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The intestine and endocrine pancreas of the African elephant: a histological, immunocytochemical and immunofluorescence study

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ABSTRACT

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Histological, immunocytochemical and immunofluorescence methods were employed to study the intestine and endocrine pancreas of the elephant. The histological findings were in line with those in monogastric mammals. In the mucosa of intestine, endocrine cells were immunoreactive to somatostatin, gastrin, CCK, GIP, secretin, motilin, glucagon and NPY. Nerve cells immunoreactive to somatostatin, substance P, VIP, PHI, NPY, bombesin and CGRP were detected. No immunoreactivity to neurotensin was observed. Islets of the pancreas had insulin cells in their cores and glucagon and somatostatin cells in their mantles. The antisera employed failed to demonstrate PP cells in the pancreas, but NPY-immunoreactive cells were present.

Keywords: African elephant, endocrine pancreas, histological, immunocytochemical, immunofluorescence, intestine

INTRODUCTION

The African elephant, *Loxodonta africana,* has a voracious feeding habit (300 kg green food per day). Although it is an endangered species elsewhere, its numbers in the Kruger National Park have to be regulated by culling, which provides an opportunity to learn more about its digestive system.

In a previous publication (Van Aswegen, Schoeman, De Vos & Van Noorden 1994), the structure of the oesophagus and stomach, and the immunocytochemical localization of hormones and neuropeptides in these areas were described. In this paper, the findings in the intestine and pancreas are described.

MATERIALS AND METHODS

During a culling expedition, samples of tissue were promptly taken from the duodenum, jejunum, ileum, caecum, colon, rectum and pancreas of three adult specimens.

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TABLE 1 Primary antisera used

Antisera	Code	Dilution	
		PAP	IF
Somatostatin[a]	KB18	1:1000	
Somatostatin 14 (synthetic)[b]	744	1:1000	1:1000
Gastrin (synthetic porcine)[a]	B36	1:200	
CCK (synthetic porcine 1 39)[b]	899		1:500
CCK (synthetic midportion)[b]	280	1:4000	1:200
Glucagon (porcine pancreatic)[a]	B37	1:2000	
Secretin (natural porcine)[a]	B33	1:2000	
Neurotensin (synthetic bovine)[b]	810	1:1000	1:800
Neurotensin (synthetic bovine)[a]	B44	1:2000	
GIP (natural porcine)[b]	378	1:2000	
NPY (synthetic porcine)[b]	2005	1:400 (ABC)	
VIP (porcine)[b]	652	1:2000	1:2000
PHI (synthetic)[b]	938		1:900
Substance P (synthetic porcine)[b]	910		1:500
Bombesin (synthetic amphibian)[b]	627		1:400
CGRP (synthetic rat)[b]	1208		1:200
met-Enkephalin[b]	869		1:400
Galanin[b]	1152		1:1000
Motilin(porcine)[c]	M37B	1:2000	
Pancreatic polypeptide (bovine)[d]	Lance	1:2000	
Pancreatic polypeptide (bovine)[e]	422	1:500 (ABC)	
Pancreatic polypeptide (avian)[d]		1:1000	
Insulin (porcine)[e]	GP7	1:8000	

[a] Purchased from MILAB
[b] Supplied by Prof. J.M. Polak, London
[c] Purchased from Quadrologic Inc.
[d] Supplied by Prof. J.R. Kimmel, Kansas City
[e] Purchased from Lilly Research Laboratories

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 histology and 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 pre-treated with poly-L-lysine (Huang, Gibson, Facer, Gu & Polak 1983).

For immunofluorescence on frozen sections, samples were immersed for 2–3 h in 0,4% p-benzoquinone freshly dissolved in 0,01 M of phosphate-buffered normal saline, pH 7,0 (PBS) at ambient temperature. They were then transferred to PBS containing 15% sucrose and 0,1% sodium azide and kept in this buffer, with several changes at 4 °C, and were then cut into suitable blocks and snap-frozen for sectioning in the cryostat (Bishop, Polak, Bloom & Pearse 1978).

Histology

Paraffin sections were stained with haematoxylin and eosin and, alternatively, with phloxine and tartrazine for Paneth cells, Verhoeff's stain for elastic fibres, ac-cording to Masson's trichrome and periodic-acid Schiff (PAS) method. All sections were dehydrated and mounted with DPX.

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 M of Tris-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 most of the bioactive peptides. Pancreatic NPY was shown by an avidin-biotin technique (ABC) (Hsu, Raine & Fanger 1981). The reaction sites were revealed by the method of Graham & Karnovsky (1966). Details of the primary antisera employed are listed in Table 1.

Immunofluorescence for enteric neuropeptides

Cryostat sections at 10 µm were mounted on slides pre-coated with poly-L-lysine and allowed to dry for 1–3 h. They were then treatedfor 10 min with non- immune goat serum diluted 1:20 in PBS and subsequently incubated overnight at 4 °C with the primary antiserum (see Table 1 for details), rinsed in three changes of PBS and incubated for 1 h at room temperature with fluorescein isothiocyanate-conjugated goat anti-rabbit immunoglobulin (Miles Laboratories) diluted 1:200. After the sections had been rinsed, they were mounted in buffered glycerine and viewed in a fluorescence microscope.

Controls for immunocytochemistry

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

RESULTS

Histology

The mucosal surface was enlarged by a prominent spiral fold (Fig. 1), and well developed villi and intes-

TABLE 2 Distribution of immunoreactive endocrine cells and nerves in the elephant intestine and pancreatic islets

Antiserum	Duodenum		Jejunum		Ileum		Caecum		Colon		Rectum		Pancreas	
	C	N	C	N	C	N	C	N	C	N	C	N	C	N
Somatostatin	++	–	+	+	+	–	+	–	+	–	+	–	+++	–
Gastrin	+	–	+	–	–	–	–	–	–	–	–	–	–	–
CCK	+	–	–	–	–	–	–	–	–	–	–	–	–	–
GIP	++	–	++	–	+	–	–	–	–	–	–	–	–	–
Secretin	++	–	+	–	–	–	–	–	–	–	–	–	–	–
Motilin	+	–	–	–	+	–	–	–	–	–	–	–	–	–
Glucagon	++	–	+	–	++	–	+	–	+	–	+	–	++++	–
Neurotensin	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Substance P	–	+	–	+	–	+	–	+	–	++	–	+	–	–
VIP	–	+	–	+	–	+	–	+	–	++	–	+	–	–
PHI	–	+	–	+	–	+	–	+	–	+	–	+	–	–
NPY	+	+	+	+	–	–	–	–	+	+	–	–	++	–
Bombesin	–	+	–	+	–	+	–	+	–	+	–	+	–	–
CGRP	–	+	–	+	–	–	–	–	–	+	–	–	–	–
Galanin	–	–	–	–	–	–	–	–	–	–	–	–	–	–
met-Enkephalin	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Pancreatic poly peptide	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Insulin	–	–	–	–	–	–	–	–	–	–	–	–	+++++	–

C = cells; N = nerves

tinal crypts were observed in the small intestine. In the duodenum the villi were broad, and towards the distal end of the small bowel they became more slender. The surfaces of the villi and crypts were lined with simple columnar epithelium. These cells had basally situated, round to oval nuclei and a conspicuous, striated border. PAS-positive goblet cells were observed throughout the intestinal tract, and their numbers markedly increased towards the caecum, colon and rectum. Paneth cells were sparsely distributed in the basal parts of the crypts of the small intestine only (Fig. 2). Crypts were typically seen in the caecum and large bowel (Fig. 3).

The intestinal lamina propria consisted of loose connective tissue containing mainly relatively coarse collagen, as well as some elastic and smooth muscle fibres. Abundant eosinophils, diffuse lymphocytic infiltration and lymphatic follicles were seen. The latter were solitary as well as aggregated, and the larger ones occupied both the mucosa and submucosa.

The muscularis mucosae were discontinuous and the muscle fibres were not arranged in longitudinal and circular layers. Diffuse lymphocytic infiltration was observed in this layer.

Typical loose connective tissue was seen in the submucosa of the intestine. It contained diffuse lymphocytic infiltration, lymphatic nodules, submucosal plexus and some adipose tissue. In the duodenum, branched tubular duodenal glands were detected.

A thick tunica muscularis was observed, containing a myenteric plexus, and it differentiated into inner circular and outer longitudinal layers. The serosa consisted of loose connective tissue with abundant collagen fibres, and was lined with typical mesothelium.

Immunocytochemistry

The immunostaining results are summarized in Table 2. Absorption controls showed that all positive immunoreactions could be prevented by preabsorption of the diluted antibody with its homologous antigen.

Gut endocrine cells

Specific staining for somatostatin was observed in all the regions of the intestine. Immunoreactive cells were numerous in the duodenum, but relatively sparse in the other regions. Some of the cells reached the lumen, and some had basal extensions (Fig. 4).

In all the regions of the bowel glucagon cells were seen. They were numerous in the ileum, but very sparse in the other regions (Fig. 5). Cells immunoreactive for secretin were observed in the duodenum and the proximal part of the jejunum only (Fig. 6). A few scattered motilin-immunoreactive cells were seen in the duodenum and ileum (Fig. 7).

Endocrine cells, weakly positive for neuropeptide Y (NPY), were present in the duodenum and jejunum, and at the base of the crypts of the colon. This immunoreactivity was not abolished by preabsorption with pancreatic polypeptide (PP).

Antiserum for glucose-dependent insulinotropic peptide (GIP), stained endocrine cells in the duodenum, jejunum and ileum. The cells became less numerous towards the ileum. Gastrin cells were present in the

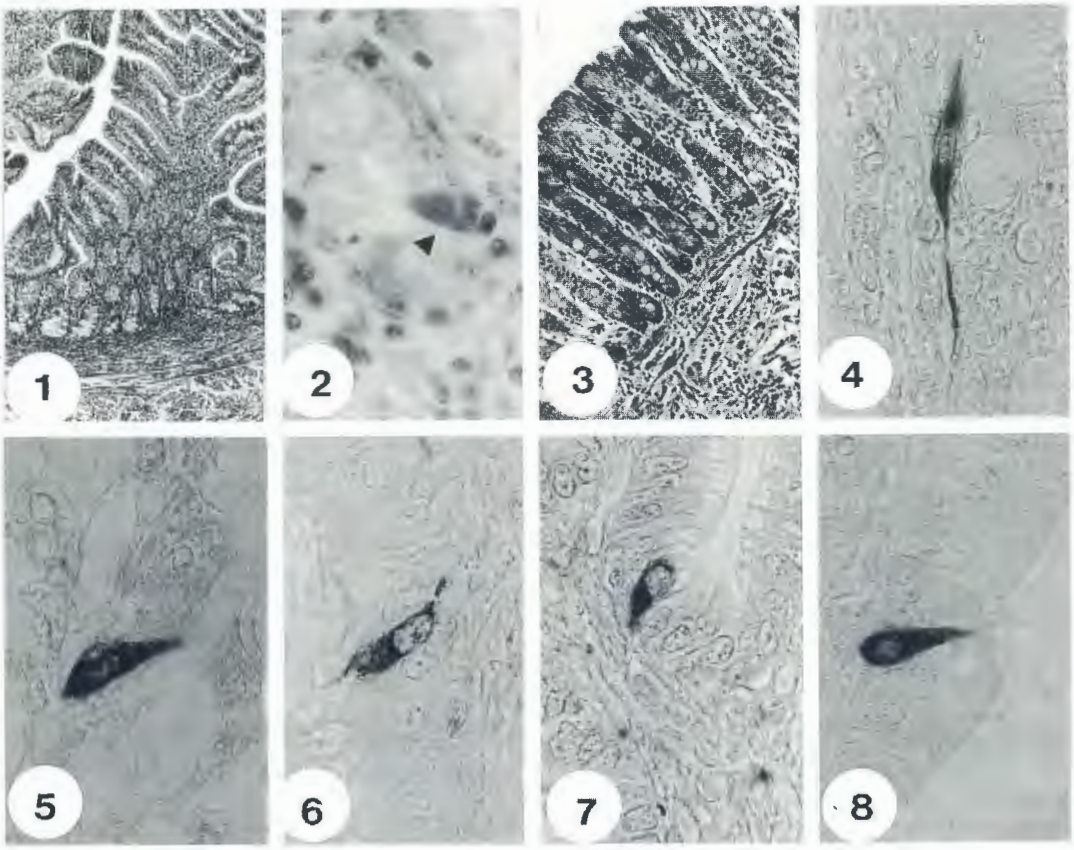

FIG. 1 A part of the jejunal spiral fold covered with villi
200 x haematoxylin and eosin

FIG. 2 Base of a crypt containing a Paneth cell (arrow head)
800 x phloxine and tartrazine

FIG. 3 Crypts in the colon studded with goblet cells
200 x haematoxylin and eosin

FIG. 4 Somatostatin cell in the duodenal mucosa showing a cytoplasmic extension at the basal end
800 x PAP

FIG. 5 Glucagon cell in the mucosa of the ileum
800 x PAP

FIG. 6 Secretin cell in the duodenal epithelium
800 x PAP

FIG. 7 Motilin cell in a crypt of the ileum
800 x PAP

FIG. 8 Gastrin cell in the jejunum mucosa
800 x PAP

mucosa of the duodenum and jejunum (Fig. 8), and the antiserum specific for cholecystokinin (CCK) demonstrated cells in the duodenum only.

Antisera specific for neurotensin failed to demonstrate cells in the intestine.

Nerves

Very strong substance-P immunoreactivity was seen in the nerves of the muscle layer and lamina propria throughout the intestine, but most particularly in the colon (Fig. 9). Nerve fibres immunoreactive for somatostatin 14 were seen in the lamina propria of the jejunum only.

Vasoactive intestinal polypeptide (VIP)-immunoreactive nerves were present in the lamina propria and muscle layers throughout the intestine, particularly in the colon (Fig. 10). Ganglion cells were seen in the plexus. Nerves immunoreactive to peptide histidine isoleucine (PHI) had a distribution similar to that of VIP.

Nerve fibres immunoreactive for somatostatin 14 were seen in the lamina propria of the jejunum only, but they were not very prominent.

Antiserum to bombesin, demonstrated nerves in the muscle layer of the intestine. Cell bodies were not positive, but the ganglia contained bombesin-immunoreactive nerve fibres. Calcitonin gene-related peptide (CGRP)-immunoreactive nerves were seen in the mucosae of the duodenum, jejunum and colon (Fig. 11).

No immunoreactivity was observed for the antisera to galanin, neurotensin and metenkephalin.

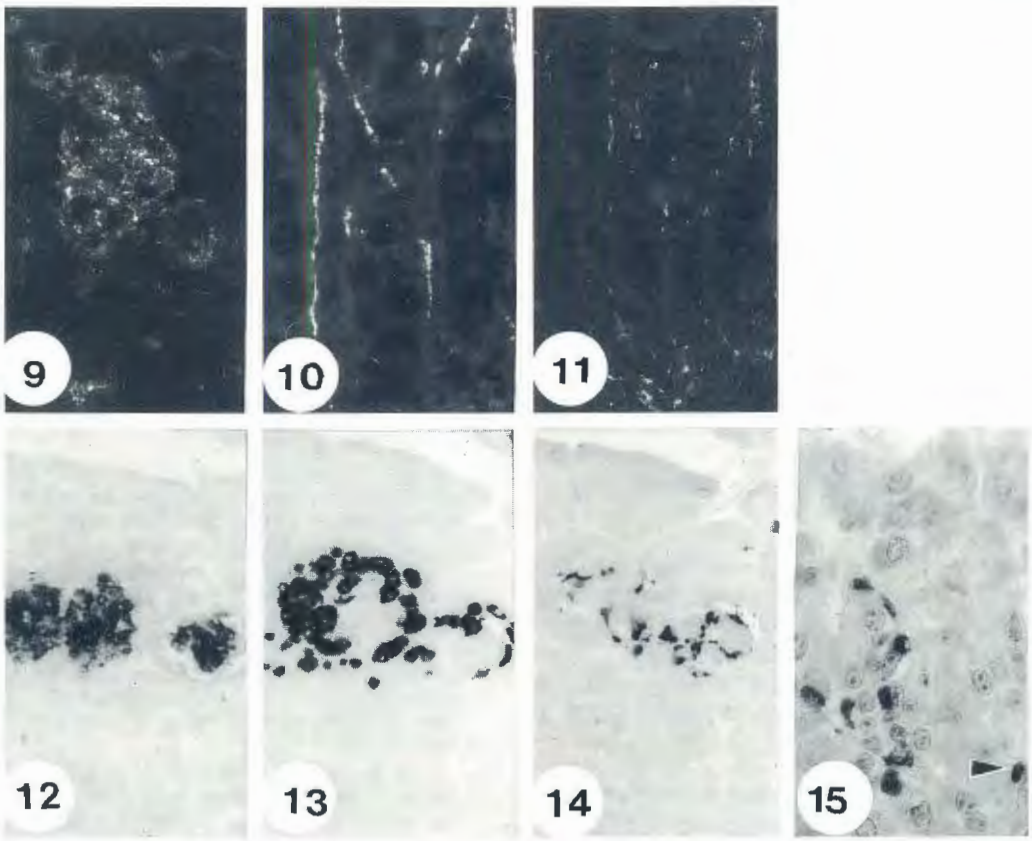

FIG. 9 A plexus immunoreactive for substance P in the muscle layer of the colon
1570 x FITC

FIG. 10 Nerve fibres immunoreactive for PHI in the mucosa of the large bowel
1570 x FITC

FIG. 11 CGRP immunoreactive nerve fibres in mucosa of the colon
100 x FITC

FIG. 12 An islet in the body of the pancreas stained for insulin cells
200 x indirect peroxidase

FIG. 13 A consecutive section showing the same islet stained for glucagon
200 x indirect peroxidase

FIG. 14 The same islet as above stained for somatostatin
200 x indirect peroxidase

FIG. 15 NPY-immunoreactive cells in a pancreatic islet and one in the parenchyma (arrow head)
400 x ABC. Counterstained with haematoxylin

Endocrine pancreas

In all the regions examined, the insulin cells were located in the centers of the islets (Fig. 12). Glucagon cells surrounded their insulin counterparts (Fig. 13). Within the mantle of glucagon cells, somatostatin cells occurred (Fig. 14). They were less numerous in the islets of the duodenal lobe (head) than those of the splenic lobe (body and tail). One of the antibodies to bovine PP demonstrated very sparce positive cells in occasional islets or in the parenchyma. Anti-NPY, however, revealed numerous cells in the islets. They were small and slender compared with the glucagon cells and preferentially located towards the periphery (Fig. 15). In order to demonstrate these cells, the antibody was used at a low dilution and revealed with an avidin-biotin-peroxidase reaction, more sensitive than the PAP reaction used for other peptides.

No difference was noted between the head and tail regions.

In the ductular epithelium of the pancreas insulin, glucagon and somatostatin cells were sparsely distributed.

DISCUSSION

Histologically, the small and large bowel of the elephant are in line with those of monogastric mammals (Banks 1986). The abundant eosinophils in the lamina propria were seen in all the samples, as they were in the stomach (Van Aswegen, Schoeman, De Vos & Van Noorden 1994).

The occurrence of somatostatin endocrine cells throughout the entire gut, is typical (Larsson 1985).

One would expect these cells to contain somatostatin 28 (Baskin & Ensink 1984). However, as the region specificity of antiserum KB 18 was not known, further investigation is needed to ascertain the molecular form of this peptide in endocrine cells of the elephant intestine.

Distribution of glucagon cells in the intestine of the elephant is in line with that of mammals in general (Larsson, Holst, Hakanson & Sundler 1975). Because some endocrine cells in the intestine were stained by the antiserum to NPY (although NPY is strictly a neuropeptide) (Wang, McDonald & Wyatt 1987), and because anti-NPY—but not anti-PP—stained cells in the pancreatic islets, it seems probable that "elephant PP" resembles NPY in its molecular structure, rather than it does the pancreatic polypeptide members of the family.

The distribution of secretin and motilin cells in the intestine of the elephant corresponds with that of mammals (Larsson, Sundler, Alumets, Hakanson, Schaffalitzky de Muckadell & Farenkrug 1977). Likewise, gastrin and CCK have a distribution pattern typical for mammals (Sundler, Böttcher, Ekblad & Hakanson 1989).

Both the antisera employed to demonstrate neurotensin, failed to stain endocrine cells and nerves. As neurotensin cells occur in the gut of mammals (Sundler, Hakanson, Hammer, Alumets, Carraway, Leeman & Zimmerman 1977), it would be most surprising if this peptide were absent from the gut of the elephant. We suggest that it may have a sequence not recognized by the antisera used in this study.

The anatomic juxtaposition of endocrine cell types within the islets of the elephant, is in line with the situation in other mammals (Samols, Bonner-Weir & Weir 1986). There is no reason to believe that PP is absent from the elephant pancreas, but it may be of an NPY-like structure, as suggested by the immunostaining results. The PP of some fishes is more like NPY than PP (Conlon, Bjenning, Moon, Youson & Thim 1991). As the sequence homology of PP is well conserved in the various mammalian species (Hazelwood 1993), it will be interesting to determine the sequence of this peptide in elephant.

The presence of endocrine cells in the ductular epithelium, seen in this investigation, is a known phenomenon in mammals (Dorn, Lorenz & Koch 1977).

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