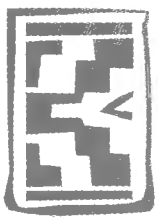

Distribution of endocrine cells in the gut of the impala (*Aepyceros melampus*)

J.H. SCHOEMAN[1], V. DE VOS[2] and G. VAN ASWEGEN[3]*

ABSTRACT

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Immunocytochemical methods were employed to demonstrate endocrine cells, containing peptides and serotonin, in the gut of the impala. Cells immunoreactive to serotonin, somatostatin, gastrin, cholecystokinin, glucagon, neurotensin, secretin, glucose-dependant insulinotropic peptide and motilin were detected. Antisera raised to substance P and pancreatic polypeptide failed to stain any cells. The distribution of these peptide-containing cells is more in line with the situation in sheep than other ruminants. In contrast, the distribution and abundance of serotonin cells in the gut of the impala parallels the situation seen in game herbivores.

Keywords: *Aepyceros melampus,* endocrine cells, gut peptides, impala, serotonin

INTRODUCTION

Impala are distributed in woodlands of southern Africa (Skinner & Smithers 1990). They are grazers and browsers (Kay, Engelhardt & White 1979) and their diet may vary depending on the habitat and season (Skinner & Smithers 1990). In spite of their abundance in southern Africa (Skinner & Smithers 1990), and particularly in the Kruger National Park (Nicholls, Viljoen, Knight & Van Jaarsveld 1996), little is known of the digestive physiology of this species.

Recently, considerable attention has been focused on the neuroendocrine modulation of the functions of the gut (Sundler, Böttcher, Ekblad & Hakanson 1989). Some gut endocrine functions have been elucidated, for example, secretin evoking pancreatic secretion and motilin which elicits gut motility (Holst & Schmidt 1994). Endocrine/paracrine modulation of gut secretion, absorption and mucosal blood flow is not yet fully understood (Holst & Schmidt 1994). As research progresses the evidence for the integration of nervous and endocrine control of digestive functions is increasing (cf. Cooke 1987; Sundler *et al.* 1989). The gut endocrine system is complex, and acts in various ways, viz. autocrine, endocrine, paracrine and neuroendocrine processes (cf. Rehfeldt 1990). The field of gut regulatory peptides is therefore exciting and comparative studies, involving species other than those traditionally employed in the laboratory, may contribute to a better understanding of gut physiology.

Reports on the gut endocrine cells of some ruminants have contributed to this effort, including bovine species (Kitamura, Yamada, Calingasan & Yamashita 1985; Weyrauch, Schnorr & Glaser 1989), the sheep (Calingasan, Kitamura, Yamada, Oomori & Yamashita 1984), the lesser mouse deer (*Tragulus javanicus*) (Angungpriyono, Yamada, Kitamura, Yamamoto, Said, Sigit & Yamashita 1994) and the fallow deer (*Dama dama* L.) (Ceccarelli, Pedini & Gargiulo 1995).

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In this investigation the distribution of endocrine cells containing bioactive peptides and serotonin was demonstrated in the gut of the impala, and compared to the situation in other ruminants.

MATERIALS AND METHODS

During a culling expedition samples of tissue were promptly taken from six adult impala including the abomasum, duodenum, jejunum, ileum, caecum, colon and rectum.

Fixation and processing

All tissue samples were rinsed vigorously in Ringer's solution for 30 s to remove sand and other particles from the luminal surface. For immunoperoxidase staining, the samples were fixed in Bouin's fluid for 12 h at room temperature. The tissue blocks were dehydrated in ethanol and embedded in paraffin wax. Sections (5 μm) were cut and floated on slides pretreated with poly-L-lysine (Huang, Gibson, Facer, Gu & Polak 1983).

Immunoperoxidase staining for mucosal endocrine cells

Paraffin sections were dewaxed and treated with 0,3 % hydrogen peroxide in methanol for 30 min to block all endogenous peroxidase activity. The sections were hydrated through a series of ethanols and transferred to 0,05 MTris-saline. To reduce non-specific staining, the sections were incubated with 10 % normal swine serum (Burns 1979). Either the indirect peroxidase or the peroxidase anti-peroxidase (PAP) (Sternberger 1979) methods were employed to identify immunoreactivity for the bioactive peptides and serotonin. The reaction sites were revealed by the method of Graham & Karnovsky (1966). Details of the primary antisera employed are listed in Table 1.

Controls for immunocytochemistry

As positive controls, sections of tissues from other species (swine and dog), known to contain the peptides in question, were employed. Negative controls consisted of substitution of non-immune rabbit serum for the primary antibody and pre-absorption of the diluted antibody with 10 nmol or at least 20 μg per mℓ of the homologous peptide or serotonin.

RESULTS

All the antisera were absorbed by their parent antigens. No immunoreactivity was observed for the antisera for pancreatic polypeptide and substance P (Fig. 1).

TABLE 1 Primary antisera used

Antisera	Code	Dilution
Somatostatin 14 (synthetic)[b]	744	1:1000
Gastrin (porcine)[a]	B35	1:1000
CCK (synthetic midportion)[b]	280	1:1000
Glucagon (porcine pancreatic)[a]	B27	1:2000
Secretin (natural porcine)[a]	B37	1:2000
Neurotensin[b]	810	1:1000
Neurotensin[e]	–	1:500[d]
GIP (natural porcine)[b]	378	1:1000
Motilin (porcine)[c]	M37B	1:1000
Serotonin[d]	–	1:500
Pancreatic polypeptide (bovine)[f]	Lance	1:2000
Substance *P* (synthetic porcine)[b]		

[a] – purchased from MILAB
[b] – supplied by Prof. J.M. Polak, London
[c] – purchased from Quandrologic Inc.
[d] – supplied by Prof. J.M. Lander, Chapel Hill
[e] – supplied by Prof. C. Shaw, Belfast
[f] – purchased from Lilly Research Laboratories

Except for the caecum and rectum, somatostatin immunoreactive cells were observed in all the regions of the gut. They were particularly numerous in the pyloric region (Fig. 2). Some cells had basal cytoplasmic extentions.

Cells immunoreactive for gastrin were abundant in the pyloric region (Fig. 3), less abundant in the duodenum and sparse in the small intestine. They were absent from the large bowel. In all the regions where these cells occurred, none of them reached the lumen. Cholecystokinin (CCK)-immunoreactive cells were confined to the small intestine (Fig. 4), and their numbers declined from the proximal duodenum to the ileum. Very few of these cells were seen to reach the lumen.

No glucagon immunoreactive cells were observed in the abomasum. In the entire small intestine they were quite sparse, but in the duodenum, colon and rectum they were slightly more abundant. They were confined to the basal part of the mucosa (Fig. 5).

Antiserum 810 failed to demonstrate endocrine cells. At a dilution of 1:500 the antiserum supplied by Prof. C. Shaw stained a few cells immunoreactive for neurotensin in jejunum and ileum. They were seen in the crypts and villi.

The antiserum to secretin stained cells in the mucosa of the duodenum only (Fig. 6). They were present in the crypts and villi, and many of them were of the open type. Similarly, the distribution of glucose-dependant insulinotropic peptide (GIP)-immunoreactivity (Fig. 7) was limited to the duodenal mucosa. The GIP-endocrine cells were preferentially located towards the basal part of the mucosa.

Cells immunoreactive to motilin were sparsely scattered in the duodenum and jejunum (Fig. 8). The antiserum to serotonin (5HT) demonstrated endocrine

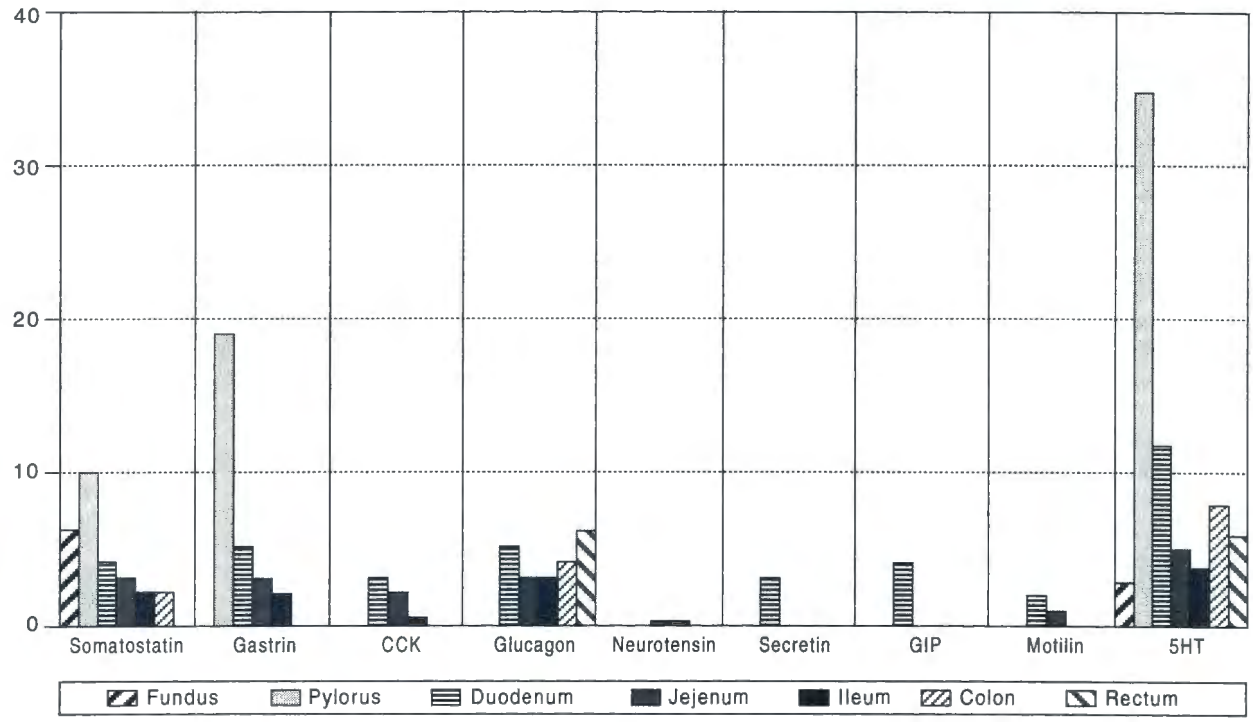

FIG. 1 Histogram showing the relative abundance of cells per 0,25 mm²

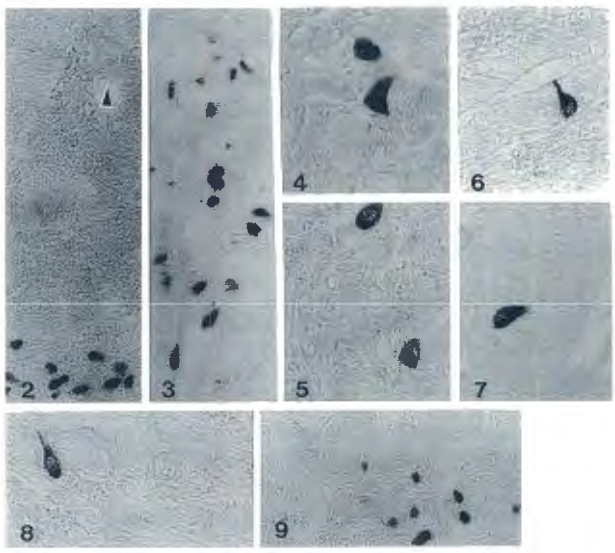

FIG. 2 Somatostatin cells in the basal part of the pyloric region. Arrow head pointing towards the lumen
200 x Indirect peroxidase

FIG. 3 Gastrin cells in the pyloric region
200 x Indirect peroxidase

FIG. 4 CCK-immunoreactive cells in the jejunum
350 x PAP

FIG. 5 Glucagon cells in the ileum
350 x PAP

FIG. 6 A cell immunoreactive to secretin in the duodenum
350 x PAP

FIG. 7 A GIP-immunoreactive cell in the duodenum
350 x PAP

FIG. 8 A motilin cell in the jejunum
350 x PAP

FIG. 9 Cells immunoreactive to 5HT in the rectum
200 x Indirect peroxidase

cells in all the regions of the gut under investigation. They were relatively scarce in the fundus, somewhat more abundant in the jejunum and ileum, but markedly numerous in the pyloric region. If compared to the small intestine, their numbers progressively increased in the colon and rectum (Fig. 9). In the regions of the gut where they were sparse, they were randomly distributed, but as their numbers increased they were increasingly confined to the basal part of the mucosa.

DISCUSSION

The distribution and abundance of somatostatin and gastrin endocrine cells in the gut of the impala are in line with the general situation seen in smaller ruminants, such as goat and sheep (Calingasan *et al.* 1984; Weyrauch, Blähser & Perschbacher 1987), the lesser mouse deer (Angungpriyono *et al.* 1994) and the fallow deer (Ceccarelli *et al.* 1995). In contrast to the situation in the lesser mouse deer (Angungpri-

yono *et al*. 1994), the GIP- and secretin-containing cells of the impala are confined to the proximal small bowel only, which is in agreement with findings on the cow (Kitamura *et al*. 1985) and confirms the presence of the typical mammalian entero-pancreatic axis of the impala (Sundler *et a*l. 1989) in this region of the gut.

In contrast to sheep (Calingasan *et al*. 1984), but in line with the situation in the lesser mouse deer (Angungpriyono *et al*. 1994) and cow (Kitamura *et al*. 1985), endocrine cells containing motilin were restricted to the proximal small intestine of the impala. CCK-endocrine cells seem to be preferentially located in most of the regions of the small intestine of the impala and small ruminants (Calingasan *et al*. 1984; Ceccarelli *et al*. 1995). The paucity of neurotensin cells seen in the impala is typical for herbivores (Sundler, Hakanson, Hammer, Alumets, Carraway, Leeman & Zimmerman 1977) and is in agreement with the situation in sheep (Calingasan *et al*. 1984).

In contrast to the findings on other ruminants (Calingasan *et al*. 1984; Kitamura *et al*. 1985; Angungpriyono *et al*. 1994) it seems that either endocrine cells immunoreactive for substance P and pancreatic polypeptide do not occur in the gut of the impala, or their sequences are so different that they escaped detection by the antisera employed.

The topographical distribution and abundance of serotonin endocrine cells in gut of the impala follow the situation in the fallow deer (Ceccarelli *et al*. 1995) more closely than that of the lesser mouse deer (Angungpriyono *et al*. 1994). These cells seem to be markedly more abundant in game herbivores, if compared to their domesticated counterparts. Judging from their abundance, it seems that serotonin plays an important role in the digestive physiology of the impala and the fallow deer (Ceccarelli *et al*. 1995). It is known that serotonin, together with other gut peptides, modulates the digestive processes (Gonella 1981). The abundance of serotonin cells, and its action in the gut of the impala, may allow it to thrive on a harsh and much varied seasonal and habitat diet (cf. Pietersen 1991). Cross-reaction between substance P and serotonin (Rawdon & Andrew 1994) is not considered in this study, because no substance P immunoreactivity was observed.

The absence of glucagon endocrine cells in the stomach of the impala was also seen in the fallow deer (Ceccarelli *et al*. 1995), and cow (Kitamura *et al*. 1985), whereas the distribution of these cells in the intestine of the impala is parallel to the findings on glicentin in sheep (Calingasan *et al*. 1984). The possibility that the antiserum employed to detect glucagon cross-reacted with glicentin, should not be ignored. The absence of glucagon immunoreactive cells, notably in the fundus of the impala and cow, is not surprizing, because they are known to occur in the fundic regions of carnivores (cat and dog) (Larsson, Holst, Hakanson & Sundler 1975; Grimelius, Holst, Buffa, Polak, Pearse & Solcia 1976). In omnivores (rat and man) these cells are sparsely distributed (Grimelius *et al*. 1976). The situation in the impala and cow therefore seems to be typical for herbivores as in the case of the elephant (Van Aswegen, Schoeman, De Vos & Van Noorden 1994).

One of the main features of the topographical distribution of endocrine cells in gut of the impala is that most were situated in the basal part of the mucosa and therefore implicate a paracrine function (Larsson, Golterman, De Magistris, Rehfeld & Schwartz 1979).

This study is the first to report on the gut endocrine cells of an African game ruminant. Furthermore, it shows that the distribution patterns of the endocrine cell types in the gut of the impala resemble those of sheep more closely than other ruminants, but are different enough to conclude that they are unique for the species, particularly the abundance and distribution of serotonin endocrine cells. The influence of diet on the gut endocrine cells of the impala is not considered here, because it may be very difficult to ascertain, as its diet varies from one habitat or season to another (Skinner & Smithers 1990).

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