b). Note the increased staining density and doubling of the carpal cartilages (c) in the PTE-treated animal.

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