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The oesophagus and stomach 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 oesophagus and stomach of the elephant. The histological findings were in line with the situation in monogastric species like swine and man. In the mucosa of the stomach, endocrine cells were immunoreactive to gastrin, somatostatin, chromogranin A and serotonin. Nerve cells immunoreactive to somatostatin, bombesin, VIP, PHI and CGRP were detected in the submucosal and myenteric plexus of the stomach. In the stomach, the absence of glucagon cells and the presence of endocrine cells immunoreactive to PYY, are in contrast to the situation in mammals and need further investigation. Small gastric ulcers were observed in some of the specimens.

INTRODUCTION

The Kruger National Park is one of the few remaining areas where the African elephant (*Loxodonta africana*) is over-abundant and where regular culling has to be performed (De Vos, Bengis & Coetzee 1983; Smithers 1986). Elsewhere, this species is considered endangered and all knowledge that can be gathered about it is needed as background for rational management decisions in order to guarantee its preservation.

African elephants are opportunistic feeders and their diet includes grass and shrubs as well as foliage and the bark of trees (Van Hoven 1983; Smithers 1983). Their ability to digest food is rather poor, therefore an elephant consumes about 300 kg green food per day (Smithers 1983). Although the diet of the elephant has received attention, no data exist on the morphology and physiology of the gut of the African elephant (Van Hoven 1983).

On a structural basis there are two main types of monogastric mammals, those which have a non-glandular proximal stomach and those in which the entire stomach is glandular (Banks 1986). Of the latter, the elephant is the largest terrestrial example. Van Hoven (1983) examined ten adult specimens and found the volume of the gastric lumen to be 60 ± 5 dm^3.

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Recently, attention has been focused on the neuroendocrine system of the gut, because it modulates digestive functions (Sundler, Böttcher, Ekblad & Hakanson 1989). In this study, serotonin and bioactive peptides of the neuroendocrine system of the stomach of the elephant were investigated.

MATERIALS AND METHODS

During a culling expedition samples of tissue were promptly taken from three adult specimens and included the distal third of the oesophagus and the cardiac, fundic, body and pyloric regions of the stomach.

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 pretreated 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-bezoquinone freshly dissolved in 0,01 M 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, with the Grimelius silver-impregnation (Grimelius 1968) or the periodic acid-Schiff (PAS) method. All sections were dehydrated and mounted with DPX. The diameter of the eosophageal epithelium was measured with an ocular micrometer.

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 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 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.

TABLE 1 Details of primary antibodies used

Antisera	Code	Dilution	
		PAP	IF
Gastrin, C-terminal (synthetic human)[a]	B36	1:2000	
Somatostatin 14 (synthetic)	744	1:1000	1:1000
Cholecystokinin (mid-portion 9–20)	1937	1:1000	
PYY	900	1:4000	
Chromogranin A[b]			
Mouse monoclonal, clone LK2H10	1199021	1:500	
Serotonin	644	1:1000	
Glucagon	1383	1:500	
Bombesin (synthetic amphibian)	627		1:400
VIP (whole molecule)	652		1:2000
PHI (synthetic porcine, C-terminal)	938		1:900
CGRP (synthetic rat)	1208		1:200
NPY	820	1:4000	1:400
Neurotensin (synthetic bovine)	810	1:800	
Met-enkephalin	869		1:400
Galanin	1152		1:1000

[a] Purchased from MILAB
[b] Purchased from Boehringer Mannheim

All other primary antibodies were supplied by Professor J.M. Polak, Royal Postgraduate Medical School, London

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 treated for 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 they had been rinsed, the sections were mounted in buffered glycerine and viewed in a fluorescence microscope.

Controls for immunocytochemistry

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

RESULTS

Histology

Oesophagus

The wall of the oesophagus was about 2 cm thick; however, its bulk was due mainly to a well-developed

TABLE 2 Distribution of immunoreactive endocrine cells and nerves in the elephant stomach

Antibody	Cardiac region		Fundic region		Pyloric region	
	Cells	Nerves	Cells	Nerves	Cells	Nerves
Gastrin	–	–	–	–	+++	–
Somatostatin	++	+	++	+	+	+
Cholecystokinin	–	–	–	–	–	–
PYY	±	–	±	–	–	–
Chromogranin A	+	–	+	–	+++	–
Serotonin	+	–	+	–	++	–
Glucagon	–	–	–	–	–	–
Bomesin	–	+	–	++	–	+
VIP	–	++	–	+	–	+
PHI	–	++	–	+	–	+
CGRP	–	+	–	+	–	+

tunica muscularis consisting of a typical inner circular and outer longitudinal layer of visceral muscle. The non-keratinized, stratified, squamous epithelium of the mucosa was about 480 µm thick. Papillae of the *lamina propria* were seen to run deep into the epithelium (Fig. 1). Slightly PAS-positive mucosal and submucosal glands were observed. They were structurally similar and separated by thick strands of the *muscularis mucosae*. Frequently fibres of the *muscularis mucosae* were seen between groups of alveoli of the submucosal glands. Both the submucosal and mucosal glands had pale-staining cells with basally situated nuclei. Groups of lymphocytes were common within the oesophageal glands (Fig. 2).

The submucosa was a relatively thin layer. In five of the samples examined, plant material was observed in the submucosa. These foreign objects were usually surrounded by neutrophils and some lymphocytes (Fig. 3).

Stomach

Both the cardiac and pyloric glands had deep and slender gastric pits and consisted mainly of pale-staining, mucous-secreting cells. The apical parts of these cells were intensely PAS positive (Fig. 4). In the pyloric region the basal parts of the glands were coiled. The fundic glands had typical pale-staining cells in their neck portions, and parietal and chief cells in their body portions. Frequently, the parietal cells occurred in groups, and the basal parts of the glands were coiled (Fig. 5). Mucosal endocrine cells shown by Grimelius silver impregnation were present in all areas, and were particularly abundant in the pyloric glands.

In the *lamina propria* of the gastric mucosa there were various degrees of eosinophil infiltration. Scattered lymphocytes and lymphoid nodules were observed throughout the mucosa. Samples from two elephants exhibited, in some places, lesions associated with marked infiltration of inflammatory cells consisting mainly of neutrophyls. These ulcers were seen to erode the gastric glands (Fig. 6). No foreign objects were observed in them.

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.

Endocrine cells

No immunoreactive endocrine cells were seen in the mucosa of the oesophagus.

Immunostaining for the general endocrine marker, chromogranin, demonstrated endocrine cells in the mucosa of all areas of the stomach.

Specific staining for gastrin was confined to the pyloric region, mainly in a band running through the basal third of the mucosa (Fig. 7). All the gastrin-positive cells were embedded in the pyloric glands and some could be seen to reach the lumen.

Somatostatin-immunoreactive cells were found in all regions of the stomach. In the cardiac region these cells were sparse, but in all the other regions they were numerous. They occured in the gastric glands (Fig. 8), and in the fundus they had very obvious basal extensions. In the pyloric region some of the somatostatin cells were seen to reach the lumen.

Scattered cells were immunoreactive for peptide YY (PYY) in the cardiac and fundic regions, but not the pyloric region. Serotonin-positive cells were found in all regions.

Nerves

Nerve fibres immunoreactive for somatostatin 14 were seen in the *lamina propria* of pylorus, but they were not very prominent. Vasoactive intestinal peptide (VIP)-positive nerves were seen in the *lamina propria* and muscle layer of the stomach, particularly in the

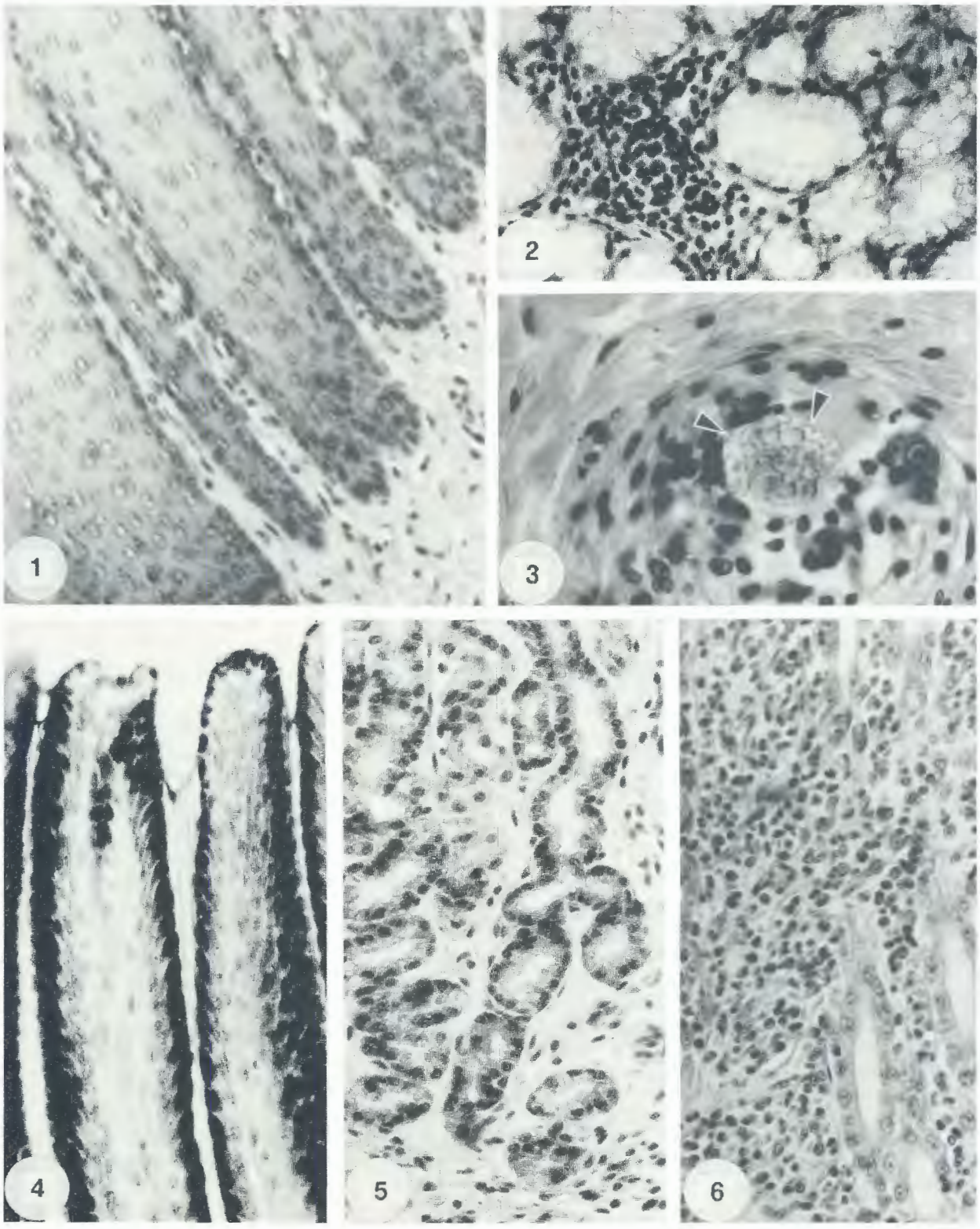

FIG. 1 Papillae of the *lamina propria* of the oesophagus running into the epithelium (x 825)
FIG. 2 Lymphocyte infiltration of the submucosal glands of the oesophagus (x 825)
FIG. 3 An inflammatory site in the submucosa of the oesophagus surrounding a piece of plant material (arrow heads) (x 1300)
FIG. 4 Surface epithelium of the pylorus showing a PAS-positive reaction (black) (x 825)
FIG. 5 Coiled basal part of fundic glands. Parietal cells are pale (x 825)
FIG. 6 A site of inflammation in the pyloric mucosa, invading the glandular tissue which is visible on the right hand side of the figure (x 825)

cardiac region. Ganglion cells were seen in the plexus (Fig. 9). Nerves immunoreactive for peptide histidine isoleucine (PHI) had a distribution similar to that of VIP, and were particularly noticeable around the vessels of the glandular layer of the stomach (Fig. 10). Nerves immunoreactive for substance P (SP) were seen in the *lamina propria* of all the regions of the stomach (Fig. 11), and nerve-cell bodies were positive in the muscle plexus. Bombesin-containing nerves were seen in the muscle layer of the stomach and were particularly clear in the fundic region (Fig. 12). The cell bodies were not positive, but the ganglia contained bombesin-immunoreactive nerve fibres. Nerves and plexus immunoreactive for calcitonin gene-related peptide (CGRP) were seen in the muscle wall of cardiac and fundic regions only (Fig. 13). No immunoreactivity was observed with the antisera to glucagon, galanin, neurotensin, met-enkephalin and neuropeptide Y (NPY).

DISCUSSION

Histologically, the oesophagus of the elephant is in line with that of monogastric domestic mammals (Banks 1986). It seems that the oesophagus of the elephant is not well protected against sharp objects because plant material penetrated the submucosa. No lesions were seen in the mucosa, probably because they were not in the sectioning plane. Plant material was so frequently observed in the submucosa that it may be considered normal for this species.

The gastric mucosa of the African elephant is comparable to that of swine (Banks 1986) and man (Neutra & Padykula 1983), because the entire stomach is glandular and the glands of the different regions are comparable. Although the eosinophil infiltration of the *lamina propria* was marked, it seems to be normal for this species, as it was not confined to the lesions. The small inflammatory sites in the mucosa are not considered to be true peptic ulcers, because in none of them was the entire mucosa lost (Walter 1989).

Stomach ulceration is a consistent feature of elephants slaughtered during culling operations in the Kruger National Park (De Vos, personal observations). In a previous study, 15 out of 22 elephant carcasses showed ulcers of the stomach which were confined mainly to the anterior portion of the greater curvature of the stomach (Basson, McCully, De Vos, Young & Kruger 1971). The ulcers varied from very superficial ulcerations to loose, hyperplastic lesions with predominantly granulation tissue, and ranged in size from approximately 3–50 mm in diameter. The older and bigger lesions invariably contained a yellowish exudate and numerous very slender nematodes identified as *Parabronema africanum*. These lesions can, under certain adverse conditions, become bleeding ulcers (De Vos, personal observations). Bleeding ulcers were found in young elephant calves shortly after they had been taken from the wild and held in captivity; even to the extent of causing death. Stress suffered by these animals immediately after capture is probably the trigger mechanism for these lesions to be converted to fully fledged bleeding ulcers with severe consequences, and this fits the descriptions of Selye (1950), and Glavin, Murison, Overmier, Pare, Bakke, Henke & Hernandez (1991).

Lesions of the stomach have been associated with parabronemiasis before. Evans (1910) reports that as early as 1882, Cobbold associated *Parabronema indicum* of the Indian elephant, with small tumours in the stomach. Sutherland, O'Sullivan & Ohman (1950) recorded a large abscess in the stomach of an elephant containing many *P. indicum*, while Condy (1974) also associated parabronemiasis with stomach ulcers in elephants.

The topographic distribution of gastrin cells in the stomach of the elephant is in line with the situation in monogastric mammals (Kitamura, Yamada, Yamashita & Yanaihara 1982; Tobe, Chen, Henmi & Fukuchi 1976).

Somatostatin cells in the pyloric region of the stomach of the elephant were confined to basal parts of the glands—as they are in the cat (Kitamura *et al.* 1982). Cytoplasmic projections were observed only in the somatostatin cells of the fundus. This is in line with observations made on other species, viz. mouse, dog and man (Larsson 1985). The distribution of serotonin-containing cells is in line with the situation found in the rat stomach (Rubin & Schwartz 1983).

This study of the content and distribution in the elephant stomach of peptides of the diffuse neuroendocrine system indicates that the elephant is, in many respects, a typical mammal. However, the absence of immunoreactivity for glucagon differentiates it from several mammals in which this peptide occurs in endocrine cells of the gastric fundus, notably the cat and the dog (both carnivores) (Larsson, Holst, Hakanson & Sundler 1975; Grimelius, Holst, Buffa, Polak, Pearse & Solcia 1976). In rat and man (both omnivores), glucagon cells are only sparsely present, though they are more prominent in the human fetus (Grimelius, Holst, Buffa, Polak, Pearse & Solcia 1976). The presence, albeit infrequent, of PYY-immunoreactive cells is intriguing, since PYY is found co-localized with glucagon in enteric endocrine cells (Ali-Rachedi, Varndell, Adrian, Gapp, Van Noorden, Bloom & Polak 1984), but has not so far been reported in the stomach. Whether elephant glucagon differs from other mammalian glucagons to the extent that it does not react with the antibodies used in this investigation, or whether the absence of glucagon and the presence of PYY is connected with the complex

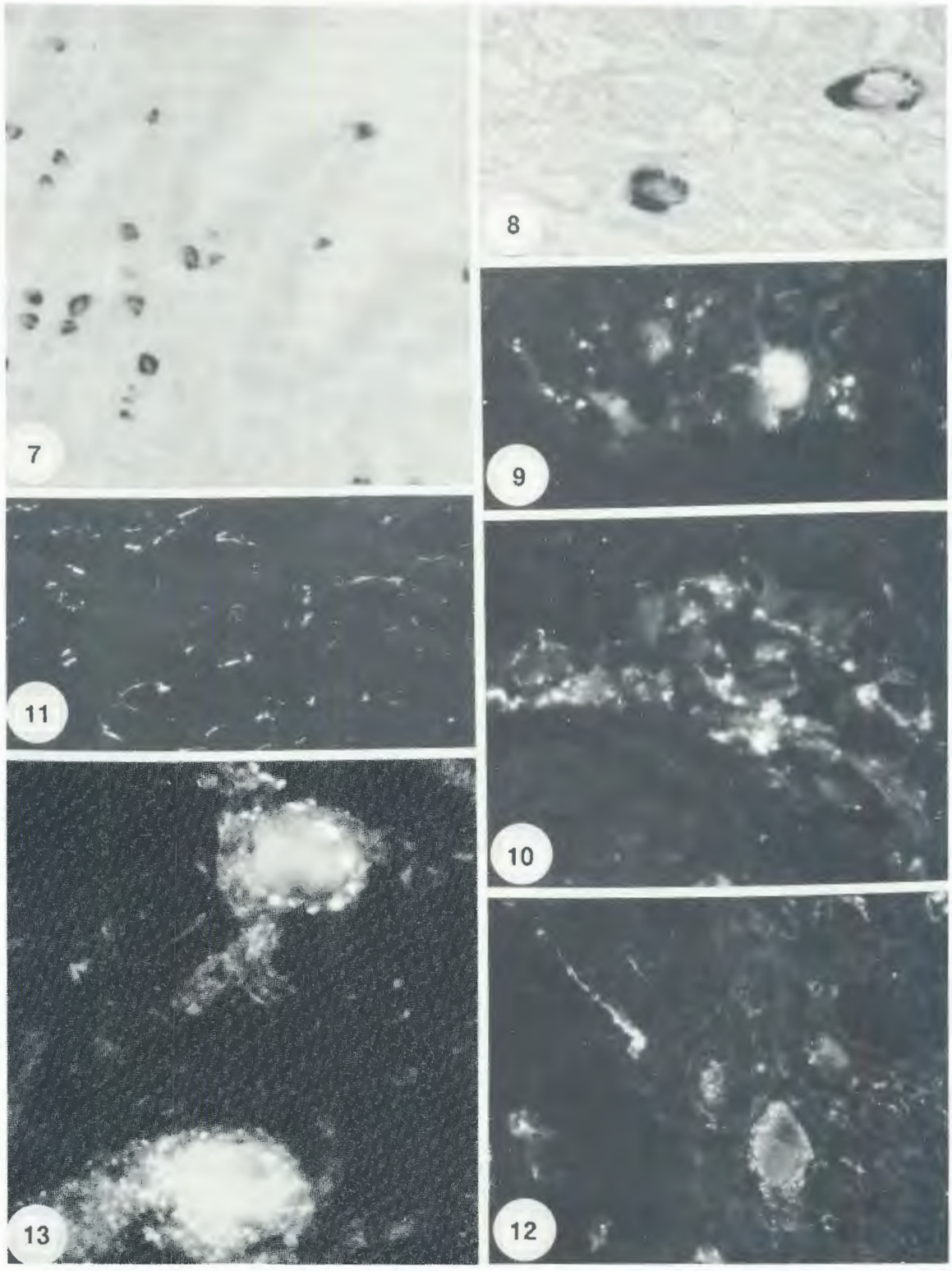

FIG. 7 Immunostained gastrin cells in the pylorus (x 825)
FIG. 8 Immunostained somatostatin cells within the gastric glands (x 1300)
FIG. 9 Cell bodies and nerves in the myenteric plexus of the cardiac stomach immunoreactive for VIP (x 4400)
FIG. 10 A submucosal plexus of the cardiac stomach immunostained for PHI (x 4400)
FIG. 11 Substance P-immunoreactive fibres in the mucosa of the cardiac stomach (x 900)
FIG. 12 A cell body and nerves containing bombesin immunoreactivity in the submucosal plexus of the fundus (x 2750)
FIG. 13 Cell bodies surrounded by nerves in myenteric plexus of the pylorus, staining positively for CGRP (x 4400)

digestive needs of the elephant's entirely herbivorous diet, requires further investigation, e.g. studies in other regions of the elephant gut and comparative studies in other species.

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