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Histochemical investigations on the secretory cells in the oesophagogastric tract of the Eurasian green toad, *Bufo viridis*

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Summary

The secretory cells of the oesophagogastric tract of the Eurasian toad, *Bufo viridis*, were examined using standard histochemical methods and lectin histochemistry. Two goblet cell types were found in the oesophageal epithelium, differing in their morphology and the histochemical features of the secretory granules. These contained mainly acidic glycoconjugates, both sulphated and carboxylated, and a small amount of pepsinogen. Type I goblet cells contained stable class-III mucosubstances, which were absent in Type II. No pluricellular oesophageal glands were found. The oesophagogastric junction had a superficial epithelium similar to that of the oesophageal epithelium, with alveolar pluricellular glands, secreting stable class-III mucins, and few oxynticopeptic cells. The gastric mucosa presented secretory cells both in the surface epithelium and in the gastric glands. Superficial and foveolar cells produced neutral mucins with Galβ1,3GalNAc residues. Neck cells, oxynticopeptic cells and endocrine cells were found in the gastric glands. Neck cells produced stable class-III mucosubstances. A functional gradient was observed in the oxynticopeptic cells from the oral to the aboral fundus, with a decrease in pepsinogen secretion towards the aboral fundus and a possible increase in HCl secretion. In the pyloric mucosa, the oxynticopeptic cells disappeared and the glands produced only neutral mucins, without stable class-III mucosubstances.

Introduction

There is some disagreement in the literature about the histological organization of the mucosa of the anuran oesophagogastric tract, in particular about the localization of the cells producing pepsinogen (Taylor & Tyler 1986). In general, it is assumed that in non-mammalian vertebrates most of the pepsinogen and hydrochloric acid are produced by one type of cell, the oxynticopeptic cell, clustered in glands in the gastric mucosa (Smit 1968, Helander 1981). This condition is found in a number of Anura (Giraud & Yeomans 1981, Bani 1983, Oinuma *et al.* 1991, Bani *et al.* 1992). Peptic cells have been found in several genera of Ranoidea clustered in oesophageal glands with the gastric glands producing mainly hydrochloric acid from oxyntic cells (Norris 1959, Hirji 1982, Hirji & Nikundiwe 1982, Bani 1983, Bani *et al.* 1992, Gallego-Huidobro *et al.* 1992, Gallego-Huidobro & Pastor 1996, Ferri *et al.* 2001). Thus, it would seem that the presence of oesophageal glands in Anura is limited to the superfamily, whereas in other Anura, oesophageal glands are lacking and the oxynticopeptic cells of the gastric mucosa produce both pepsinogen and hydrochloric acid. However, data from the Bufonidae family are somewhat contradictory. In the genus *Bufo,* Noble (1931) reports that oesophageal glands occur 'just before the stomach' (no species are given), but no oesophageal glands are found in *B. gutturalis* from Africa (Hirji & Nikundiwe 1982) nor in *B. melanostictus* from eastern Asia (Loo & Wong 1975). Ruiz *et al.* (1993) and Taylor and Tyler (1986) have revealed the presence of pepsin by biochemical methods in the oesophagus of the American cane toad, *B. marinus*. An oro-aboral secretory gradient has been found in some non-mammals in which both pepsinogen and hydrochloric acid are produced by oxynticopeptic cells. In particular, the secretion of pepsinogen decreases from the oral to the aboral fundus, as has been found using histological methods in a number of species, such as the river stingray *Potamotrigon* sp. (Gabrowski *et al.* 1995) and the lizards *Tiliqua scincoides* (Giraud *et al.* 1979), *Chalcides chalcides* (Ferri *et al.* 1999) and *Podarcis sicula* (Ferri & Liquori 1994, Ferri *et al.* 1999). A pepsin gradient has been found in Anura using biochemical methods in the American cane toad, *Bufo marinus* (Taylor & Tyler 1986, Ruiz *et al.* 1993). In contrast, the secretion of hydrochloric acid seems to increase from the oral to the aboral fundus (Gabrowski *et al.* 1995).

The other main products of the exocrine cells in the oesophagogastric mucosa are mucosubstances. The type of mucosubstances produced along the alimentary tract varies with the region, cell type, developmental stage and species in relation to different functions, such as protection against gastric juice or lubrication during the passage of food (Suganuma *et al.* 1981, Oinuma *et al.* 1991, Ferri *et al.* 2001) and, probably, to phylogenetic relationships (Suganuma *et al.* 1981).

In this paper, we present the results of a histological and histochemical investigation on the oesophagogastric tract of the Eurasian green toad, *B. viridis* (Laurenti 1768), with three main purposes.

(1) Identifying the cells elaborating pepsinogen and hydrochloric acid and assessing the presence/absence of oesophageal peptic glands. This is of great interest from a phylogenetic point of view, because *Bufo* is included in the family Bufonidae, superfamily Bufonoidea, that are regarded as the sister-group of Ranoidea (Ford & Cannatella 1993, Hay *et al.* 1995, Ruvinsky & Maxson 1996). The presence of these glands in some species would indicate that they appeared repeatedly and independently, may be even within the same genus (*Bufo*). Otherwise, their absence would support the hypothesis that this feature is a synapomorphy of the Ranoidea.
(2) Verifying the existence of an oro-aboral gradient in the production of pepsinogen and hydrochloric acid. For this purpose, we tested a lectin, DBA, that can give indirect information on hydrochloric acid secretion by oxyntic-copeptic cells. This lectin specifically binds to α-GalNAc residues on the intracellular canalicular membranes that in mammalian parietal cells produce hydrochloric acid (Peschke *et al.* 1983, Ito *et al.* 1985, Kessimian *et al.* 1986).
(3) Detecting regional differences in the composition of the mucous layer in relation to its different functions, using standard histochemical methods and lectin histochemistry.

Material and methods

Two adult green toads, *B. viridis*, of each sex were collected from areas around Bitonto, Bari (Italy). The animals were sacrificed with ether and their digestive tracts quickly removed. The samples were fixed in 10% formalin, dehydrated through graded ethanols, and embedded in paraffin wax. Serial sections, 4 μm thick, were cut. Rehydrated sections were stained using the periodic acid-Schiff (PAS)–haemalum method, Alcian Blue (AB) at pH 2.5 and 1.0, or with high iron diamine (HID) (Spicer 1965). The periodic acid-borohydride–potassium hydroxide–periodic acid-Schiff method (PB–KOH–PAS) was also performed to demonstrate O-acylated sialic acid (Culling *et al.* 1974). Zymogen granules were identified with Bowie's modified method according to Bonucci (1981), or with a combined PAS–Bowie to stain both mucus and zymogen granules simultaneously.

Six peroxidase-labelled lectins (Sigma, St. Louis, USA) were used to determine the nature and the distribution of glycosidic residues in the oesophagogastric mucosa. The lectins employed, their concentrations and their sugar specificities are summarized in Table 1. Binding with PNA and SBA was performed with and without pretreatment with sialidase (neuraminidase Type V from *Clostridium perfrigens* [Sigma, St. Louis, USA]). Lectin binding was performed as previously reported (Ferri & Liquori 1997). Briefly, rehydrated sections were exposed to 3% hydrogen peroxide for 10 min to inhibit endogenous peroxidase activity, and then incubated for 30 min at room temperature with solutions of peroxidase-labelled lectins. The horseradish peroxidase label was then visualized histochemically with 3,3′-diaminobenzidine (DAB)–hydrogen peroxide medium (Graham & Karnowsky 1966) for 10 min. Finally, without counterstaining, the sections were dehydrated, cleared and mounted with DPX.

The control tests for lectin staining included:

(a) Incubation of untreated sections with DAB–H_2O_2 to check the endogenous peroxidase activity;
(b) Substitution of the respective peroxidase-labelled lectin with phosphate-saline buffer (PBS).
(c) Incubation in the peroxidase-labelled lectin with addition of the appropriate inhibitory sugar to confirm the specificity of lectin staining. The concentrations of the inhibitory sugars are reported in Table 1.

Sialidase digestion was performed by overlaying hydrated sections for 30 min at 37 °C, in a damp Petri dish, with a drop of a 0.05 M acetate buffer, pH 5.5, containing 1 U/10 ml sialidase and 0.1% calcium chloride (Leathern & Atkins 1983) before treatment with hydrogen peroxide as above.

Various treatments carried out prior to the Con A method have been found to affect staining and have permitted differentiation of three main classes of complex carbohydrates in the mammalian alimentary tract. Class I mucosubstances lose Con A reactivity while classes II and III gain Con A reactivity after periodate oxidation. Class II mucosubstances lose reactivity whereas class III gain or increase their reactivity with a

Table 1. Characteristics of the plant lectins utilized.

Lectin	Source	Binding specificity	Lectin concentration (μg/ml)	Inhibitory sugar
Con A	*Canavalia ensiformis*	D-mannose D-glucose	50	0.1 M MαM
WGA	*Triticum vulgaris*	(GlcNAcβ1,4)*n*	20	0.01 M TACT
SBA	*Glycine max*	GalNAc	20	0.2 M GalNAc
PNA	*Arachis hypogaea*	Galβ1,3GalNAc	60	0.2 M Gal
WPA	*Lotus tetragonolobus*	L-Fucα1,6GlcNAc and L-Fucα1,2Galβ1,4 [L-Fuc1,3] GlcNAcβ1,6R	100	0.2 M L-fuc
DBA	*Dolichos biflorus*	α-GalNAc	20	0.2 M GalNAc

Abbreviations: Gal – galactose; GalNAc – N-acetylgalactosamine; GlcNAc – N-acetylglucosamine; L-Fuc – L-fucose; MαM – methyl-α-mannopyranoside; TACT – N,N′,N″-triacetylchitotriose; R – remainder of oligosaccharide residues.

reduction step interposed between oxidation and Con A staining (PCS staining) (Katsuyama & Spicer 1978). This staining was performed to attempt to identify mucous neck cells that, in mammals, strongly react with this method.

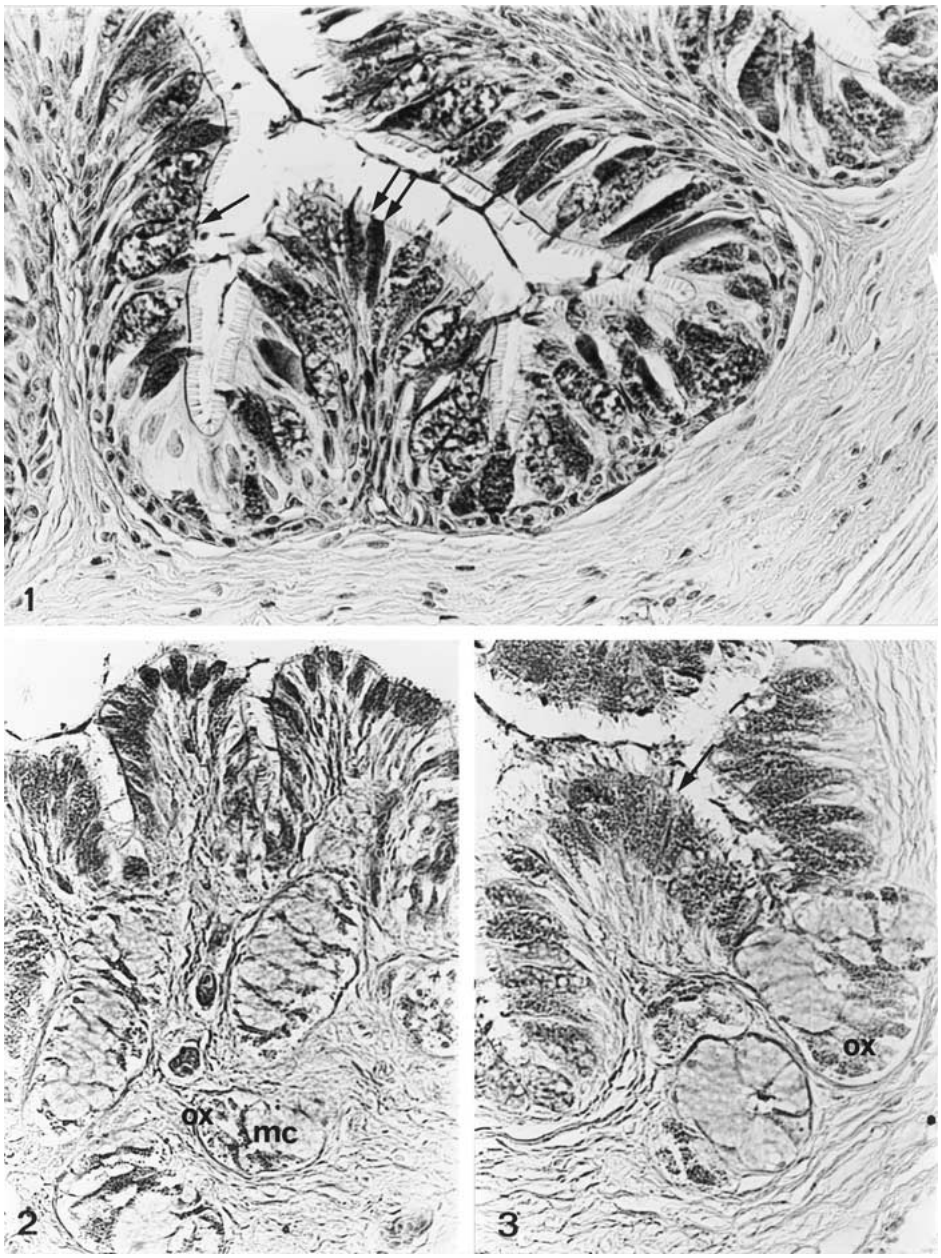
Lectin-binding was also performed after β-elimination with 0.2 M KOH in dimethylsulphoxide–H_2O–ethanol (50 : 40 : 10) for 1 h at 45 °C and subsequent neutralization with 10 mM HCl and washing in PBS (Downs *et al.* 1973). Only the O-linked glycanes are removed from glycoproteins by this method.

Some sections were stained with the Masson–Fontana silver method to detect argentaffin endocrine cells (Pearse 1972).

The animals received humane care in compliance with the current national laws.

Results

(a) *Oesophagus*. The oesophagus of *B. viridis* was lined with a ciliated columnar epithelium containing widespread



Figures 1–3. Oesophageal and oesophagogastric junction mucosae of *B. viridis* stained by different histochemical methods. (1) Oesophagus, PAS–haemalum. The oesophageal epithelium consists of two goblet cells types, which are both PAS positive, scattered among ciliated cells. The mucosa does not contain oesophageal glands. Arrow, Type I goblet cells; double arrows, Type II goblet cells. 1500×. (2) Oesophagogastric junction, PAS–Bowie staining. In this area, the first gastric glands appear and consist mainly of mucous cells (mc), weakly PAS positive, with a low number of oxynticopeptic (ox) cells. 1500×. (3) Oesophagogastric junction, Bowie's staining. Goblet cells (arrows) and oxynticopeptic cells show a number of secretory granules stained by Bowie's reaction. 1500×.

mucous goblet cells (Figure 1). The mucosa was in longitudinal folds, and did not contain oesophageal glands. Two different types of goblet cells could be distinguished (Figure 1). Type I were flask-shaped cells, with flattened basal nuclei and a supranuclear cytoplasm containing large secretory droplets. These cells showed strong PAS positivity and reacted with AB at pH 1.0 and at pH 2.5. They revealed affinity for HID and stained black with the HID–AB staining sequence. Moderate staining was observed with the PB–KOH–PAS method. Type II goblet cells were thinner, with numerous small secretory granules in the supranuclear cytoplasm. They showed the same basic histochemical features as Type I, except for a lower affinity for AB pH 1.0 and HID, but differed from them in their lectin binding pattern.

(b) *Oesophagogastric junction*. This area marks the transition from the oesophagus to the stomach, and the mucosa presented the features of both organs. The epithelium was still of the oesophageal type, with ciliated Type I and Type II goblet cells having the same staining properties as their oesophageal homologues (Figure 2). In this area, the first gastric glands appeared (Figures 2 and 3). They were mostly alveolar and consisted mainly of muco-secreting cells, with a low number of oxynticopeptic cells (Figures 2 and 3). Mucous glandular cells were moderately PAS positive and did not react with AB. Towards the stomach, goblet cells showed an increasing number of secretory granules stained by the Bowie's reaction (Figure 3).

(c) *Stomach*. The stomach of *B. viridis* is subdivided into a wide corpus, or fundus, and a short *pars pylorica*, containing fundic and pyloric glands, respectively, both emptying into gastric pits. The luminal surface of the stomach and the gastric pits were lined by a single layer of mucus-secreting cells, indicated as superficial and foveolar, respectively. The fundic glands were of the simple or branched tubular type, and consisted of mucous neck cells located in their upper third, argentaffin endocrine cells and, mainly, oxynticopeptic cells. The tubules were longer and oxynticopeptic cells were more abundant in the oral fundus (Figure 4). The pyloric glands were shorter than the fundic glands. They consisted of muco-secreting cells, and numerous endocrine cells.

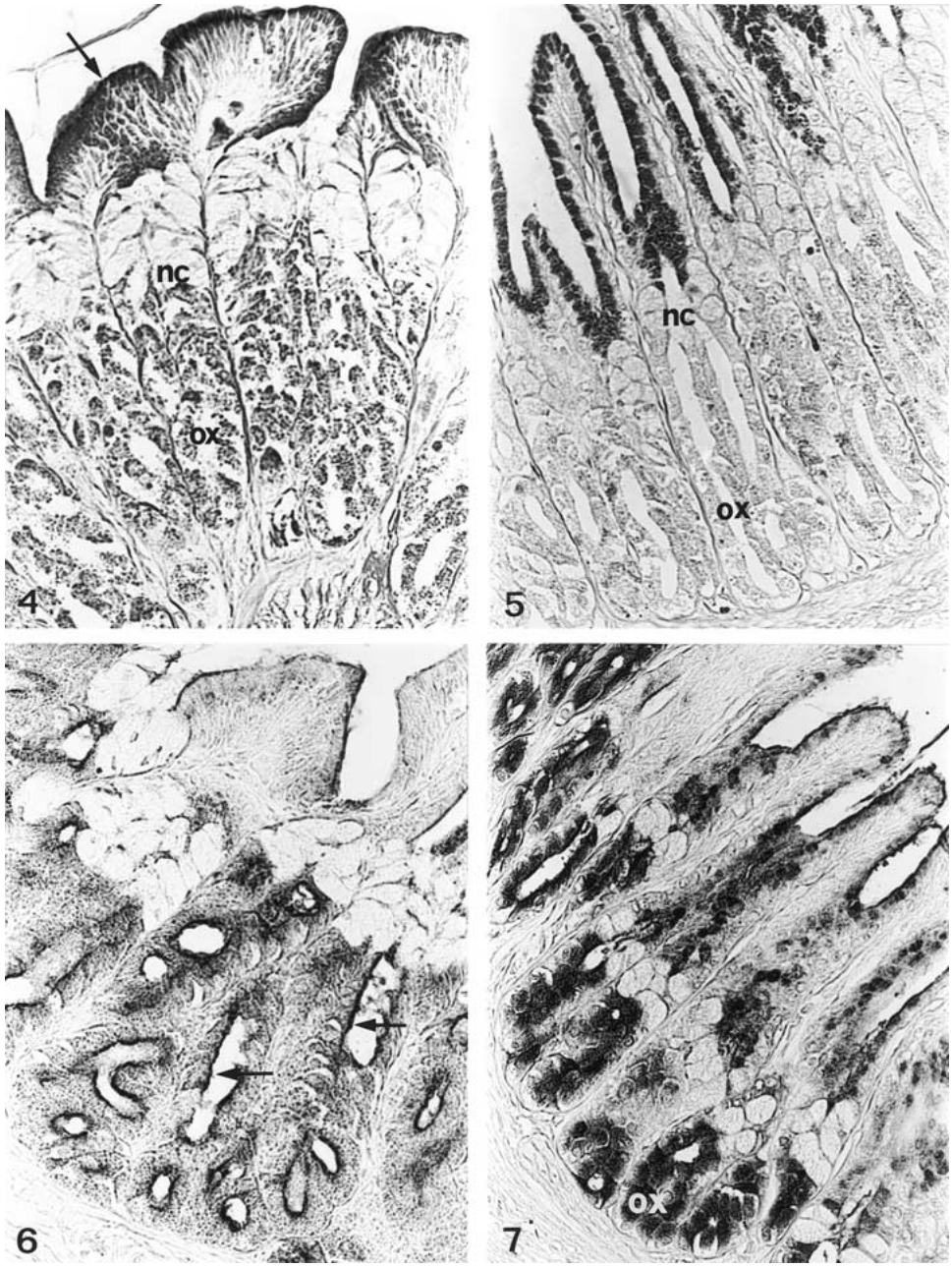
The superficial and the foveolar cells were PAS positive but did not react with AB. The mucous neck cells were moderately PAS positive and did not react with Alcian Blue. The oxynticopeptic cells in the oral fundic glands were filled with many large secretory granules that stained intensely with Bowie's reaction (Figure 4) whereas in the aboral fundus, the secretory granules in the oxynticopeptic cells reduced in number and size (Figure 5). In the oral area, these cells moderately stained with DBA lectin, particularly in their apical areas (Figure 6) whereas in the aboral region DBA reactivity increased and extended to the whole cell (Figure 7).

The epithelial superficial cells of the pylorus were PAS positive and AB negative. The mucous cells in the pyloric glands displayed the same basic histochemical features as the fundic neck cells but differed in the lectin-binding pattern. No cells with Bowie-positive granules were observed.

(d) *Lectin histochemistry of mucous cells*. The secretory product of the Type I oesophageal goblet cells did not react with WPA, DBA or SBA (Figure 8). Pretreatment with sialidase imparted a strong reactivity with SBA (Figure 9), but did not affect the intense staining observed with PNA (Figure 10). These cells reacted less intensely with WGA and Con A, also after periodate oxidation–borohydride reduction (Paradoxical Con A). β-elimination caused a decrease in affinity with PNA and WGA, but not with Con A. Secretory granules of Type II goblet cells stained weakly with WGA and only after sialidase digestion with SBA (Figure 9) and PNA, whereas they did not react with WPA, DBA or Con A, even after periodate oxidation–borohydride reduction. In the oesophagogastric junction, mucous glandular cells showed affinity for PNA, but not for the other lectins tested. They stained positive with Con A only after periodate oxidation–borohydride reduction. The secretory product of superficial and foveolar cells of the stomach showed affinity for PNA (Figure 11), that was reduced after β-elimination. No binding was observed with the other lectins tested. The cells did not stain with Con A after periodate oxidation–borohydride reduction. The mucous neck cells of the fundic glands moderately stained only with PNA (Figure 11) and with Con A only after periodate oxidation–borohydride reduction. Affinity for PNA was reduced after β-elimination. In the pyloric region superficial cells stained weakly only with the PNA lectin, while the secretory product of the glandular mucous cells stained with SBA and, mainly, with WGA (Figure 12), but not with paradoxical Con A. β-elimination reduced the affinity for WGA. In control sections, exposure to DAB–H_2O_2 medium after substitution of PBS for lectin–HRP conjugates produced no specific staining. Lectin staining was significantly inhibited by addition of the corresponding hapten sugar.

Discussion

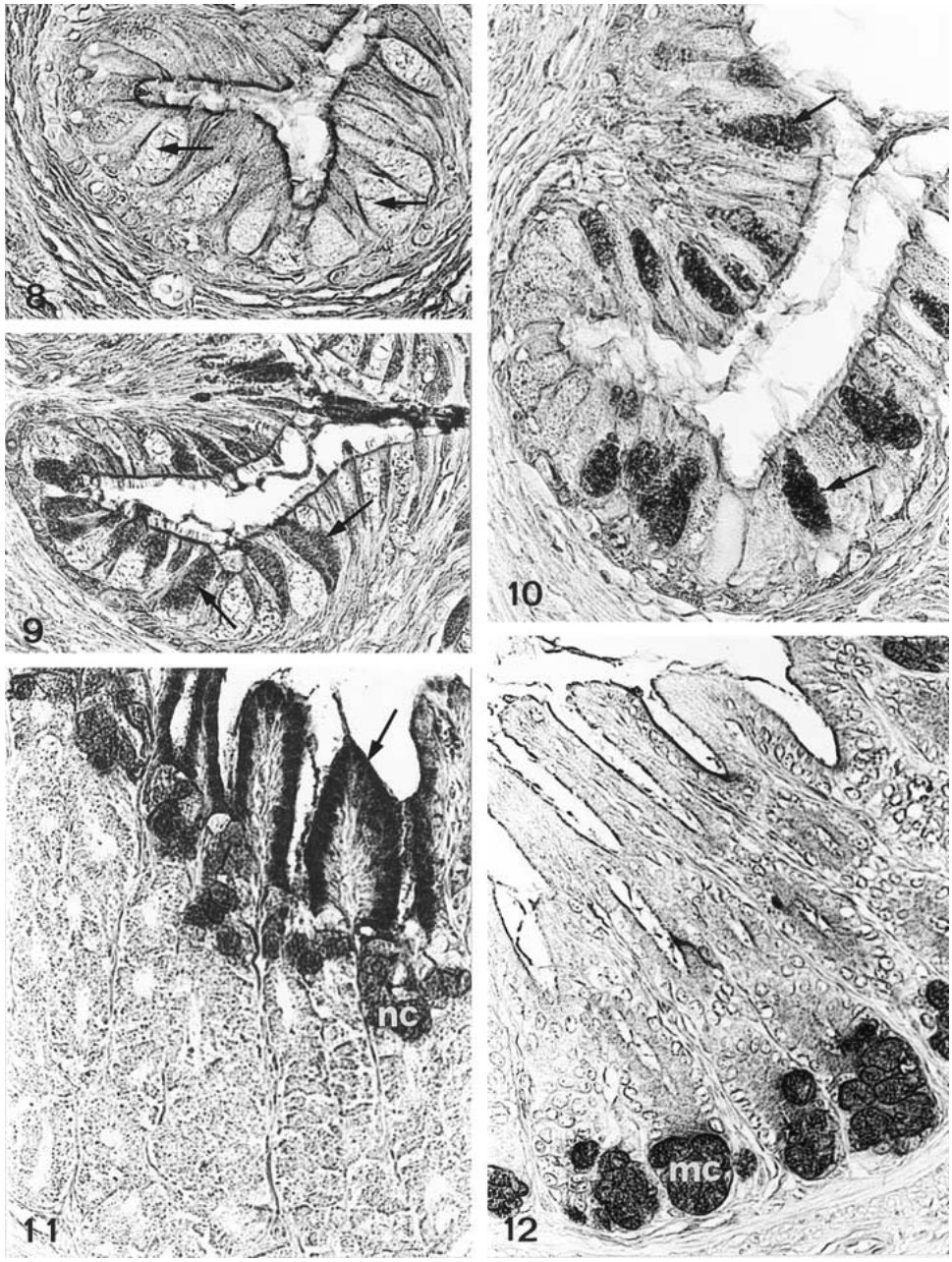
The oesophagogastric tract of *B. viridis* presents the general features of most non-mammalian vertebrates. As reported for other species of *Bufo* (Loo & Wong 1975, Hirji & Nikundiwe 1982), the oesophageal mucosa of *B. viridis* lacks any pluricellular gland. In contrast, Ranoidean frogs present true oesophageal glands with mucous and peptic cells producing most of the pepsinogen in the gastric juice (Norris 1959, Hirji 1982, Bani 1983, Bani *et al.* 1992, Gallego-Huidobro *et al.* 1992, Gallego-Huidobro & Pastor 1996, Ferri *et al.* 2001). In *B. viridis*, the first pluricellular glands appear in the oesophagogastric junction, and are alveolar, mainly muco-secreting, with few oxynticopeptic cells. The shape of these glands changes to tubular towards the gastric mucosa, and the number of oxynticopeptic cells increases. A moderate positivity to Bowie's staining was seen in the oesophageal goblet cells and hence it cannot be excluded that a small amount of pepsinogen is produced. Immunohistochemical techniques should confirm this finding. Pepsinogen secretion by oesophageal superficial epithelial cells has been demonstrated using Bowie



Figures 4–7. Gastric mucosa of *B. viridis* stained by different histochemical methods. (4) Oral fundus, PAS–Bowie. Surface mucous cells (arrow) stain intensely with PAS, while mucous neck cells (nc) which are voluminous and numerous, react weakly. Oxynticopeptic cells (ox) are filled with many granules which stained with Bowie's reaction. 1500×. (5) Aboral fundus, PAS–Bowie. The oxynticopeptic cells (ox) show few secretory granules stained with Bowie's reaction. Mucous neck cells (nc) are small and few in number. 1500×. (6) Oral fundus, DBA lectin. The apical cytoplasm of the oxynticopeptic cells (arrows) is stained with this lectin. 1500×. (7) Aboral fundus, DBA lectin. The DBA reactivity is extended to the whole cytoplasm of the oxynticopeptic cells (ox). 1500×.

staining in the 'rock snake' (Imai *et al.* 1991: no scientific name is given). Pepsin has been detected in the oesophagus of the toad *B. marinus* using biochemical methods (Taylor & Tyler 1986, Ruiz *et al.* 1993).

In the gastric mucosa of *B. viridis* tubular glands are seen. The tubules consist of mucous cells, oxynticopeptic cells and endocrine cells. The morphology of the oxynticopeptic cells changes from the oral to the aboral fundus. The maximum number and size of pepsinogen granules is reached in the oral fundus. Gradually, smaller and rarer granules are found towards the aboral fundus and oxynticopeptic cells disappear completely in the pylorus. In contrast, DBA-positivity increases from the oral to the aboral fundus. DBA specifically binds to the α-GalNAc residues in the intracellular canalicular membranes, the extension of which seems to be related to the production of HCl in the parietal cells of mammals (Peschke *et al.* 1983, Ito *et al.* 1985, Kessimian *et al.* 1986). In non-mammals, oxynticopeptic cells do not contain intracellular canaliculi, but show a tubulo-vesicular membranous system, the extension of which appears to



Figures 8–12. Oesophageal and gastric mucosae of *B. viridis* stained by different lectins. (8) Oesophagus, SBA. The oesophageal goblet cells (arrows) do not react with SBA. 1500×. (9) Oesophagus, Sialidase–SBA. In some Type I and Type II goblet cells (arrows) pretreatment with sialidase imparted strong reactivity with the same lectin. 1500×. (10) Oesophagus, PNA. Type I goblet cells (arrows) stained strongly with PNA. 1500×. (11) Oral fundus, PNA. Superficial mucous cells (arrows) and mucous neck cells (nc) bind PNA. 1500×. (12) Pylorus, WGA. The mucous glandular cells (mc) show affinity with this lectin. 1500×.

correlate with hydrochloric acid production (Liquori *et al*. 2000). DBA staining could indirectly confirm that HCl production increases in *B. viridis* from the oral to the aboral fundus.

The morphological variation of the oxynticopeptic cells from the oral to the aboral fundus in *B. viridis* is very similar to that of the seps, *Chalcides chalcides* (Liquori *et al.* 2000) and of the ruin lizard, *Podarcis sicula* (Ferri *et al.* 1999). Using biochemical methods, in the cane toad Taylor and Tyler (1986) and Ruiz *et al.* (1993) found a maximum pepsinogen content in the oral fundus, and a decrease towards the pylorus, that is in accordance with our histochemical findings. Thus, even if the oxynticopeptic cells in the gastric glands of *B. viridis* are involved in the secretion of both pepsinogen and hydrochloric acid, they produce mainly pepsinogen in the oral fundus and mainly hydrochloric acid in the aboral fundus. Our finding of gastric pepsinogen and an HCl gradient in an amphibian is in accordance with the pattern already observed in cartilagineous fish (Gabrowski *et al.* 1995) and in some squamates (Giraud *et al.* 1979, Ferri & Liquori 1994, Ferri *et al.* 1999), and can be probably extended to most non-mammalian vertebrates with oxynticopeptic cells in their

gastric glands. The symmetric pepsinogen–hydrochloric acid gradient probably allows the food to be first surrounded by pepsinogen in the oral fundus, then pepsinogen is converted to pepsin in the acid environment of the aboral fundus and proteolytic activity begins. This organization recalls that of parietal and chief cells within the gastric glands of mammals, where pepsinogen produced by chief cells in the glandular bottom is then converted to pepsin by HCl secretion of parietal cells in the isthmus and neck regions (Ito 1967). In Ranoidean frogs the functional separation between pepsinogen and hydrochloric acid secretion is achieved by concentrating the zymogenic activity in the oesophageal peptic cells, whereas in the fundic glands the oxynticopeptic cells produce mainly hydrochloric acid (Norris 1959, Sedar 1961a,b, Geuze 1971, Bani 1983). The cited functional variations along the oesophagogastric mucosa probably induce differences in composition of the mucus covering, related to lubrication, mechanical protection, defense against pathogens and gastric juice injuries. The mucus covering the oesophagogastric epithelium of *B. viridis* is produced by a number of cells and differs from one tract to another in quantitative expression of acidic and neutral glycoconjugates, as well as in lectin-binding patterns. Goblet cells in the oesophagus produce mainly acidic glycoconjugates, both sulphated and carboxylated. Acidic mucosubstances are also predominant in the oesophageal mucosa of *B. melanostictus* (Loo & Wong 1975), as well as in many other vertebrates (Suganuma *et al.* 1981, Ferri *et al.* 2001). Two types of goblet cells are seen. Type I goblet cells contain stable class-III mucosubstances (Katsuyama & Spicer 1978) which are both O-linked and N-linked glycoproteins, with Galβ1,3GalNAc sequences, β1,4GlcNAc oligomers, D-mannose and/or D-glucose, sulphated esters on internal residues, and abundant non-O-acylated terminal sialic acid bound to penultimate GalNAc. Type II oesophageal goblet cells mainly differ from Type I cells in that they contain class-III mucosubstances, D-mannose and/or D-glucose, and terminal Galβ1,3GalNAc sequences.

Oesophageal goblet cell types differing in granular ultrastructure and histochemical features have been found in other amphibians as well (Suganuma *et al.* 1981, Setoguti *et al.* 1987, Gallego-Huidobro *et al.* 1992, Ferri *et al.* 2001). In other anurans Type I cells also stain with PCS, but not Type II (Suganuma *et al.* 1981, Ferri *et al.* 2001).

In the stomach of *B. viridis* mucus is secreted by three cell types, superficial, foveolar and neck cells. It consists of neutral mucins with Galβ1,3GalNAc residues. Neutral mucins above were also detected in the gastric mucosa of *B. melanostictus* (Loo & Wong 1975), whereas in *Rana aurora* small amounts of acidic mucins were also observed with GalNAc residues (Ferri *et al.* 2001). The neck cells of *B. viridis* presented stable class-III mucosubstances, as has been observed in the gastric glands of a number of amphibians, reptiles and mammals (Suganuma *et al.* 1981, Ferri *et al.* 2001), but not in fish, birds or most saurians (Gabe & Saint-Girons 1972, Suganuma *et al.* 1981, Domeneghini *et al.* 1998). According to Suganuma *et al.* (1981), amphibians were the first vertebrates to evolve true mucous neck cells, which are typically Paradoxical Con A positive. The mucous glandular cells in the oesophagogastric junction of *B. viridis* have the same histochemical features as the gastric neck cells and include stable class-III mucosubstances, so that it may be assumed that they are homologous.

Like fundic neck cells, pyloric mucous glandular cells also produce neutral mucins, but these differ in that they lack stable class-III mucosubstances and Galβ1,3GalNAc residues, whereas they present GalNAc and GlcNAcβ1,4 residuals in O-linked oligosaccharides chains. Stable class-III mucosubstances are present in the pyloric mucous glandular cells of some amphibians and other vertebrates (Suganuma *et al.* 1981, Ferri *et al.* 2001).

Differences in the composition of mucus between the oesophagus and the stomach are probably linked to different functions. Oesophageal mucus is probably involved in lubricating and protecting the epithelium from mechanical injuries and pathogenic organisms, whereas gastric mucus protects against mechanical injuries, pathogens and aggressive pepsin, as well as supporting surface neutralization of acid by mucosal bicarbonate (Ferri *et al.* 2001).

B. viridis differs from the Ranoidean frogs in its histological, histochemical and lectin-binding patterns. In general, it would seem that *B. viridis* retains some primitive features, such as the oesophagus without peptic glands and the stomach with oxynticopeptic cells in the fundic glands. These features are shared with a number of non-Ranoidean frogs (Bani 1983, Hirji & Nikundiwe 1982, Bani *et al.* 1992) and suggest that they are symplesiomorphies of Anura. Ranoidean frogs on the other side present true oesophageal glands and a stomach with oxyntic cells in the fundic glands, that could be regarded as autoapomorphic characters. A lot more data from a greater number of species are needed to support this view, and careful examinations of serial sections of the oesophagus and of the oesophagogastric junction should confirm both epithelial pepsinogen production and lack of peptic glands.

Such studies would also indicate whether different patterns in mucus composition between species, like those found in *B. viridis* in comparison to *Rana aurora* (Ferri *et al.* 2000), have a functional and/or a phylogenetic significance.

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