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# Ultrastructure and apical secretion of peripheral epithelial microreceptors in trout embryos

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SUMMARY

We have found epithelial microreceptors to be mainly distributed on the cephalic epidermis around the ear and eye of trout embryos. The same types of cells as those described by other authors were identified in taste buds. The basal cells do not extend a process into the taste pore, whereas supporting and chemosensory cells do extend into it. Nerve elements reach the basal and apical pores of the epithelial microreceptors, the latter communicating with the exterior medium. With SEM, the epidermal surface shows several pits corresponding to the pores. The appearance of epithelial microreceptors in fish occurs relatively early on embryonic development. In early embryos of *Salmo trutta* (34 days postfecundation) they are found resting on the developing basement membrane. The epidermis is nonstratified and thin, and taste buds are composed of a few cells. Innervation is not yet established. During these stages of development, receptors show the same characteristics as those observed in the adults.

**Key words:** Trout embryos - ultrastructure -lectin - taste buds

## INTRODUCTION

In recent years, numerous studies concerning the morphology of vertebrate taste buds (TBs), their ultrastructure and function have been carried out (Murray, 1971, 1973, 1978, 1986; Roper, 1989), most of them focused on teleosts (Reutter, 1978, 1980, 1982; Tucker, 1983; Caprio, 1988; Jakubowski, 1983; Jakubowski and Whitear, 1990). Although the different types of cells comprising TB have been described, difficulty still remains when establishing a clear nomenclature for the epithelial sensory cells, primarily based on their staining properties. Hara (1992) described two different types of cells in the sensory epithelium of the TB: the so-called light or sensory cells, and the dark or supporting cells.

Transmission and scanning electron microscopy studies have provided some clues for the morphological and functional differentiation of both types of cells. Furthermore, lectin histochemistry (Witt and Reutter, 1988, 1990) reveals some differences between sensory cells and supporting cells (light and dark) and constituent TB cells. This technique has also been valuable in identifying the mucus substances covering the TB receptor field (Reutter, 1980).

We found taste receptors in trout embryos at a very early stage of development (34 day postfecundation), many of them located in the head epidermis, mainly around the eyes, ears and nostrils. There are no ultrastructural differences between these and those located in the mouth cavity, the pharynx, the proximal oesophagus, the branchial region, and on the gill arches.

One question is whether the gustatory sense (typically visceral) spreads into a cutaneous somatic territory. The present study focuses on the ultrastructure of peripheral sensory organs with a view to contributing with ultrastructural and histochemical characteristics to the differentiation of the types of cells that these sensory organs consist of.

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## Materials and methods

### *Embryos*

Fertilized eggs of *Salmo trutta* were collected from a fish farm in León (Spain) and fixed "in situ" using the fixative described below. Once they were cover-released and microdissected under the stereomicroscope, they were postfixed using 4% paraformaldehyde, and 0.25% glutaraldehyde in 0.1 M PBS pH 7.2, for 1 h at 4°C and finally washed three times in PBS.

### *Scanning microscopy*

Small sections from the cephalic region of these embryos were postfixed in 2% osmium, dehydrated in graded concentrations of ethanol, ending in pure acetone. They were subjected to the critical point method with $CO_2$ and sputter-coated with gold-palladium. The scanning electron microscope study was performed with a JEOL JSM 6400.

### *Transmission microscopy*

Tissue samples were postfixed in 1% osmium tetroxide in PBS, progressively dehydrated in graded concentrations ethanol and propylene oxide, and embedded in a mixture of Epon Araldite resins. Semithin sections (1 µm) were stained using methylene blue-azur II for light microscopy. Ultrathin sections were stained with uranyl acetate and lead citrate. Photographs were taken under a JEOL 840 transmission electron microscope at 60 kV.

### *Lectin histochemistry*

After fixation, specimens were stored in PBS buffer for 2 days at 4°C, before paraffin embedding. Sections were obtained at 5 µm and stained by the Alcian Blue (pH 2.5) periodic-acid-Schiff method.

The information regarding the lectin-horseradish peroxidase (HRP) conjugates used, their concentrations, and their nominal specificities is listed in Table 1. The sources of conjugates were as follows: *Glycine maxima* (SBA), *Canavalia ensiformis* (Con A), *Dolichos biflorus* (DBA), *Ulex europaeus* (UEA I), *Arachis hypogaea* (PNA) and *Triticum vulgaris* (WGA) from Sigma Chemical Co. (St. Louis, MO).

The tissue sections were deparaffinized. Endogenous peroxidase was blocked by a 15 min. immersion of sections in 3% $H_2O_2$ in distilled water. After rinsing in phosphate-buffered saline (pH 7.2), sections were incubated with a lectin-HRP conjugate for 1 hr. at room temperature. Each conjugate was diluted with 0.1M phosphate buffer (pH 7.2). After exposure to the conjugate, sections were rinsed again, incubated for 8 min. in 3-3'-diaminobenzidine-$H_2O_2$ tetrahydrochloride substrate purchased from Sigma and counterstained with haematoxylin.

Control sections were incubated in parallel for 1 hr. at room temperature in a mixture of the lectin-HRP conjugate and the appropriate hapten sugar (Table 1) at a concentration of 0.2 M. The hapten sugars were purchased from Sigma (St. Louis, MO). An additional control was made according to the following procedure: exposure to 3-3'-diaminobenzidine-$H_2O_2$ tetrahydrochloride substrate without previous lectin-HRP conjugates. Sections were viewed and photographed using an Olympus Vanox H3M microscope.

**Table 1.–** Lectins used in this study and their specificities.

| Lectin | Specificity[1] | C[2] | IS[3] |
|---|---|---|---|
| *Canavalia ensiformis* Con A | αMan>αGlc>GlcNac | 10 | Manop |
| *Triticum vulgare* WGA | GlcNac(ß1,4GlcNAc)$_{1\text{-}2}$>ßGlcNAc>Neu5Ac | 10 | GlcNac |
| *Dolichos biflorus* DBA | GalNacα1,3GalNAc >GalNAc | 20 | GalNAc |
| *Glycine maxima* SBA | GalNAcα1,3GalNAc >GalNAc | 20 | GalNAc |
| *Arachis hypogaea* PNA | Galß1,3GalNAc>αßGal | 20 | Gal |
| *Ulex europeus* UEA I | αL-Fuc | 20 | αL-Fuc |

[1] After Goldstein and Poretz (1986)
[2] Concentration: µg/ml
[3] Concentration of inhibiting sugars: 0.2 M

## Results

### *Electron microscopy*

Soon after the cephalic neural folds become fused and lose contact with the ectoderm, cell populations derived from the neuroepithelium converge, giving a variety of derivatives. Among them, the epithelial microreceptors (so-called taste buds) can be found, just dorsal and lateral to the cephalic region and next to the lateral line (Fig. 1).

This cell population is part of the peripheral nervous system originating in the neural crest cells. This group of cells makes up isolated sensory organs sometimes close each other in the epidermal tissue. These microreceptors contact the basement membrane (B) of the epidermis. Moreover, in a closer view, they seem to remain in contact with the underlying connective tissue, where mesenchymal cells still persist in the undifferentiated stage.

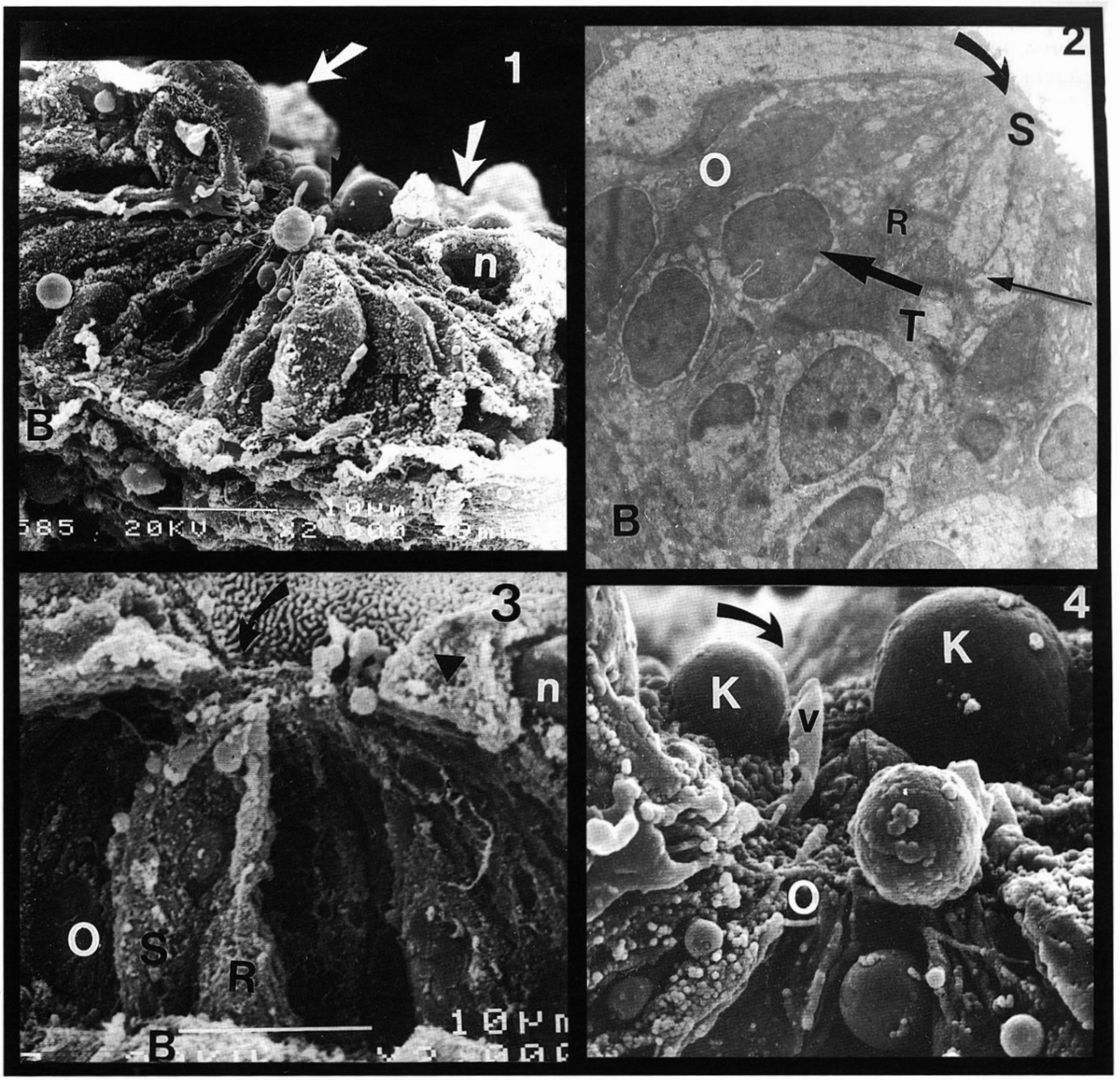


**Fig. 1.**–Transverse section of two epithelial microreceptors (straight arrow), close to each other in the integument, opening to the surface through a gustatory pore (curved arrow) and surrounded by mucous cells (n) and cuboidal epithelial cells (triangle). The different types of cells they consist of are located on the basement membrane (B). x 1,700.

**Fig. 2.**–TEM cross section where the pore (curved arrow) can be observed as well as the groups of different cells (T) resembling the staves of a barrel, and reminiscent of the properly called TBs of other vertebrates; (B) basement membrane; (O) Dark cells; (R) Receptory sensorial cell in the centre of the microreceptor; Light cell cytoplasm (thin arrow); Dark cell nucleus (thick arrow). x 3,500.

**Fig. 3.**–Microreceptor and underlying connective tissue. Mucous cells (n); Secretory pore (arrow); Epithelial pavement cells (triangle); The limiting basement membrane (B) and the light (S) and dark (O) stained cells can be observed, the latter showing a secretory aspect and possibly involved in the synaptic process. x 3,400.

**Fig. 4.**–A higher magnification view showing the pores of the epithelial microreceptors; Secretion products (K) in the proximity of the pore. Depending on membrane specialization, pores can have many microvilli belonging to dark cells (O) and wider microvilli resembling degenerated cilia (V). x 21,600.

Studies based on comparative anatomy show the skin of lower vertebrates to be well innervated. Teleost epithelial microreceptors have nerve endings specialized in detecting thermal, ionic, tact, pain, and pressure changes. In a 34-day old embryo, their structure at the head region level resembles that of the taste buds located at the mucosa of the bucal cavity, pharynx, proximal oesophagus, branchial region and on the gill arches.

Epithelial microreceptors are ovoid, consisting of groups of elongated cells resembling the staves of a barrel (T), extending from the basement membrane (B) to the apex of the TB or pore (arrow) and forming a small concavity in the surrounding epithelium (Figs. 2 and 3). The sensory epithelium of a TB is surrounded by epithelial and mucous (n) cells (Figs. 1 and 3), rendering it less vulnerable to friction and abrasion.

The sensory epithelium of the microreceptors basically consists of sensory receptor cells (R) (Figs. 2 and 3) located at the center of the sensory organ extending to the lumen with pore form. This is most probably the location where sensory transduction takes place.

These sensory epithelium cells can be classified in two different types according to their staining characteristics under light, transmission and scanning electron microscopy: light (S) or neuroepithelial type II cells, and dark (O) or supporting type I cells. We have completed this classification using the three-dimensional morphological description provided by SEM, the ultrastructural findings from TEM, and lectin conjugate functionality studies (Table 2).

Light cells (Fig. 5) resemble chloride cells owing to the presence of some organelles responsible for ion transport. Thus, they can be identified by their elongated mitochondria, more abundant in the apical cytoplasm; an electron-dense smooth endoplasmic reticulum, which differentiates these cells from the dark ones, and many vesicles in close association with the Golgi complex. At the apical pole they terminate in some microvilli (C) that are larger than those observed in the dark cells, resembling degenerated cilia (V).

**Table 2.**–Staining patterns shown by the lectins used in this study.

| Lectin | TB(H)[1] | TB(PH)[2] |
|---|---|---|
| *Canavalia ensiformis* (Con A) | - | - |
| *Triticum vulgaris* (WGA) | + | + |
| *Dolichos biflorus* (DBA) | + | ++ |
| *Glycine maxima* (SBA) | + | ++ |
| *Arachis hypogaea* (PNA) | - | - |
| *Ulex europeus* (UEA I) | - | - |

[1] Intensity of the lectin-carbohydrate reaction: - (negative); + (light staining); ++ (heavy staining).

[2] Abbreviations: TB Epitelial Microreceptor; H Hatching stages; PH Post-hatching stages.

The ultrastructural differences between both types of cells are quite clear: dark cells (Fig. 14) have a greater number of organelles in their cytoplasm, especially more intermediate filaments (F) organized into bundles running longitudinally through the cell, as well as more microtubules. Among the dark cells there are desmosomes and tight junctions typically located at the apical region.

The third type of cells are the basal cells (Z) (Figs. 5, 6, 7 and 8), oriented transversely to the longitudinal axis of the TB and directly in contact with the basement membrane (B), (Fig. 9). The apical plasmalemma of these disk-like cells has impressions of nerve fibres and sensory cell processes and occasionally extends into the nerve plexus to form microvillus-like structures. The nuclei (P) tend to be lobed and, like very specialized embryonary cells, consist of abundant disperse heterochromatin, while euchromatin areas are located more centrally (E). The nuclear envelop forms perinuclear cisternae and some nuclear pores (arrow).

Their cytoplasm is rich in mitochondria, and shows a very prominent and active rough endoplasmic reticulum (re). Abundant ribosomes free in the cytoplasm as well as attached to the membrane can be visualized, pointing to an important function of protein synthesis.

The richness in intermediate filaments (F) can be seen in their assembly in a mesh, which determines the morphology and polarity of these cells, as well as the nature of their ascendent movements from the basal lamina. The polarity is reinforced by microtubules in close association (Figs. 7 and 8).

Marginal cells (W) (Figs. 10 and 12) are located peripherally to the sensory epithelium, just at the border between a TB and the stratified squamous epithelium, and arranged following the elongated and bent shape of the microreceptor. These cells attach through desmosomes and interdigitations. Apically, marginal cells are rounder and more elevated over the epidermal surface level, forming a small hillock with a TB in its center (arrow). They also contain abundant Golgi systems and vesicular structures with secretory and limiting functions.

Light cells synapse onto the fibres of the nerve fibre plexus, to be joined by the ganglionary cell sensory fibre endings of the VII cranial nerve (Weber branch in fishes), IX and X. Their pathways would be somatic-afferent, thus having somatic sensitivity, and would comprise fibres with exteroceptive sensitivity locates in the integument. The basal processes of sensory cells are intermingled between the mass of nerve fibres. Synapses are established at the basal part of the cell. Light cells synapse directly onto the fibres of the nerve plexus. These synapses are rich in small clear vesicles (t) (Fig. 13).

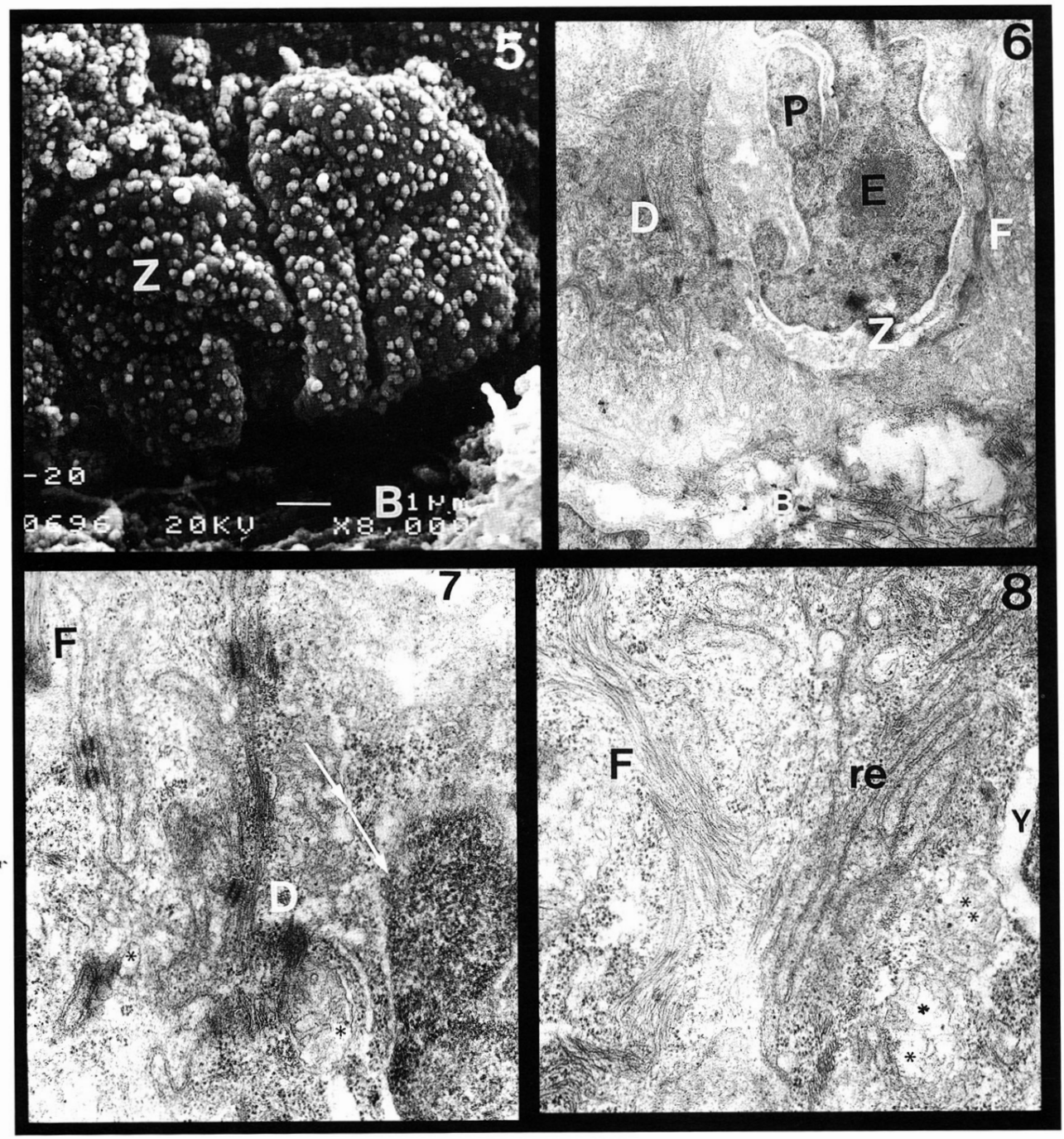


**Fig. 5.**–SEM view of adjacent basal cells (Z) directly in contact with the basement membrane (B), showing indented cytoplasms. x 14,000.
**Figs. 6, 7 and 8.**–Basal cells (Z). Mitochondria (*), abundant endoplasmic reticulum (re), ribosomes, numerous filaments (F), and microtubules can be observed. The lobed nucleus (P) consists of distinct areas of heterochromatin. (E) eucromatin area located centrally in the nucleus. The perinuclear membrane forms cisternae (Y) and numerous nuclear pores (arrow); (D) junctions by desmosomes are abundant. x 20,000.

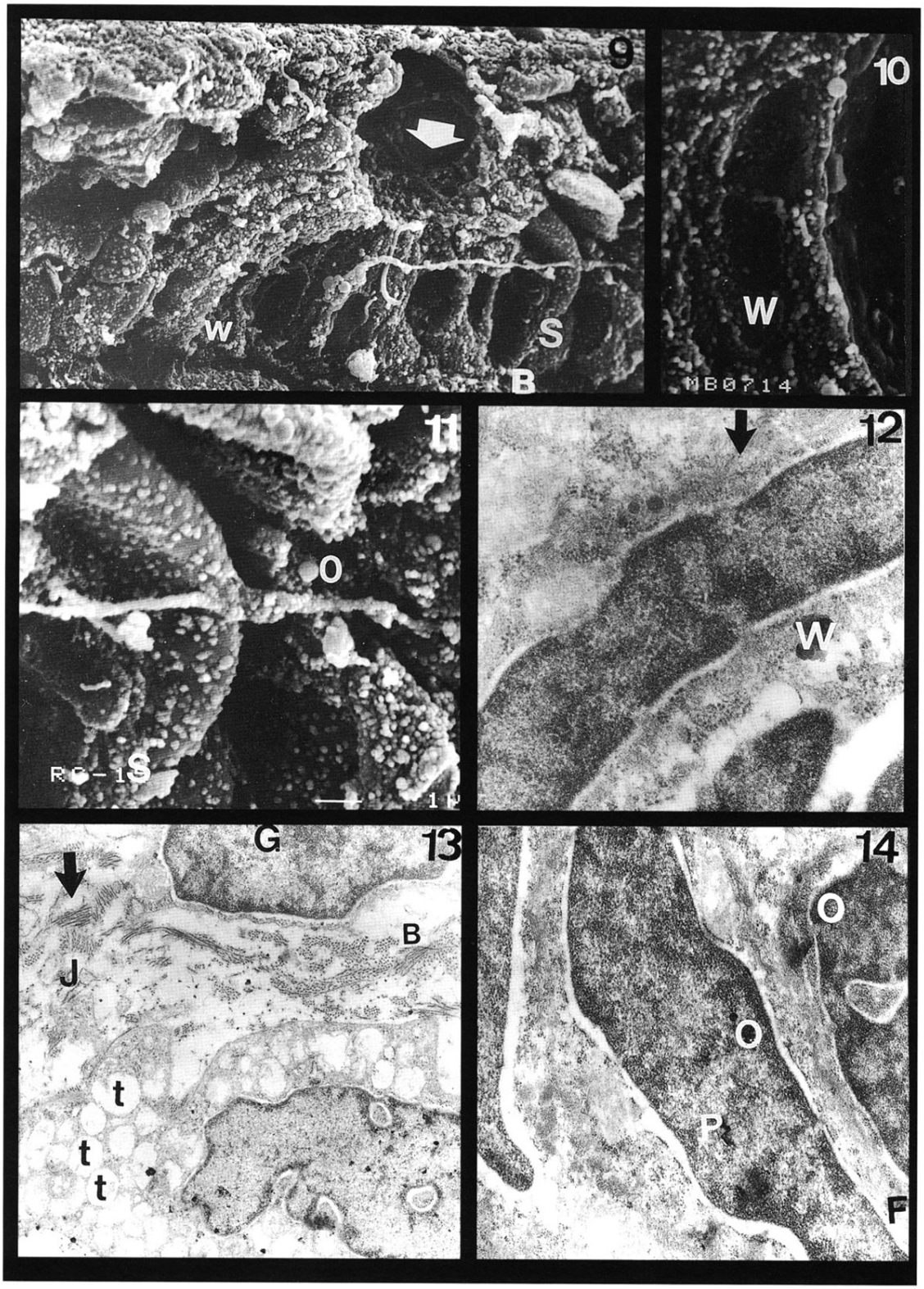


**Fig. 9.**–Photograph showing sensory light cells (S) in close connection with the basement membrane (B). This could provide a three-dimensional image of the synapse. Note the basement membrane where numerous collagen and elastin fibers are located. Two processes arise from the cytoplasm, establishing interconnections with neighbouring cells. The white arrow shows the pore of the microreceptor. x 4,500.

**Fig. 10.**–Marginal cells (W) limiting the epithelial microreceptor at its edge. x 14,000.

**Fig. 11.**–Group of dark (O) and light (S) cells affording a three-dimensional view of the synapse. Light cells synapse directly onto the nerve endings. Cytoplasmic processes interdigitate intensively with neighbouring cells. x 18,000.

**Fig. 12.**–TEM image of marginal cells (W). The elongated nucleus shows a disperse, granular, and fine chromatin. Note many small fibres (arrow) in the cytoplasm. The marginal cells could play a role in supporting the organ as a whole. x 75,000.

**Fig. 13.**–Very active fibroblast (G) differentiated from the embryonic connective tissue forming bundles of collagen fibres (J) and elastin meshes that anastomose (arrow) to give an amorphous appearance. Abundant fundamental substance as a result of the nature of embryonic tissue. These cells with elongated and branched cytoplasm form the basement membrane (B). A cell with a secretory aspect can be observed; this could participate in chemical synaptic processes (t). x 8,500.

**Fig. 14.**–Dark cells (O), elongated nuclei (P), abundant microfilaments (F) parallel to the nuclear membrane and microtubules. x 75,000.

Dark cells seldom synapse onto the nerve fibres of the plexus, but have lobe-like processes that ensheath the apical parts of the light cells; interdigitations, desmosomes and tight junctions can also be found at different levels between both types of cells. Dark cells would thus stabilize indirect communication with dendritic neuronal endings.

These sensory cells are innervated by the facial nerve (VII), glosopharyngeal nerve (IX) and vagus nerve (X), depending on where they are located. The sensory cells of the epithelial microreceptors have no axons and the same is the case of cells forming the TB located in the mouth and the pharynx.

### *Lectin histochemistry*

The results obtained are shown in Table 2. In embryonic stages prior to hatching, reactivity to lectins is principally localized along the primitive gut and the epidermis. The positive reactive lectins for TB were *Glycine maxima* (SBA) and *Dolichos biflorus* (DBA). Both identify the same saccharide residue, N-acetylgalactosamine. A moderate reactivity was found when the lectin from *Triticum vulgare* (WGA) was used, which binds to N-acetylglucosamine residues. These lectins stained the cytoplasmic content of the cell. The positive reaction of TB to DBA and SBA is weak compared with the staining of mucous cells in the epithelium. TB did not show any reactivity at all for lectins UEA I, PNA and Con A. PAS staining was positive for the cytoplasmic vesicles of TB, which did not react with Alcian Blue.

The light and dark cell projections constitute the receptor field (Fig. 4), which is covered by mucopolysaccharides (K) coming from the dark and marginal cells, reacting positively to WGA and Concanavalina A lectins. Using SEM and TEM, it was possible to observe a glycocalix cover (Fig. 4), microvilli, and dense substances between them.

After hatching, the only change that was noticed in lectin histochemistry was an increased binding to DBA and SBA in the epithelial microreceptors.

## Discussion

Controversy arises when comparing sensory microreceptors and taste buds, mainly due to their possible equivalence in different species separated on the zoological scale. In most vertebrates, taste buds are located in the epithelium of the mouth and pharynx.

According to Beaumont and Cassier (1972), the sense of taste is well developed in fishes. Taste buds extend out of the mouth to the epithelium of the head and body trunk. In the most teleosts, this extension of the typically visceral gustatory sensitivity to a cutaneous somatic territory can be compared to the inverse phenomenon: the extension of the cutaneous extereoceptive tactile somatic sensitivity to a visceral territory, i.e. when tactile buds invade the mouth converting the tongue into a tactile organ.

This simple extrapolation leads to some confusion when the sensory afferent components are established. We suggest that they would be somatic afferent exteroceptive elements of the tegument, while those located in the mucosa of the mouth, oesophagus, and gill arches would be gustatory visceral afferent elements.

The above authors described taste buds as barrel-shaped structures, close to the neuromast and slightly inside a gustatory channel that opens through a gustatory pore. Neuromast consists of different cellular categories whose functions are not still well known. This description could again lead to confusion with the neuromast on the lateral line that follows that description, but their structure and morphology is clearly different when studied under TEM and SEM.

It is commonly believed that the somatic senses include pain, ambient temperature, gross touch or pressure, and light touch able to make precise discriminations in space and time. These are probably the sensations detected by the cutaneous sensory microreceptors, and not sweet, salty, bitter or acid sensations.

The dendritic ending of the afferent ganglionary neuron transmits stimuli to the central nervous system and can appear as a ciliate epithelial cell specialized in the perception of certain stimuli. The cilia of these cells usually have a non- typical structure type 9+0. This means that they consist of nine doublets of peripheral fibres without a central doublet.

Considerable controversy remains about the differentiation between light and dark cells in the sensory epithelium of the gustatory organs. Jakubowski and Whitear (1990) differentiate between light or sensory cells, and dark or supporting cells. Light cells could also have a gustatory function and may participate in ion transport. These authors classified them as interoceptive while others call them "special", defining them as chemoreceptive.

Hara (1992) attributes a sensory function to both types of cells, but more developed in the light cells. Moreover, they carry out other functions such as secretion, nutrition, and support. Our results confirm this hypothesis.

Using TEM, we visualized significant differences in their organelles content. Both types of cells seem to establish synaptic interconnections with the nerve fibre plexus. Basal cells also synapse onto the nerve fibre and they have thus

been considered interneurons (Reutter 1978; 1980) although their functions remain to be determined.

Using electron microscopy lectin histochemistry we detected positive reactions of the sensory epithelium to Con A and WGA lectins. Con A belong to the group of mannose-binding lectins, and preferentially interact with the mannose type N-linked oligosaccharides of glycoproteins (Debray et al., 1981; Shibuya et al., 1988). Con A binds to cytoplasmatic membranes of sensory cells located at the base of the organ and also to the apical microvilli of the sensory epithelium. Wheat-germ agglutinin (WGA) is a lectin with binding specificity for N-acetylglucosamine and sialic acid (Goldstein et al., 1975). This lectin reacted with sensory microvilli and the apical cytoplasm of sensory cells. We detected positive binding to the glycocalix of the surrounding mucous cells. Witt and Reutter (1988, 1990) demonstrated positive lectin binding sites at the mucous layer above the receptor field, describing as massive the lectin binding to the apical part of the dark cells, in agreement with our results.

Marginal cells exhibit a positive apical binding to these lectins as evidence of their secretory function. The positive WGA and Con A binding to the microvilli of the receptor field indicates the presence of D-mannose and N-acetylglucosamine residues.

Concerning the rest of lectins used, we found DBA and SBA to bind exclusively to the sensory microvilli of the epithelial microreceptors. These lectins identify the same radical (N-acetylgalactosamine). The reaction to these lectins was increased in post-hatching stages. These findings indicate that there is an increase in the release of N-acetylgalactosamine residues after hatching.

UEA I (specific to L-fucose) binds microvilli only in later stages of embryonic development.

It could be argued that a variety of carbohydrate radicals form the sensory microvilli, qualitative variations in the sugar composition of sensory epithelial cells occurring as development progresses. This could be related to morphofunctional changes of cell specialization in the receptor bud.

## Conclusion

The present findings offer a sufficiently detailed morphological and functional description on which it is possible to differentiate these receptors as cutaneous or tegumental sensory microreceptors instead of taste buds and distinguish them from those located in the mouth, pharynx, oesophagus and gill arches, which have a true gustatory function.

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