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Short Communication

Fine Structure of the Gastric Mucous and Endocrine Cells of the Toad, *Bufo marinus*

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Summary. The ultrastructure of the mucous and endocrine cells of the gastric mucosa of the cane toad (*Bufo marinus*) has been examined. Surface mucous cells line the entire gastric mucosa and pits. Many of their secretory granules contain an electron-dense core that remains unreactive after cytochemical testing for glycoproteins. A second spatially and structurally discrete population of mucous cells is present in the gastric glands. These glandular mucous cells are probably homologous with the antral gland and mucous neck cells of mammals; their secretory granules also contain non-glycoprotein cores. Three distinct populations of endocrine cells show structural homologies with gastric hormone-storing cells of higher vertebrates.

Key words: Amphibia – Ultrastructure – Gastric mucosa – Cytochemistry – Secretory granules

The light microscopic morphology of the amphibian gastric mucosa has been well documented (Lim 1922; Norris 1959; Reeder 1964; Loo and Wong 1975; Tsukahara et al. 1978). Most fine structural studies have focused on the oxynticopeptic cell, particularly its subcellular changes during and after acid secretion (Vial and Orrego 1960; Sedar 1961, 1962, 1965; Forte et al. 1969). In contrast, the mucous and endocrine cells have been given less attention. Geuze (1971a, b) and Helander et al. (1972, 1973, 1975) have briefly described the morphology of the mucoid cells of two anuran species, while Kataoka (1973) has documented the fine structure of some endocrine cells in the stomach of one species of frog.

In the present paper, the fine structure of these cell types in the gastric mucosa of the cane toad is described, with particular emphasis on their secretory granules. The

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carbohydrate composition of mucous granules has been examined cytochemically in order to determine whether the gastric mucous granules of the toad contain more than one secretory product, as has been established for other vertebrates (Yeomans and Giraud 1980).

Materials and Methods

Adult cane toads (*Bufo marinus*) were maintained in open glass aquaria at 22° C, and fed assorted insects. Water was freely available. Food was witheld for 24 h prior to tissue fixation. Toads were anesthetized with pentobarbitone (100 mg/kg body weight intraperitoneally). The stomachs were either removed, diced into pieces and fixed by immersion, or fixed in situ by vascular perfusion via an aortic arch. The primary fixative was 3% glutaraldehyde in 0.15 M cacodylate buffer (pH 7.4); when used for perfusion, 2% Dextran T40 was added (Rostgaard and Behnke 1966). Perfusion was continued for 20–30 min; pieces of the stomach were then fixed by immersion in the perfusion fluid for another 2 h. All tissue was postfixed for 1 h in 1% OsO_4 in 0.15 M cacodylate buffer, and embedded in Araldite.

Thick (1 μm) sections were stained routinely with toluidine blue, or by the PAS-Bowie method for the dual identification of mucus and zymogen (Moxey and Yeomans 1976). Thin sections were stained with uranyl acetate and lead citrate, or with the periodic acid-chromic acid-silver methenamine (PA-CrA-SM) method of Rambourg et al. (1969) for the cytochemical demonstration of glycoproteins. Grids were incubated on silver methenamine for 60–90 min at 45° C; control grids were similarly treated except for the omission of the oxidation steps.

Results

Surface Mucous Cells (SMC)

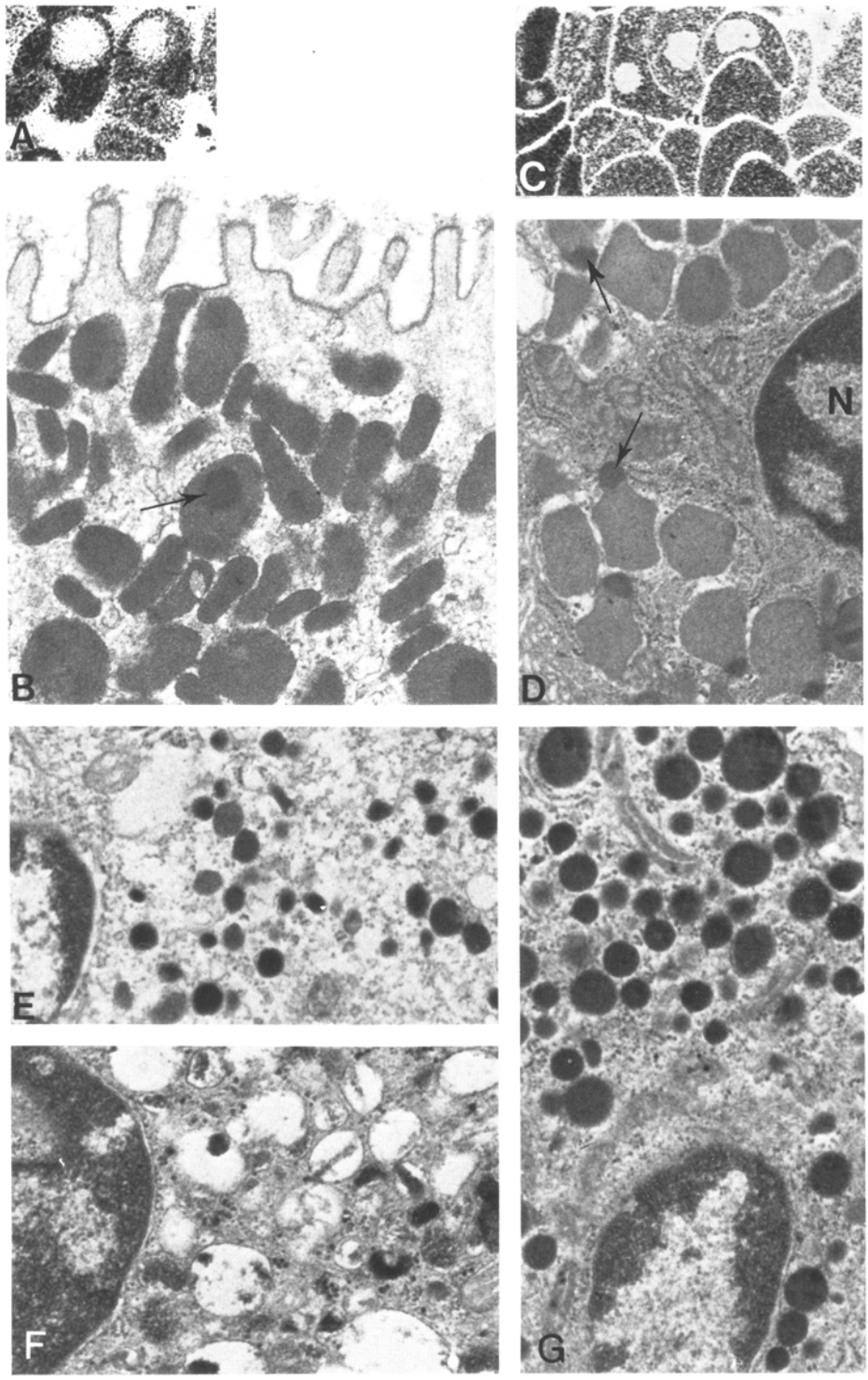
Surface mucous cells line the entire gastric surface and pits. The nucleus lies close to the folded basal lamina. Above it are Golgi complexes and a small number of light-staining inclusions, considered to be immature secretion granules. Membrane bounded mature secretory granules aggregate in the apical third of the cells. They are ovoid to dumb-bell shaped.

Some granule profiles, stained with uranyl acetate/lead citrate, reveal an electron-opaque core (Fig. 1 B). In thin sections reacted for glycosyl groups with the PA-CrA-SM method, a positive reaction was obtained over the granule cortices but *not* over the cores (Fig. 1 A), indicating that the core is unlikely to contain mucus.

Glandular Mucous Cells (GMC)

Glandular mucous cells, a second morphologically and spatially discrete population of mucus-secreting cells in the gastric mucosa of the toad, are located

Fig. 1A–D. Mucous cells. **A** Surface mucous cell granules stained by the PA-CrA-SM method for mucus. Granule cortices are heavily stained, but cores remain unreactive. ×15.000. **B** Apical cytoplasm of surface mucous cell stained with uranyl acetate and lead citrate (UaPb). Granule cores (*arrow*) stain more densely than cortices. ×15,000. **C** Cytochemical demonstration of glycoprotein in glandular mucous cell granules. Many granules stain evenly; others contain unstained cores. PA-CrA-SM. ×12,000. **D** Portion of a glandular mucous cell. Some granules contain eccentric electron-dense cores (*arrow*). *N* nucleus. UaPb. ×12,000. **E–G.** Endocrine cells. **E** Paranuclear cytoplasm of type 1 endocrine cell. Secretory granules are small and vary in shape. UaPb ×24,000. **F** type 2 cell. Granules appear vacuolated; some contain a dense, inner portion surrounded by an electron-lucent zone. UaPb ×24,000. **G** Type 3 cell. Secretory granules round and dark-staining. UaPb ×24,000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below the level of the gastric pits, in the gastric glands. These are large, polymorphous cells, with a prominent nucleus, containing many randomly distributed secretory granules. After conventional staining the granules are highly variable in shape and electron-density. Most granules are evenly electron dense, some stain palely, and others contain a small, discrete, peripheral body of more electron-dense material (Fig. 1 D). In sections treated with the PA-CrA-SM procedure, most secretory granules are uniformly covered by silver grains, but some contain peripheral unstained regions corresponding to the dense areas described above (Fig. 1 C).

Endocrine Cells

Three distinct types of endocrine cells have been distinguished. Type 1 cells are present in gastric glands throughout the stomach. They contain many pleomorphic, electron-dense granules (mean diameter 120 nm) distributed throughout the cytoplasmic (Fig. 1 E). Type 2 cells are restricted to the gastric glands of the proximal and middle thirds of the stomach. This cell type commonly features a bilobed nucleus as well as numerous round secretory granules which appear vacuolated or contain variable amounts of electron-dense material (Fig. 1 F). The granules have a mean diameter of approximately 330 nm. Type 3 cells (Fig. 1 G) are present in smaller numbers than either of the previously described cell types. Their secretory granules (150 nm in diameter) are oval to round and moderately to densely electron-opaque.

Discussion

The *surface mucous cells* of the stomach of adult toads have almost the same ultrastructure as those of other vertebrates, specifically fish (Noaillac-Depeyre and Gas 1978), reptiles (Giraud et al. 1979) and mammals (Ito 1967). One particular aspect of this shared property is the presence of non-glycoprotein, electron-dense cores in the mucous granules. These have been noted previously in other vertebrate classes (Yeomans 1974; Noaillac-Depeyre and Gas 1978; Giraud et al. 1979). Fox et al. (1972) have previously described dense cores in gastric surface-cell granules of *Xenopus laevis* larvae. In parallel with this observation, fetal rats show non-glycoprotein, electron-dense material in the greater part of the secretion granules of the surface cells (Yeomans 1978). The nature of the core substance is not yet known, although its widespread occurrence in vertebrate ontogeny and phylogeny hints at a significant biological role.

The structure of the toad's *glandular mucous cells* is also very similar to that of the GMC of lizards (Giraud et al. 1979), and of the two glandular mucous cells (mucous neck and antral gland cells) of mammals (Ito 1967). There is some evidence that mammalian GMC contain pepsinogen (Yasuda et al. 1966; Samloff 1971; Samloff and Liebman 1973), localized in granule cores (Zeitoun et al. 1972). It is probable that toad GMC granules also contain pepsinogen, since in distal mucosa, where oxynticopeptic cells are absent, pepsinogen is assayable in homogenates (Giraud 1980), and demonstrable in glandular cells by immunofluorescence (Reese et al. 1979).

The distinction among three types of *endocrine cells* in the toad, based primarily on the criterion of granule morphology, parallels that in mammals (Solcia et al. 1975). There are marked similarities between the type 1 cell of *Bufo* and that of the frog *Rana nigromaculata nigromaculata* (Kataoka 1973), as well as the EC cell of mammals (Solcia et al. 1978), and the type I cell of lizards (Giraud et al. 1979). Likewise, the type 2 cell of *Bufo* corresponds closely in both distribution and ultrastructure to the type 2 cell of Kataoka (1973), the reptilian type II cell (Giraud et al. 1979) and the mammalian ECL cell (Solcia et al. 1978). The affinities of the toad's type 3 cell are more obscure, but its granules resemble those of the frog type 3 cell (Kataoka 1973) and the bombesin-like immunoreactive cell (Lechago et al. 1979).

Homologies of structure therefore exist between individual endocrine cell types of amphibians belonging to different genera, and between those of amphibians and higher vertebrates. Morphological similarities alone are insufficient grounds to establish parallelism of function (Solcia et al. 1978); nevertheless it appears likely that the amphibian stomach produces a variety of hormones, and that at least two of these may be similar to hormones produced by the mammalian stomach.

References

Forte GM, Limlomwongse L, Forte JG (1969) The development of intracellular membranes concomitant with the appearance of HCl secretion in oxyntic cells of the metamorphosing bullfrog tadpole. J Cell Sci 4:709–727

Fox, H, Bailey E, Mahoney R (1972) Aspects of the ultrastructure of the alimentary canal and respiratory ducts in *Xenopus laevis* larvae. J Morphol 138:387–406

Geuze JJ (1971a) Light and electron microscope observations on the gastric mucosa of the frog (*Rana esculenta*) I. Normal structure. Z Zellforsch 117:87–102

Geuze JJ (1971b) Light and electron microscope observations on the gastric mucosa of the frog (*Rana esculenta*) II. Structural alterations during hibernation. Z Zellforsch 117:103–117

Giraud AS (1980) Phylogeny of the gastric mucosa: structure and aspects of function. Ph.D. Thesis: Melbourne University

Giraud AS, Yeomans ND, St John DJB (1979) Electron microscopic structure and cytochemistry of the gastric mucosa of a reptile (*Tilique scincoides*). Cell Tissue Res 197:281–294

Helander HF, Sanders SS, Rehm WF, Hirschowitz BI (1972) Quantitative aspects of gastric morphology. In: Sachs G, Heinz E (eds) Gastric secretion. Academic Press, New York, pp 69–88

Helander HF, Rehm WS, Sanders SS (1973) Influence of fixation on physiological properties of frog gastric mucosa. Acta Physiol Scand 88:109–122

Helander HF, Sanders SS, Shanbour LL, Rehm WS (1975) Reversibility of effects of very hypertonic fluids on in vitro frog gastric mucosa. A functional and physiological study. Acta Physiol Scand 95:353–363

Ito S (1967) Anatomic structure of the gastric mucosa. In: Code CF, Heidel W (eds) Handbook of Physiology, Sect 6, Alimentary Canal, Vol 2, Secretion, American Physiological Society, Washington

Kataoka K (1973) An electron microscope study of the gastro-enteric endocrine cells of the frog, *Rana nigromaculata nigromaculata*. In: Gastro-entero-pancreatic endocrine system. A cell-biological approach. Fujita T (ed) Igaku Shoin Ltd, Tokyo, pp 39–48

Lechago J, Crawford BG, Walsh JH (1979) Bombesin(like)-producing cells in frog gastric mucosa: immunoelectronmicroscopic identification. Gastroenterology 76:1182

Lim RKS (1922) The gastric mucosa. Q J Micros Sci 66:187–212

Loo SK, Wong WC (1975) Histochemical observations on the mucins of the gastrointestinal tract in the toad (*Bufo melanostictus*). Acta Anat 91:97–103

Moxey PC, Yeomans ND (1976) Identification of cell types in semithin epoxy sections of gastric fundic mucosa. J Histochem Cytochem 24:755–756

Noaillac-Depeyre J, Gas N (1978) Ultrastructural and cytochemical study of the gastric epithelium in a freshwater teleostean fish (*Perca fluviatilis*). Tissue Cell 10:23–37

Norris JL (1959) Normal histology of the esophageal and gastric mucosae of the frog, *Rana pipiens*. J Exptl Zool 141:155–174

Rambourg A, Hernandez W, Leblond CP (1969) Detection of complex carbohydrates in the Golgi apparatus of rat cells. J Cell Biol 40:395–414

Reeder WG (1964) The digestive system. In: Moore JA (ed) Physiology of the amphibia. Academic Press, New York, pp 99–150

Reese JH, Lechago J, Samloff IM (1979) Localization of pepsinogens in stomach and duodenum of several animal species by immunofluorescence. Gastroenterology 76:1307

Rostgaard J, Behnke O (1966) Perfusion fixation and its application in electron microscopic, morphological and histochemical studies. J Ultrastruct Res 14:416–417

Samloff IM (1971) Cellular localization of group I pepsinogens in human gastric mucosa by immunofluorescence. Gastroenterology 61:185–188

Samloff IM, Liebman WM (1973) Cellular localization of the group II pepsinogens in human stomach and duodenum by immunofluorescence. Gastroenterology 65:36–42

Sedar AW (1961) Electronmicroscopy of the oxyntic cell in the gastric glands of the bullfrog, *Rana catesbiana*. II. The acid-secreting gastric mucosa. J Biophys Biochem Cytol 10:47–57

Sedar AW (1962) Electronmicroscopy of the oxyntic cell in the gastric glands of the bullfrog (*Rana catesbiana*). III. Permanganate fixation of the endoplasmic reticulum. J Cell Biol 14:152–156

Sedar AW (1965) Fine structure of the stimulated oxyntic cell. Fed Proc 24:1360–1367

Solcia E, Capella C, Vasallo G, Buffa R (1975) Endocrine cells of the gastric mucosa. Int Rev Cytol 42:223–280

Solcia E, Polak JM, Pearse AGE, Forssmann WG, Larsson LI, Sundler F, Lechago J, Grimelius L, Fujita T, Creutzfeldt W, Gepts W, Falkmer S, Lefranc G, Heitz Ph, Hage E, Buchan AMJ, Bloom SR, Grossman MI (1978) Lausanne 1977 classification of gastroenteropancreatic endocrine cells. In: Bloom SR (ed) Gut hormones. Churchill Livingstone, London, pp 40–48

Tsukahara M, Tatematsu M, Katsuyama T (1978) Comparative and embryological mucosubstance histochemistry of the gastric mucous neck cell. In: Sixth World Congress of Gastroenterology Proceedings, Madrid. Editorial Garsi

Vial JD, Orrego H (1960) Electron microscope observations on the fine structure of parietal cells. J Biophys Biochem Cytol 7:367–372

Yasuda K, Suzuki T, Tanako K (1966) Localization of pepsin in the stomach revealed by fluorescent antibody technique. Okajimas Folia Anat Jpn 42:355–367

Yeomans ND (1974) Ultrastructural and cytochemical study of mucous granules in surface and crypt cells of rat gastric mucosa. Biol Gastroenterol (Paris) 7:285–290

Yeomans ND (1978) Secretory granules in differentiating mucous cells in rat fundic mucosa. Gastroenterol Clin Biol 2:925–928

Yeomans ND, Giraud AS (1980) Nonglycoprotein "cores" in gastric mucous granules. Gastroenterology 78:1658–1659

Zeitoun P, Duclert A, Liautaud F, Potet F, Zylberberg L (1972) Intracellular localization of pepsinogen in guinea-pig pyloric mucosa by immunohistochemistry. Histochemical and electron microscopic correlated structures. Lab Invest 27:218–225

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