

## BIOACTIVE PEPTIDES AND SEROTONIN IN THE GUT ENDOCRINE CELLS OF THE CROCODILE, *CROCODYLUS NILOTICUS* (LAURENTI 1768): AN IMMUNOCYTOCHEMICAL STUDY

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### ABSTRACT

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Little is known about peptide-storing endocrine cells in the gut of the Nile crocodile. As in the case of other reptiles, particularly the alligator, a limited range of peptide-storing cells was found in the gut of the crocodile. They were somatostatin, glucagon, gastrin, neurotensin and pancreatic polypeptide. The topographical distribution of cells immunoreactive to somatostatin and gastrin in the gut of the crocodile is comparable to the situation in the alligator. Glucagon and neurotensin immunoreactive cells have a much wider distribution in the gastro-intestinal tract of the crocodile compared to the alligator. Cholecystokinin and bombesin cells previously reported in the small intestine of the alligator were not detected in this study. This is the first report to demonstrate pancreatic polypeptide and serotonin immunoreactivity in the gut of a crocodilian specie.

### INTRODUCTION

Commercial crocodile farming aimed at the production of hides, has made a substantial contribution to the economic status of these animals (Smith & Marais, 1990). As our knowledge of reptile gut physiology is still fragmentary (Skoczylas, 1978), the economic status of crocodiles has reverted the interest to its gut biology, and therefore this study was undertaken.

Various activities of the vertebrate gut are modulated by a variety of bioactive peptides and serotonin. They are secreted by a diffuse system of endocrine and paracrine cells in the epithelium of the stomach and intestine (Sundler, Böttcher, Ekblad & Hakanson, 1989). However, bioactive peptides of the alimentary tract of reptiles received little attention (Buchan, Lance & Polak, 1983). Only a few contributions were made on the gut of snakes (Buchan, 1980; Masini, 1986), two species of lizard (Reinecke, Schluter, Yanaiharu & Frossman, 1981; El-Salhy & Grimelius, 1981) and a turtle (Larsson & Rehfeld, 1977). By employing immunochemical and immunocytochemical methods gastrin/cholecystokinin-related peptides were demonstrated in the stomach and duodenum of the Nile crocodile (Dimaline, Rawdon, Brandes, Andrew & Loveridge, 1982; Rawdon, Brandes, Andrew & Loveridge, 1980).

The first full scale investigation of the gut peptides of a member of the crocodilian family was done on *Alligator mississippiensis* (Buchan *et al.*, 1983). They demonstrated six bioactive peptides with the following topographic distribution: somatostatin was found in all the regions of the gut; cells immunoreactive to glucagon and bombesin occurred in the mucosa of

the stomach only; neurotensin and gastrin immunoreactivities were observed in the stomach and small intestine, while secretin antiserum stained cells in the upper small intestine only. Furthermore, Buchan *et al.* (1983) failed to demonstrate any endocrine cells by employing antisera raised to glicentin, gastric inhibitory polypeptide (GIP), vasoactive intestinal polypeptide (VIP), substance P, CCK, motilin and met-enkephalin.

### MATERIALS AND METHODS

Three 18 month old crocodiles were used in this study. Each animal was shot in the brain, its entire gut was promptly removed and samples were taken from the following regions; oesophagus, fundus and antrum of the stomach, duodenum, ileum and colon. Tissue samples of each region were placed in a 2 l flask containing Ringer's solution designated for cold blooded animals (Humason, 1979), and shaken vigorously for 30 s to remove mucus and sand particles from the mucosa. All tissue samples were placed in Bouin's solution for 12 h at room temperature and embedded in paraffin wax. Sections (5 µm) were cut and floated on slides pretreated with poly-L-lysine (Van Noorden & Polak, 1983).

All sections were dewaxed and the endogenous peroxide activity was blocked by treating them with 0,3 % hydrogen peroxide in methanol for 30 min. (Van Noorden & Polak, 1983). The sections were hydrated in a graded series of ethanol and transferred to 0,05 M Tris-saline. To eliminate some of the non-specific staining, the sections were preincubated with non-immune serum (Burns, 1979). Both gut peptides and serotonin were demonstrated by the peroxidase-anti-peroxidase (PAP) method (Sternberger, 1979) and the primary antisera employed are listed in Table 1. Reaction sites were revealed by employing 3,3'-diaminobenzidine (Graham & Karnovsky, 1966). After dehydration the stained sections were mounted with Entellan (Merck).

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TABLE 1 Details of the primary antisera employed

Antiserum raised to	Code	Dilution	Source
Somatostatin	1191	1:1000	*J. M. Polak, London
Somatostatin	310	1:1000	*R. Benoit, Montreal
Somatostatin	928	1:1000	*J. M. Polak, London
Gastrin (C-terminal)	RPN1529	1:1000	Amersham
Gastrin (C-terminal)	655	1:1000	*J. M. Polak, London
CCK (midportion 9-20)	1937	1:1000	*J. M. Polak, London
Glucagon	RPN1602	1:1000	Amersham
Secretin	S3250	1:1000	Sigma
Substance P	910	1:500	*J. M. Polak, London
Neurotensin	116	1:1000	*C. Shaw, Belfast
Bombesin	934	1:1000	*J. M. Polak, London
Motilin	123	1:500	*J. M. Polak, London
Vasoactive intestinal polypeptide	925	1:500	*J. M. Polak, London
Gastric inhibitory polypeptide	B35	1:500	Milab
Pancreatic polypeptide (chicken)	Lance	1:1000	*J. R. Kimmel, Kansas City
Serotonin	—	1:500	*J. M. Lauder, Chapel Hill, N.C.

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Sections of tissue from the other species, known to contain serotonin and the peptides in question, were employed as positive controls. To establish the specificity of the primary antisera, each antisera was pre-absorbed by incubating with 20 µg/ml diluted antiserum of its antigen at 4 °C for 12 h.

## RESULTS

Although the authors are well aware of the fact that the antisera do not necessarily demonstrate a substance identical to the parent antigen, the stained cells are named after the parent antigen for the sake of simplicity. For example neurotensin-like immunoreactive cells are referred to as neurotensin cells.

Antisera raised to CKK, secretin, substance P, bombesin, motilin, VIP and GIP failed to stain endocrine cells in the gut of the crocodile.

### Gastrin

Both antisera employed demonstrated numerous gastrin cells in the antrum (Fig. 1) and duodenum (Fig. 2) and sparsely distributed cells in the ileum. Throughout the duodenum and ileum gastrin cells were seen to reach the lumen. In the antrum the gastrin cells were deeply embedded in the glands, and they were mainly of the closed type. No gastrin cells were seen in the colon.

### Glucagon

Except for the colon, glucagon cells stained in all the regions that were tested. In the fundus (Fig. 3) and the antrum they were sparsely distributed, but were more numerous in the duodenum and ileum (Fig. 4). Some of the glucagon cells in the stomach were seen to reach the lumen, while those in the intestine were confined to the basal part of the mucosa.

### Neurotensin

Neurotensin cells were sparsely distributed in the antrum and only a few reach the lumen (Fig. 5). In the duodenum and ileum (Fig. 6) they were more numerous and many cells were seen to reach the lumen. Slender flask-shaped neurotensin cells, of the open type, occurred in the colon (Fig. 7).

### Somatostatin

Antiserum 1191 known to detect somatostatin-28 failed to stain cells, and antiserum 928 raised to somatostatin-14 stained cells in the antrum only. Another antiserum (310) known to detect somatostatin-14 only, demonstrated cells in all the regions tested. They were sparsely distributed in the fundus (Fig. 8) and more plentiful in the antrum. In the duodenum (Fig. 9) and ileum the somatostatin cells were less numerous and confined to the basal part of the mucosa. No stained cells were observed in the colon.

### PP

The antiserum employed, which was raised to chicken PP, detected endocrine cells in all the regions tested, except the colon. In both the fundus (Fig. 10) and the antrum there was a paucity of these cells. Numerous PP-cells were observed in the duodenum (Fig. 11) and ileum. None were seen to reach the lumen.

### Serotonin

Sparsely distributed serotonin cells occurred in both the fundus (Fig. 12) and antrum, some of which were in contact with the lumen. Throughout the entire small intestine there were numerous slender flask-shaped serotonin cells (Fig. 13), most of which reached the luminal surface. Small serotonin cells were markedly abundant in the colon (Fig. 14), where they were confined to a narrow belt adjacent to the basal lamina of the epithelium.

## DISCUSSION

The gut endocrine cells found in the crocodile corresponds to a large extent with those found in the alligator (Buchan *et al.*, 1983). However, secretin and bombesin-cells recorded in the alligator gut, were not found in the present study. On the other hand PP and serotonin cells which were not sought in the alligator were demonstrated in the gastrointestinal tract of the crocodile.

The distribution of gastrin cells in the gut of the crocodile is in line with those found in the alligator (Buchan *et al.*, 1983). The results of this study is in

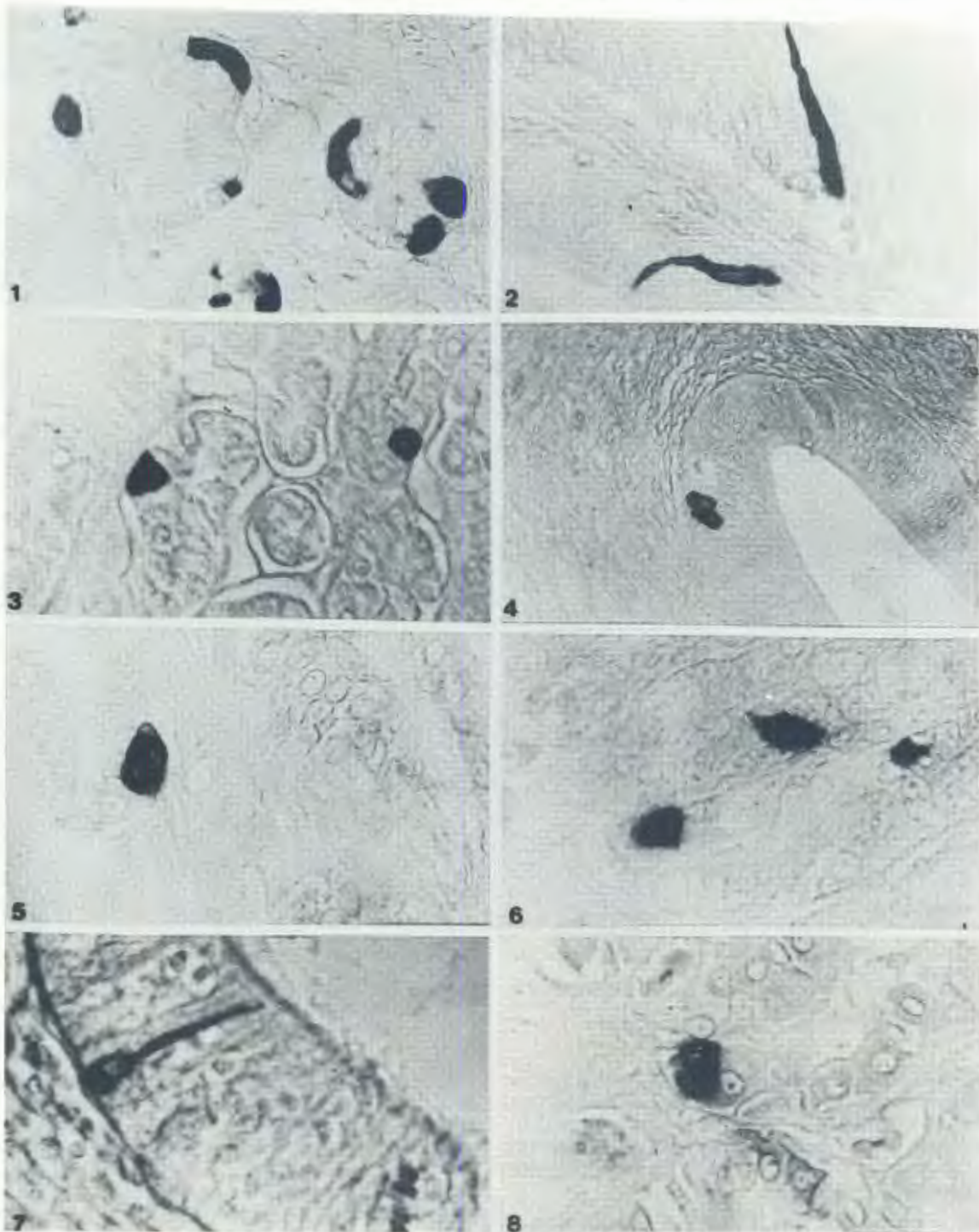


FIG. 1. Gastrin cells deeply embedded in the antrum glands. 1230 ×

FIG. 2. "Open" gastrin cells in the duodenum. 1230 ×

FIG. 3. Glucagon immunoreactive cells in the fundus of the stomach. 1230 ×

FIG. 4. Two glucagon cells in the mucosa of the ileum. 616 ×

FIG. 5. A neurotensin cell in the antrum. 1230 ×

FIG. 6. Neurotensin cells in the ileum. Note the cytoplasmic process of the cell in the middle. 1230 ×

FIG. 7. A flask-shaped neurotensin cell in the colon. 1230 ×

FIG. 8. A solitary somatostatin cell in the fundus of the stomach. 1230 ×

agreement with those of Larsson & Rehfeld (1977), Buchan *et al.*, (1983) and Dimaline *et al.* (1982), demonstrating that the carboxyl-terminal of reptilian

gastrin is structurally similar to its mammalian counterpart, as cells were stained by antisera orientated to the carboxyl-terminal of gastrin only. Should

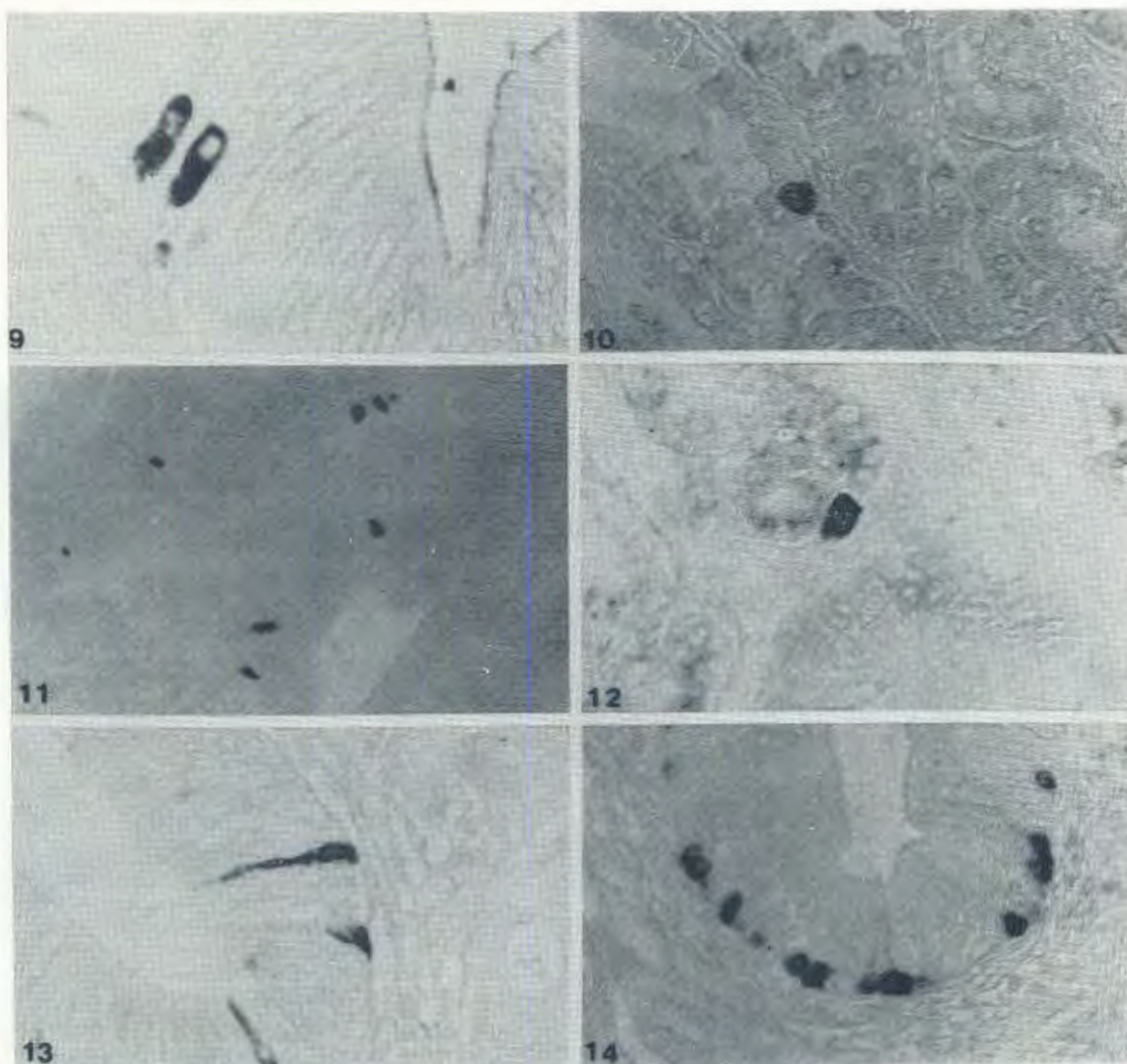


FIG. 9. Somatostatin cell in the basal part of the duodenal mucosa. 1234 ×

FIG. 10. A single PP-cell, of the closed type, in the fundus. 616 ×

FIG. 11. Numerous PP-cells in the duodenum. 308 ×

FIG. 12. A serotonin cell, situated on the basal part of a fundic gland. 616 ×

FIG. 13. Serotonin cells in the ileal mucosa. 616 ×

FIG. 14. Abundant serotonin cells in the basal part of the mucosa of the colon. 616 ×

separate CCK-containing cells exists in the gut of the crocodilian family, the peptide's midportion sequence is definitely different to its mammalian counterpart, as antisera raised to CCK-9-20 failed to stain any cells in the crocodile or the alligator (Buchan *et al.*, 1983).

Both antisera 928 and 310 are known to detect somatostatin-14 only. It is therefore not clear why antiserum 928 demonstrated somatostatin cells in the antrum only, while antiserum 310 stained cells in the fundus, antrum and small intestine. The inability of antiserum 1191 to stain cells suggests that the smaller molecular form viz. somatostatin-14, and not somatostatin 28, is present in the gut of the crocodile. Parallel to the situation in the alligator (Buchan *et al.*, 1983), somatostatin cells are abundant in the pyloric region of the crocodile and show a constant decline in numbers towards the distal small intestine.

In contrast to the findings in the alligator (Buchan *et al.*, 1983), no secretin cells were detected in the present study. As Buchan *et al.*, employed a different antiserum, the possibility that the antiserum used in this study failed to detect secretin should not be ignored. Further investigation with a variety of antisera is therefore needed to confirm the presence of secretin cells in the crocodile.

EL-Salhy & Grimelius (1981) detected PP cells in the gut of a reptile viz., the lizard *Mabuya quinquetaeniata*. Their finding is supported by this study. However, they showed PP cells to be numerous in the antrum and relatively sparse in the small intestine, which is in contrast to the situation in the crocodile where these cells were scarce in the stomach but numerous in the small intestine.

In only one instance was VIP cells recorded in the gut mucosa of a reptile (Reinecke *et al.*, 1981). Dimaline, Vaillant & Cockray (1980) employed five

antisera to VIP, each with a different regional specificity, to demonstrate VIP in gut endocrine cells and nerves of the rat. However, only one antisera stained endocrine cells which suggests that the rat may not contain the neuropeptide VIP, but an unidentified peptide. The absence of VIP endocrine cells in this study therefore seems to be justified. Furthermore the absence of substance P in gut endocrine cells of the crocodile corroborate the findings of Buchan *et al.*, (1983) who investigated the presence of substance P in the alligator. Motilin cells have only been demonstrated in one reptilian specie of reptile viz. *Mabuya quinquetaeniata* (El-Salhy & Grimelius, 1981) and appears to be absent in both the alligator (Buchan *et al.*, 1983) and the crocodile.

The first endocrine cells identified in the vertebrate gut were enterochromaffin cells containing serotonin (Sundler *et al.*, 1989). By employing conventional staining methods, Gabe (1979) demonstrated putative endocrine cells in the gut of various reptiles, including the crocodile. This study confirmed his suggestion that some of these cells may contain serotonin.

In general the findings of this study are in line with those of Buchan *et al.*, (1983) on the alligator, and thus correspond with the phylogenetic proximity of the crocodile and the alligator. However, this is the first immunocytochemical study to demonstrate PP cells in the gut of crocodylians, and serotonin cells in the gut of a reptilian specie.

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