

metachromasia, lead haematoxylin¹⁰, silver impregnation¹¹ and formaldehyde-induced fluorescence. The last of these was studied in freeze-dried material treated with formaldehyde vapour at 60 °C for 4 h, before embedding in paraffin wax.

Immunofluorescent staining with anti-gastrin I revealed 2 groups of cells (Figure 1), both entirely unstained in control preparations. The first were the cells composing one of the 2 chief types of cutaneous gland. From their cytochemical characteristics we identified these as the non-mucous, hedonic, glands described by NOBLE¹². The other group of cells, which can also be seen in Figure 1, were isolated cells, lying along, and directly beneath, the epithelium. These we could not identify positively. Only 2 types of cutaneous gland were distinguished, the non-mucous and the so-called poisonous¹² type. The latter possessed a strong yellow formaldehyde-induced fluorescence, in freeze-dried material, and this had the spectral characteristics of 5-hydroxytryptamine.

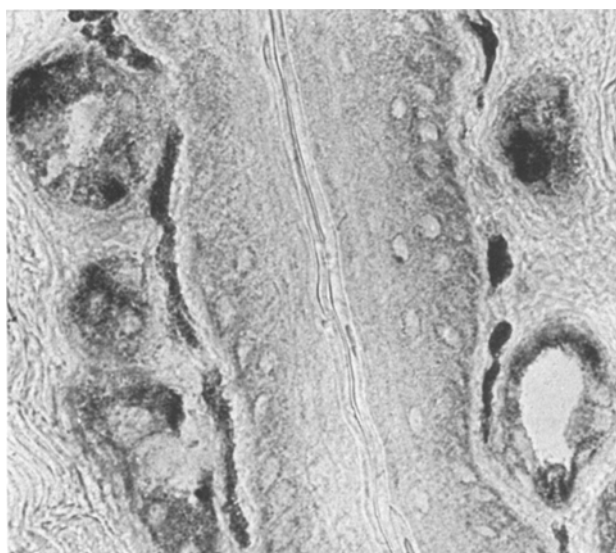


Fig. 2. Skin of *Hyla*, shows strong reaction for α -glycerophosphate dehydrogenase in non-mucous glands, and in unidentified cells lying beneath the epidermis. $\times 600$.

The presumptive caerulein-containing cells possessed the following cytochemical characteristics: masked metachromasia, positive staining with lead haematoxylin, weak argentaffinity, weak formaldehyde-induced fluorescence and a high α -glycerophosphate dehydrogenase (Figure 2). These characteristics are common to endocrine polypeptide cells in mammalian species where they are usually, but not invariably accompanied by high levels of cholinesterase or non-specific esterase, or both. Neither of these enzymes was present in the caerulein cells of *Hyla crepitans*¹³. The granules of the poisonous glands showed toluidine-blue metachromasia at pH 5 without prior acid treatment, as would be expected from their strong 5-hydroxytryptamine-binding capacity.

We conclude that the cells in the non-mucous cutaneous glands, and the isolated subepidermal cells, probably do contain caerulein. Their positive reaction for masked metachromasia presumably indicates that their storage product, like that of established APUD cells, has predominantly the random-coil conformation⁶. It is not possible to take the analogy any further on the present evidence.

Zusammenfassung. Mit indirekter Immunofluoreszenztechnik wird demonstriert, dass Anti-Gastrinserum (IgG-Fraktion) mit Hautzellen und mit zur Haut gehörenden Drüsen von *Hyla crepitans* reagiert. Diese, wahrscheinlich Caerulein enthaltenden Zellen, besitzen endokrine Eigenschaften.

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¹⁰ E. SOLCIA, C. CAPELLA and G. VASSALLO, *Histochemie*, in press.

¹¹ M. POLAK *Blastomas del sistema nervioso central y periférico* (López Liberos, Buenos Aires 1966), p. 208.

¹² G. K. NOBLE, *The Biology of the Amphibia* (Dover Publications Inc., New York 1954).

¹³ We wish to thank Prof. J. SCORZA for supplying *Hyla crepitans*, Miss MARY CLARK for the immunizations and Dr. P. A. KENDALL for purification of the immunoglobulins.

Neurosecretory Cell Types in Normal Taste Bud

Various cell types have been found in the taste bud. They represent according to the majority of researchers, evolutive stages of an original single type of cell¹. In the course of the study aimed at the investigation of the ultra-structural features of the normal taste bud, we have observed the almost constant presence of a cell type characterized by the presence of abundant vesicles of a neurosecretory type. The object of the present paper is in fact the description of the submicroscopic characteristics of this cell type.

Materials and methods. Foliate papillae of adult rabbits were removed and soaked for 3 h in glutaraldehyde 3% in 0.1 M phosphate buffer, rinsed in the same buffer and post-fixed in 2% osmium tetroxide. The sections, stained with uranylacetate and lead citrate, were then examined under a Philips EM 200 electron microscope.

Results and discussion. Following the classification of previous researchers, it is possible to observe in the taste

bud 2 types of mature cells, that is to say, type I and II. The type I is generally regarded as the classical taste cell². This cell has a spindle-shaped form and extends from the basal membrane up to the pore region. It is the one which makes the major contribution to the formation of the taste pore through the numerous microvilli which emanate from the apical region. This cell frequently assumes contact with afferent nerve fibres which it bounds in a similar way to the bounding of the axon by the Schwann's cell³. Mention must be made of the presence

¹ J. ZELENÁ, in *Progress in Brain Research* (Ed. M. SINGER and J. P. SCHADÉ; Elsevier, Amsterdam 1964), vol. 13, p. 175.

² A. J. DE LORENZO, *J. biophys. biochem. Cytol.* 4, 143 (1958),

³ W. KOLMER, in *Geschmacksorgan-Handbuch der Mikroskopischen Anatomie des Menschen* (Ed. W. v. MÖLLENDORF; Springer, Berlin 1927), vol. 3, p. 154-191.

in the cytoplasm of its apical region of characteristic dense secretory granules which at times appear to merge with the mucoid substance of RANVIER⁴. The cells which we observed in the course of this study have many characteristics in common with the type I cells, such as the presence of elongated curvilinear cisternae, the abundance of the Golgi apparatuses and the large quantity of filaments. But what distinguishes these cells from the type I cells is the relative clearness of the cytoplasmic matrix and above all the abundance of vesicles of a neurosecretory type having a dense central granule and an electrontransparent peripheral halo. Their diameter varies from 500–2000 Å. They mainly occupy the medio-basal region of the cell (Figure 1) and extend in a scattered way towards the apical region where as a rule they are rarely seen. Often they prefer areas close to the plasma membrane. In the cytoplasm of these cells we also find a variable number of smooth-edged and electron-transparent

vesicles much smaller than the previous ones (Figure 2). The numerical relationship between the 2 types observed varies greatly according to the functional moment and the cytoplasmic area examined. These cells assume frequent contact with afferent fibres, analogously to type I cells. On the other hand, some neurosecretory vesicles are also observed in type I cells⁵: the cells described can therefore represent, instead of a true and proper cell type, only a functional moment immediately prior to that peculiar of the type I cells, so that it can be suggested that the secretory granules of the apical region of the type I cells are the result of the merging together of the neurosecretory vesicles which originate in cells of less advanced differentiation, in other words in the clear cells just described. Finally it must be noted that such vesicles accumulate along the stretch of plasmatic membrane which comes into contact with the afferent nerve fibres (Figure 3), that is to say similarly to the synaptic

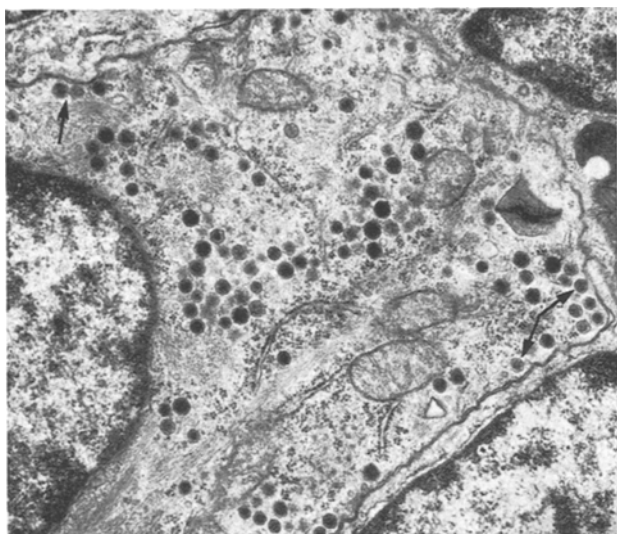


Fig. 1. Taste bud. Medio-basal zone of a cell with numerous vesicles of a neurosecretory type. Note the frequent localization of vesicles along the plasma membrane (arrows) and the abundance of filaments. $\times 15,000$.

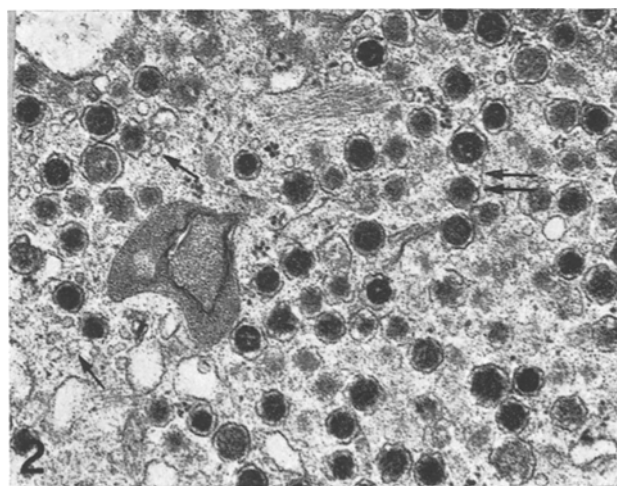


Fig. 2. Taste bud. Size variability of the neurosecretory vesicles. At some points it is possible to observe the fusion of the limiting membrane of 2 adjacent vesicles (double arrow). At the left of the figure many electron-transparent vesicles are present (single arrow). $\times 34,000$.



Fig. 3. Taste bud. Synaptic contact between a neurosecretory type cell and a nerve fibre. Note the presence of some thickenings with accumulation of vesicles (arrows) along the contact membrane. $\times 17,000$.

vesicles^{6,7}. It is possible that these neurosecretory vesicles are related to the production of acetylcholine which many authors⁸ consider to be involved in the transmission of the taste impulse. Finally, the possibility that other specific chemical transmitters are concerned with the transmission of the taste impulse cannot be ruled out⁹.

Riassunto. Nella presente nota vengono esaminate da un punto di vista ultrastrutturale particolari cellule presenti nel calice gustativo normale, per alcuni aspetti assai simili alle cellule del I tipo e contenenti numerose vescicole di tipo neurosecretorio.

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⁴ L. A. RANVIER, in *Traité Technique d'Histologie*, 2nd edition (Librairie Savy, Paris 1889), p. 724.

⁵ G. MAZZOCCHI and P. GOTTE, *Atti Soc. med.-chir. Padova* 41, 5 (1966).

⁶ E. D. P. DE ROBERTIS and H. S. BENNETT, *J. biophys. biochem. Cytol.* 1, 47 (1955).

⁷ S. L. PALAY, *J. biophys. biochem. Cytol., Suppl.* 2, 193 (1956).

⁸ S. LANDGREN, G. LILJESTRAND and Y. ZOTTERMAN, *Acta physiol. scand.* 30, 105 (1954).

⁹ C. J. DUNCAN, *Nature* 203, 875 (1964).