embryos and aleurone vacuoles contain acid phosphatase. Similar results were shown by BRANDES and BERTINI[12] in autophagic vacuoles of starving Euglena. Fractionation of vacuoles from yeast protoplasts have shown a very high specific activity of lysosomal enzymes[6,13]. The latter results indicate that the vacuoles are the only source of lysosomal enzymes within the yeast cells. The results given in the present communication also favour the notion that the vacuoles are the site of lysosomal enzyme activity.

Résumé. La localisation de la phosphatase-acide dans la levure *Cryptococcus neoformans* est étudiée ici par des méthodes cytochimiques au niveau ultrastructural. On a trouvé que l'enzyme se concentrait exclusivement dans les vacuoles, ce qui s'accorde avec les résultats que d'autres auteurs ont obtenus sur la localisation de l'enzyme dans les cellules végétales.

M. KALINA, J. J. BUBIS, Y. KLETTER, A. SHAHAR and M. ARONSON

Department of Cell Biology and Histology, Tel Aviv University Medical School; Department of Pathology, Government Hospital, Tel-Hashomer, and Israel Institute for Biological Research, Ness-Ziona (Israel), 1 September 1969.

[13] PH. MATILE and A. WIEMKEN, Arch. Mikrobiol. *56*, 148 (1967).
[12] D. BRANDES and F. BERTINI, Expl. Cell. Res. *35*, 194 (1964).

Anti-Gastrin Immunofluorescence in the Skin of *Hyla crepitans* and the Cytochemistry of the Cells Involved

In 1966 ERSPAMER et al.[1] reported on the presence of a polypeptide in the skin of Australian and South American tree frogs, especially *Hyla caerulea*. They suggested the name caerulein for the new product and described its effects as well as its chemical structure. A most important observation, by the same group of workers[2], was the recognition that the amino-acid sequence of a portion of the new decapeptide was identical with that of a corresponding portion of the gastrin molecule. Confirmation of this observation has come from the immunochemical studies of MCGUIGAN[3].

Because of our interest in polypeptide-secreting endocrine cells, especially those belonging to the APUD series[4–7], our attention was drawn to this new discovery. We report here the results of immunofluorescence studies on the skin of a South American tree-frog *Hyla crepitans*, and on the chief cytochemical characteristics of the cells giving positive immunofluorescence with anti-gastrin sera.

Skin from the ventral and dorsal surface of *Hyla crepitans* was studied. The material was processed as follows: (1) Small portions of skin were quenched in melting Arcton 22 (Freon 22) for (a) cryostat sections for immunofluorescence and enzyme cytochemistry, and (b) freeze-drying and subsequent treatment with formaldehyde vapour. (2) Other small portions of skin were fixed in glutaraldehyde or in glutaraldehyde-picric acid[8] for investigation of masked metachromasia. (3) Small blocks were fixed in glutaraldehyde and embedded in Epon for electron microscopy.

Antibodies to human synthetic gastrin I (I.C.I.) were produced by repeated immunization of white rabbits by the procedure of BOYD and PEART[9]. Antiserum obtained 14 days after the last immunization was passed through a DEAE Sephadex column to obtain a pure IgG fraction. Normal rabbit serum treated in the same way was used as a control. Cryostat sections (4 μ), unfixed and post-fixed in cold formol-calcium, were used for indirect immunofluorescence with labelled goat anti-rabbit globulin (Microbiological Association Inc.).

The controls used were: (1) fluorescent goat anti-rabbit IgG, alone: (2) normal rabbit IgG followed by fluorescent anti-rabbit IgG; (3) rabbit anti-human gastrin serum after absorption with purified human gastrin I.

Cytochemical reactions characteristically positive in cells of the endocrine polypeptide (APUD) series were also applied. They were: phosphoglycerate dehydrogenase, non-specific cholinesterase, non-specific esterase, masked

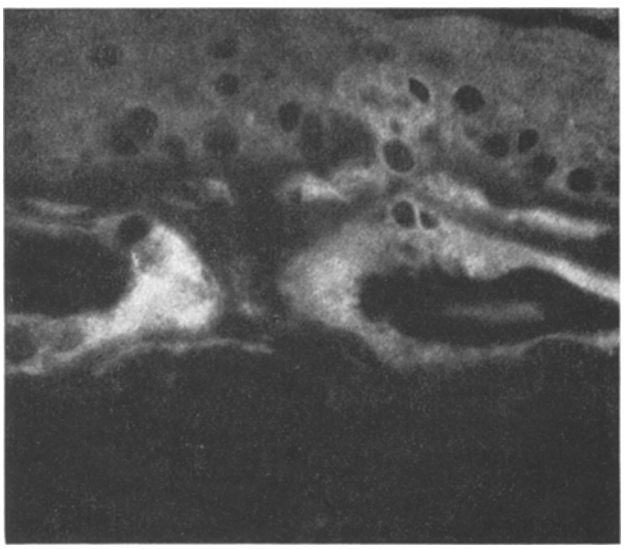

Fig. 1. Skin of *Hyla*, fresh-frozen cryostat section. Anti-gastrin immunofluorescence. Indirect method using rabbit anti-human gastrin I and fluorescein-labelled goat anti-rabbit γ-globulin. × 620.

[1] V. ERSPAMER, M. ROSEGLINI, R. ENDEAN and A. ANASTASI, Nature *212*, 204 (1966).
[2] A. ANASTASI, V. ERSPAMER and R. ENDEAN, Experientia *23*, 699 (1967).
[3] J. E. MCGUIGAN, Gastroenterology *56*, 858 (1969).
[4] A. G. E. PEARSE, Vet. Record *79*, 587 (1966).
[5] A. G. E. PEARSE, Proc. R. Soc. B. *170*, 71 (1968).
[6] A. G. E. PEARSE, Nature *221*, 1210 (1969).
[7] A. G. E. PEARSE, J. Histochem. Cytochem. *17*, 303 (1969).
[8] E. SOLCIA, G. VASSALLO and C. CAPELLA, Stain Technol. *43*, 257 (1968).
[9] G. BOYD and W. PEART, Lancet *2*, 129 (1968).

metachromasia, lead haematoxylin[10], silver impregnation[11] 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 NOBLE[12]. 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 poisonous[12] type. The latter possessed a strong yellow formaldehyde-induced fluorescence, in freeze-dried material, and this had the spectral characteristics of 5-hydroxytryptamine.

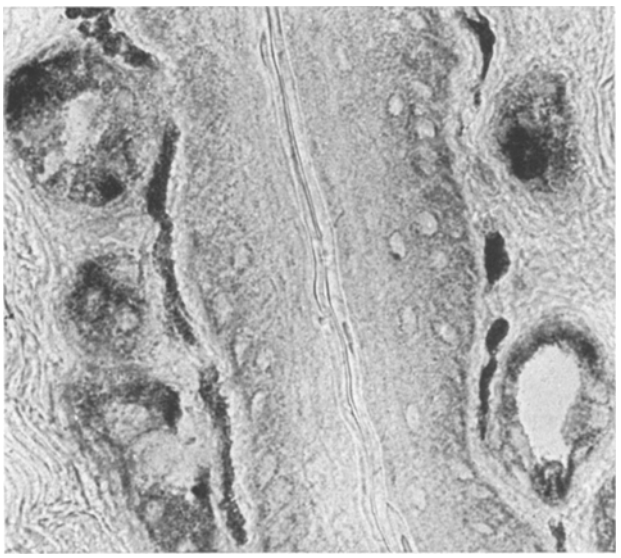

Fig. 2. Skin of *Hyla*, shows strong reaction for α-glycerophosphate dehydrogenase in non-mucous glands, and in unidentified cells lying beneath the epidermis. × 600.

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 conformation[6]. 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.

JULIA M. POLAK and A. G. E. PEARSE

Department of Histochemistry,
Royal Postgraduate Medical School, Hammersmith Hospital,
London W12 (England), 27 August 1969.

[10] E. SOLCIA, C. CAPELLA and G. VASSALLO, Histochemie, in press.
[11] M. POLAK *Blastomas del sistema nervioso central y periferico* (López Libreros, 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 cell[1]. In the course of the study aimed at the investigation of the ultrastructural 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 cell[2]. 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 cell[3]. Mention must be made of the presence

[1] J. ZELENA, 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.