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A LIGHT MICROSCOPIC AND IMMUNOCYTOCHEMICAL STUDY OF THE GASTRO-INTESTINAL TRACT OF THE OSTRICH (*STRUTHIO CAMELUS* L.)

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ABSTRACT

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Although the histological features and endocrine cells of the gastro-intestinal tract of the chicken have been well studied, little is known about these features of the gut of the ostrich. The present study was undertaken to elucidate the histology and peptide-storing endocrine cells of the ostrich.

As a rule the histological features of the gastro-intestinal tract of the ostrich corresponded to that of the fowl. However, certain differences were observed.

The superficial proventricular glands were simple, branched tubular glands, while the deep proventricular glands were restricted to a slipper-shaped area and extended into the muscularis mucosae. The gizzard had a variably developed muscularis mucosae, a feature that seems to be unique to the ostrich. The villi of the small intestine were long and branched profusely, forming a labyrinthine surface. No Paneth cells were observed. The mucosa of the ceca and the first part of the rectum was thrown in large circular folds, forming a compressed spiral. Numerous melanocytes were seen in the submucosa and the connective tissue around the bloodvessels of the muscle layers at the tips of the ceca. A well developed subserosa was present throughout the gastro-intestinal tract.

Endocrine cells immunoreactive to somatostatin, glucagon, gastrin, bombesin, neurotensin, substance P and pancreatic polypeptide were detected in the gastro-intestinal tract of the ostrich. The topographical distribution of those endocrine cells immunoreactive to glucagon, bombesin, neurotensin and substance P differed from that of the chicken. The results of this investigation inferred that at least one of the gut peptides of the ostrich (secretin) to be structurally different from its counterparts in mammal and chicken. Molecular heterogeneity of somatostatin was observed in endocrine cells situated in the deep ventricular glands of the ostrich.

INTRODUCTION

The microanatomy of the gastro-intestinal tract of the chicken has been studied by various authors (Chodnick, 1947; 1948; Clara, 1925, 1926a, 1926b; Dawson & Moyer, 1948; Eglitis & Knouff, 1962; Ferreira, 1966; Greulich, 1949; Michael & Hodges, 1973; Monesi, 1960; Overton & Shoup, 1964; Pentilla, 1968; Rosenberg, 1941; Toner, 1963, 1964a, b, 1965, 1968). Subsequently gastro-intestinal tract of the chicken has been the subject of many histochemical (Aitken, 1958; Bennett, 1969; Bennett & Cobb, 1969; Eglitis & Knouff, 1962; Holman, 1968; Hugon & Borgers, 1969; Michael & Hodges, 1973; Pentilla & Gripenberg, 1969) and immunocytochemical investigations (Alumets, Hakanson & Sundler, 1978; Andrew, 1984; Rawdon & Andrew, 1981a, b; Rawdon, 1984; Sundler, Hakanson, Hammer, Alumets, Carraway, Leeman & Zimmerman, 1979). In contrast to the relatively huge body of knowledge of the histological and histochemical features of the gastro-intestinal tract of the fowl, very little is known about the gastro-intestinal tract of the ostrich. Bezuidenhout (1986) and Macalister (1964) examined the anatomy and topography of the gastrointestinal tract, while Brock (1925) and Deurden & Brock (1924) studied the light microscopical features of the mucosa of the proventriculus and gizzard of the ostrich. Recent studies (Swart, Mackie & Hays, 1987) on the microbial digestion processes in the intestine of the ostrich suggest that the ceca, and the rectum in particular, of the ostrich may have different functions from those of their counterparts in the fowl. The present study was therefore undertaken to elucidate the histology and the peptide-storing endocrine cells of the gastro-intestinal tract of the ostrich.

MATERIALS AND METHODS

Twelve adult ostriches of both sexes and 4 age groups were employed. Birds with a body mass of 20, 45, 60 and 80 kg were anaesthetized by injecting pentobarbitone sodium. The left carotid artery was cannulated at the base of the neck and allowed to exanguinate.

Samples were promptly taken from the proventriculus, gizzard, 1st and 2nd parts of the duodenum, jejenum, ileum, proximal, middle and distal ends (tips) of the cecum, and the thick and thin parts of the rectum, for light microscopy and immunocytochemistry.

Light microscopy

The samples were fixed at room temperature in formol-saline, buffered formalin, Bouin's fluid or Zenker's fixative for at least 24 h, trimmed, dehydrated in graded ethanols and imbedded in Histosec paraffin wax. Sections (4 µm) were cut and stained with either haematoxylin and eosin, Mallory's phosphotungstic acid haematoxylin, phloxin tartrazine, by the method of Masson-Fontana, or lissamine fast red stains. The stained sections were examined with a Leitz Orthoplan microscope.

Immunocytochemistry

Tissue blocks not exceeding 123 mm^3 in volume were quickly excised from all the regions of the ostrich gastro-intestinal tract mentioned and fixed in Bouin's fluid for 12 h at room temperature. For positive controls gastro-intestinal tract tissue of the laboratory mouse (*Mus musculus*) and the bull frog (*Pixicephalus adspersus*) were fixed in the same way. All fixed tissue blocks were washed in 60 % ethanol for 1 h, processed and embedded as above. The sections (5 µm) were floated on slides pre-treated with poly-L-lysine (Van Noorden & Polak, 1983).

After dewaxing, the sections were hydrated through a graded series of ethanols and transferred to 0,05 M Tris-saline. Endogenous peroxidase activity was blocked in the sections by treating them with 0,3 % hydrogen peroxide in methanol for 30 min (Van Noorden & Polak, 1983), washed in 60 % methanol and transferred to Tris-saline. To reduce the possibility of non-specific staining, the sections were incubated at room temperature with 10 % non-immune serum for 15 min.

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The peroxidase anti-peroxidase method was employed to identify the immunoreactive sites (Sternberger, 1979). Primary antisera employed in this study are listed in Table 1. For the 2nd layer antibody immunoglobulin (DAKO) was used, and for the 3rd layer a stable peroxidase anti-peroxidase complex (DAKO) was employed. The reaction sites were revealed by 3,3′-diaminobenzidine (BDH), according to the method of Graham & Karnovsky (1966). Some sections were counter stained with haematoxylin and all the sections were dehydrated and mounted in DPX.

Controls for specificity of each of the primary antisera involved the absorption with at least 20 µg of its parent peptide per mℓ of diluted antiserum for 12 h at 4 °C, and alternatively absorbing each antiserum with closely related peptides at the same dilution.

Results

The gastro-intestinal tract showed the usual layers throughout viz, mucosa, submucosa, muscular layer and serosa, but there were marked regional differences as to their diameter and composition.

1. Tunica mucosa

It consisted of an epithelial layer resting on a thin basement membrane, a lamina propria and a muscularis mucosae throughout the gastro-intestinal tract.

Proventriculus: The mucosal lining of the proventriculus was thrown into numerous parallel folds. From the bases of these folds short superficial proventricular glands extended into the lamina propria. The superficial glands varied from simple to branched tubular (Fig. 1).

Simple columnar epithelial cells of the mucosa were filled with mucin granules, and decreased in height towards the bases of the grooves. They stained basophilic proximal and eosinophilic distal to the nucleus. In the basal region of the superficial glands the cells were cuboidal with large, round to oval vesicular nuclei and the cytoplasm stained basophilic. Scattered between the epithelial cells described were small pyramidal shaped cells with fine argyrophilic granules. Granular leucocytes infiltrated the epithelium.

Tubulo-alveolar type deep proventricular glands were confined to a slipper-shaped area along the dorsolateral wall of the proventriculus (Fig. 1). Each gland extended from a papilla on the epithelial lining into the muscularis mucosae, separating the latter into both deep and superficial layers, with strands of muscle fibres and connective tissue connecting them (Fig. 2). Deep proventricular glands constituted the bulk of the proventricular wall in the region where they occurred. They were composed of numerous rounded, angular or polymorphic lobules arranged in small groups, each of which drained into the lumen of the proventriculus via a papilla (Fig. 1). Each lobule consisted of numerous tubules radiating from a central cavity (Fig. 2). Tubules drained via short tertiary ducts into a secondary duct that formed part of the central cavity. This opened by means of the primary duct on a proventricular papilla. Tubules were lined by simple cuboidal to low columnar epithelial cells containing numerous spherical eosinophilic secretory granules, and large, round to oval vesicular nuclei. Cells packed with secretory granules, were cuboidal with a basally situated nucleus. Conversely cells devoid of granules were low columnar with a centrally situated nucleus. All the ducts were lined by a simple columnar epithelium. A short intermediate zone containing glandular and ductular epithelial cells was evident. Epithelial cells lining the primary ducts contained mucin granules, the concentration of which decreased towards the secondary ducts. Epithelial lining the secondary and tertiary ducts were mucin-free. Numerous cells containing argyrophilic granules were seen between the epithelial cells lining the ducts of the glands (Fig. 3).

The lamina propria followed the contours of epithelium and surrounded the lobules of the deep proventricular glands, forming a loosely arranged capsule. It contained fine elastic and collagen fibres, blood vessels, nerves and lymphatics. Blood vessels were particularly prominent at the bases of the superficial proventricular glands.

Poorly developed with longitudinally and circularly arranged fibres were seen in the muscularis mucosae. Muscle fibres extended into the connective tissue stroma of the proventricular folds and the deep proventricular glands *(vide supra)*.

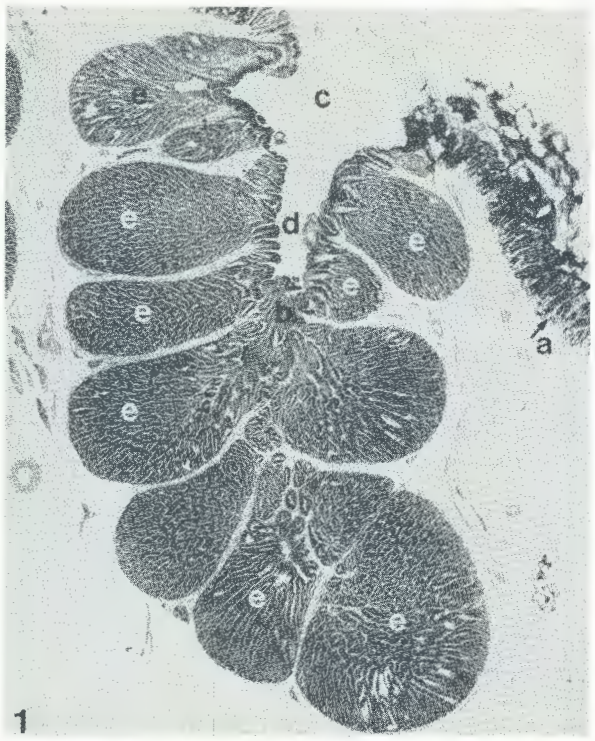
Ventriculus (gizzard): The mucosal surface was indented by shallow, broad crypts or pits into which simple to branched tubular glands, imbedded in the lamina propria, opened. A thick gastric cuticle produced by the ventricular glands covered the mucosa (Fig. 4 & 5).

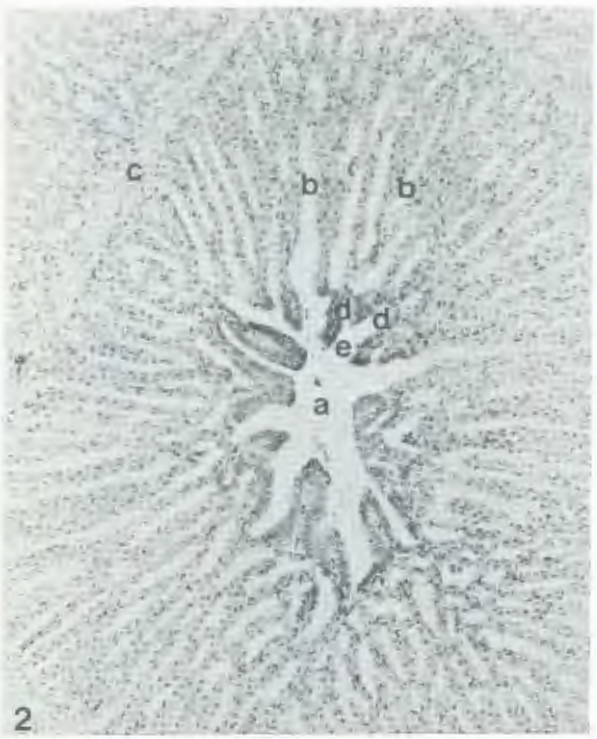
Four distinct types of cells namely, basal, chief, surface epithelial and entero-endocrine cells were identified in the simple cuboidal to columnar epithelial lining. A few cuboidal basal cells with large, round vesicular nuclei and pale-staining, clear cytoplasm were randomly distributed in the tubular glands. Low columnar chief cells with round or oval nuclei constituted the bulk of the tubular glands. The cytoplasm of the active secreting cells were filled with fine, pale-staining secretory granules distal to the nucleus and was clear, pale-staining in inactive cells. In active and inactive cells the cytoplasm consistantly stained basophilic. Surface epithelial cells lined the pits and mucosal surface of the gizzard. Epithelial cells tended to bulge at the lumen, giving the epithelial lining a honeycomb appearance on transverse sections. At the bottom of the pits the proximally situated nuclei were round to oval and pale-staining, while nuclei near the surface tended to be irregular in shape and stained more intensely. The apical portions of these cells were filled with coarse eosinophilic secretory granules, while the portions proximal to the nucleus stained basophilic. At the luminal surface of the pits the cells showed marked degenerative changes and some were desquamated. The cells of the neck portion of the tubular glands had characteristics of both the chief and surface epithelial cells. A few small, pyramidal cells containing argyrophilic granules were scattered in the basal region of the tubular glands.

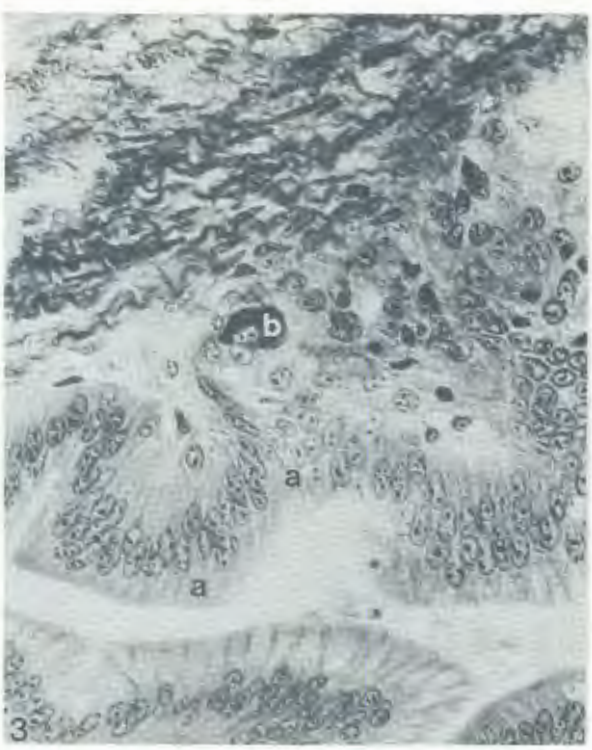
The lamina propria was well developed and consisted of loose connective tissue, blood vessels, nerves and lymphatics. It followed the contour of the epithelial lining and extended between the tubular glands.

In certain areas the muscularis mucosae was well developed, consisting of an inner circular and an outer longitudinal layer. In some regions it was poorly developed or fragmented, and consisted of isolated bundles of smooth muscle fibres only. Muscle fibres radiated from the muscularis mucosae into the lamina propria between the ventricular glands.

Intestine: Its mucosa was thrown into villi which







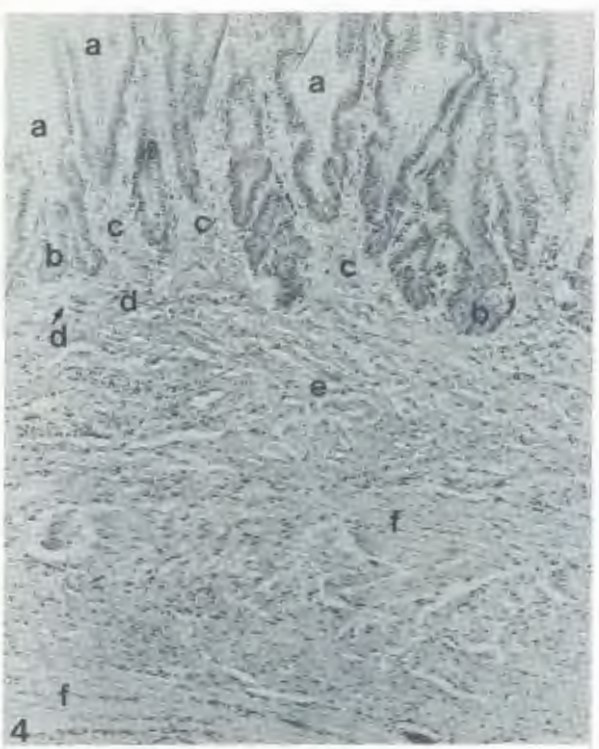


FIG. 1 A longitudinal section of the proventriculus: Mallory's phosphotungstic acid haematoxylin × 25

a = superficial proventricular glands
b = deep proventricular glands
c = papilla
d = primary duct
e = lobules of the deep proventricular glands

FIG. 2 Transverse section of a lobule of the deep proventricular gland: H E × 90

a = central cavity
b = tubules
c = muscularis mucosae
d = tertiary ducts
e = secondary ducts

FIG. 3 Deep proventricular glands. Transverse section phloxin tartrazine: × 440

a = epithelium of tertiary duct
b = entero-endocrine cell filled with argyrophilic granules

FIG. 4 Gizzard. Transverse section: H E × 30

a = gizzard crypts or pits filled with gastric cuticle
b = branched tubular glands
c = lamina propria
d = muscularis mucosae
e = submucosa
f = circular muscle layer

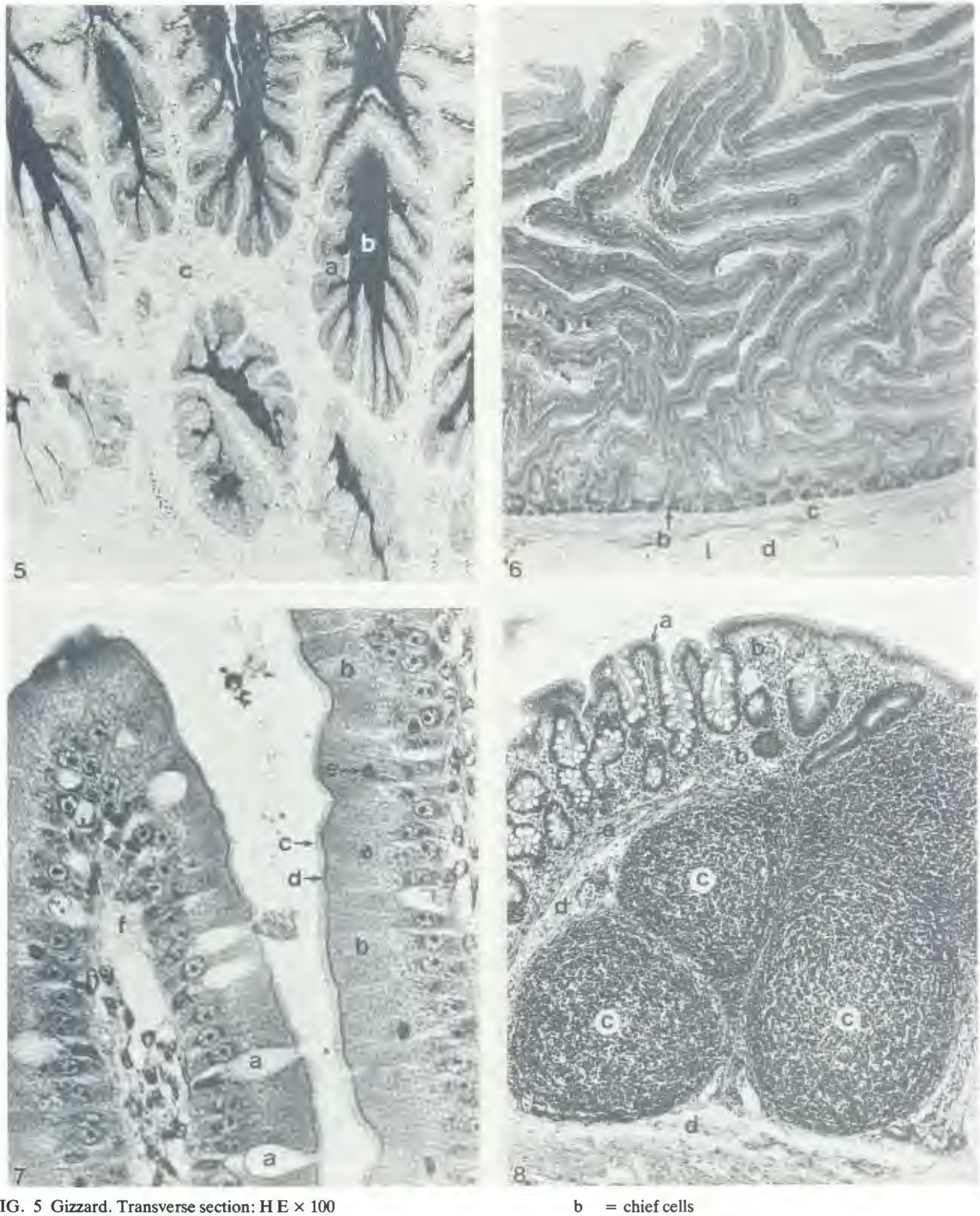


FIG. 5 Gizzard. Transverse section: H E × 100
- a = branched tubular glands
- b = gastric cuticle
- c = lamina propria

FIG. 6 Intestine (duodenum)—transverse section: H E × 30
- a = villi
- b = crypts
- c = inner circular muscle layer
- d = outer longitudinal muscle layer

FIG. 7 Intestinal villi—Longitudinal section: H E × 350
- a = goblet cells
- b = chief cells
- c = microvilli
- d = terminal bars
- e = migrating leucocytes
- f = lamina propria

FIG. 8 Cecum—transverse section: H E × 90
- a = villi
- b = lamina propria
- c = lymph nodules
- d = submucosa
- e = muscularis mucosae

showed a marked variation in density, shape and size in the different regions of the intestine. In the proximal small intestine villi were numerous and best developed, frequently branching in secondary and even tertiary ones (Fig. 6). Distally their size and numbers diminished until only a few small villi were seen in the ceca and rectum (Fig. 8). Between the villi short crypts were visible which reached the peak of their development in the proximal part of the small intestine and gradually decreased in size and number towards the large intestine. The mucosa of the ceca and proximal part of the rectum was thrown into circular folds. These became smaller and less frequent towards the distal part of the intestine and disappeared in the second half of the rectum.

The intestine was lined by a simple columnar epithelium studded with goblet cells (Fig. 7). Goblet cells demonstrated various stages of activity and were scattered amongst the chief cells. They gradually increased in frequency from the duodenum to the rectum. Distally (apically) chief cells had very well developed microvilli. Terminal bars (junctional complexes) were also observed. Migrating leucocytes were abundant between the epithelial cells. Occasionally globular leucocytes infiltrated the mucosal epithelium and the lamina propria of the crypts and less frequently the villi. Small, clear, pyramidal argyrophilic cells were found mostly in the crypts and occasionally in the villi. Their broad basal parts contained granules of various sizes and densities (Fig. 11).

Fine elastic and collagen fibres, blood vessels, nerves and lymphatics were seen in the lamina propria. Larger blood vessels were present at the bases of the villi, while smaller vessels extended into the villi. Central lacteals were absent. Blood vessels, muscle fibres and connective tissue constituted the bulk of the villi stroma. Numerous large and small lymphocytes as well as plasma cells infiltrated the lamina propria. Occasionally lymphocytes were arranged in lymphs nodules at the bases of or in the villi. Lymphocytes and plasma cells were most numerous in the duodenum and tips of the ceca, and sometimes extended into the submucosa, disrupting the muscularis mucosae (Fig. 8). Granular leucocytes were scattered in the lamina propria. The architecture of this layer remained constant throughout the intestinal tracts.

A well developed *muscularis mucosae* was observed throughout the intestinal tract. It consisted of longitudinally and circularly arranged smooth muscle fibres, and extended into the villi stroma and circular folds of the ceca and rectum. Parasympathetic ganglia were occasionally identified between muscle fibres.

2. Tela submucosa

The submucosa was a very thin layer comprised of many blood vessels, lymphatics, nerves, nerve plexusses and ganglia. It was markedly thicker in areas where ganglia of the submucosal plexus, large blood vessel and lymphatics were found. Conversely the thinnest part was observed in the ceca and rectum. The submucosa extended into the circular folds of the ceca and rectum. Large numbers of melanocytes, mostly associated with blood vessels were found in the submucosa of the tips of the ceca. Some of the larger lymphs nodules in the lamina propria disrupted the architectural structure of the muscularis mucosa and extented into the submucosa.

3. Tunica muscularis

The muscular tunic consisted of an inner circular and an outer longitudinal layer. A thin layer of reticular connective tissue that surrounded larger blood vessels, ganglia and nerves of the mesenteric plexus, separated the two layers. Throughout the gastrointestinal tract the longitudinal layer was weakly developed, and incomplete in the major part of the gizzard, while the thick circular layer formed the major part of the muscular tunic. In the proventriculus the latter was approximately 10 mm in diameter, except in areas where the deep proventricular glands occurred where it did not exceed 2 mm. In the ventriculus the circular layer was $\leqslant$ 60 mm in diameter, whereas the longitudinal layer was poorly developed and incomplete over the major part. In the intestinal tract the muscular tunic was best developed in the ileum, and became very thin in the ceca and initial part of the rectum. In the latter 2 regions, the circular muscle layer formed triangular thickenings at the basis of the circular mucosal folds *(vide supra)* and extended into the tips of the folds (Fig. 9). Large numbers of the melanocytes were present in the connective tissue surrounding the blood vessels in the muscular tunic and cecal tips.

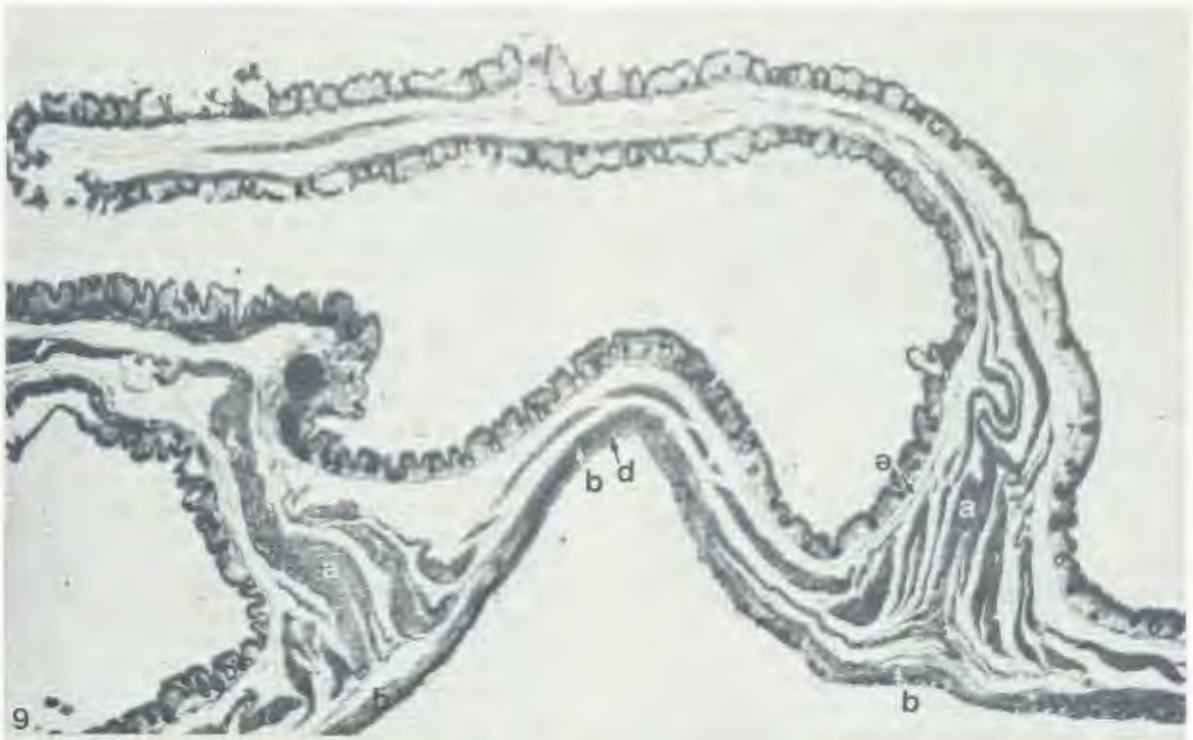
4. Serosa

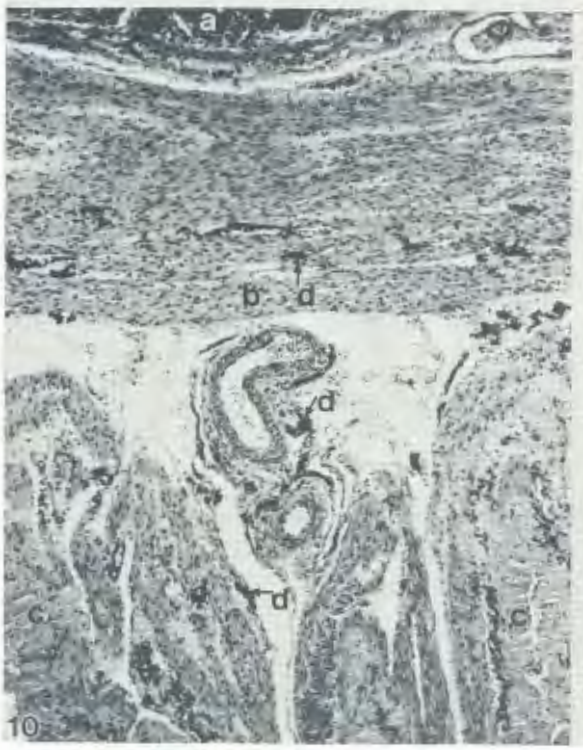
The serosa comprised of a single layer mesothelial cells resting on a basement membrane, and a well developed subserosa. The latter was present along the whole length of the gastro-intestinal tract and was composed of irregularly arranged reticular, elastic and collagen fibres. All the elements of the serosa was continuous with that of the mesenterium. Over a

TABLE 1 Details of the primary antisera employed

Antiserum raised to	Code	Dilution	Source
Somatostatin (natural porcine)	744	1:1000	J. M. Polak*, London
Somatostatin (synthetic 1–14)	RPN1612	1:2500	Amersham
Glucagon (natural porcine)	RPN1602	1:2500	Amersham
Gastrin (porcine C-terminus)	RPN1592	1:2500	Amersham
Bombesin (amphibian C-terminus)	627	1:1000	J. M. Polak*, London
Substance P	910	1:1000	J. M. Polak*, London
Neurotensin	116	1:1000	C. Shaw*, Belfast
Neurotensin	118	1:1000	C. Shaw*, Belfast
Neurotensin (C-terminus extension peptide)	GK14	1:1000	C. Shaw*, Belfast
PP (natural chicken)	B32	1:1000	Milab
Secretin (natural porcine)	53	1:1000	J. M. Polak*, London
Motilin (natural porcine)	M37B	1:1000	Quadrologic Inc.
GIP (natural porcine)	B35	1:1000	Milab
CCK(natural porcine)	RPN1742	1:2500	Amersham
CCK(Synthetic 9–20)	1937	1:4000	J. M. Pollak*, London
PHI	1200	1:1000	J. M. Polak*, London

* These gifts are gratefully acknowledged





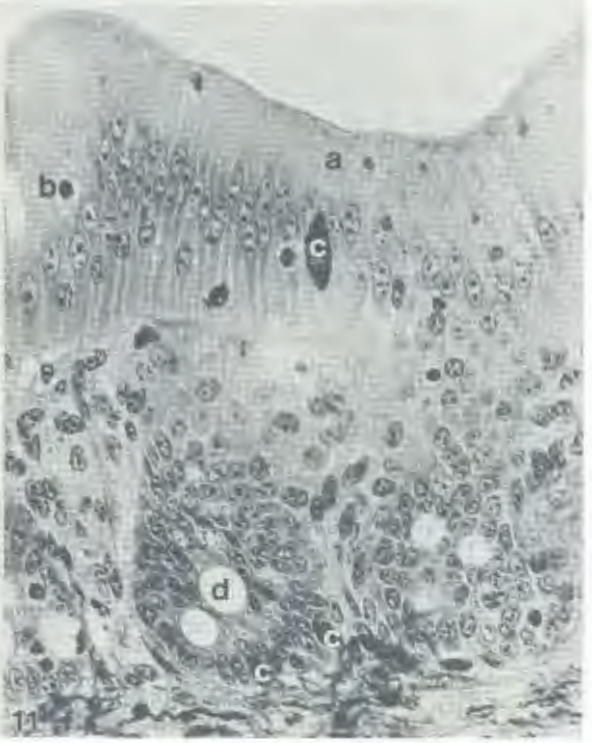


FIG. 9 Rectum—circular fold in transverse section: H E × 30

a = circular muscle layer
b = longitudinal muscle layer
c = mucosa
d = subserosa
e = muscularis mucosa

FIG. 10 Cecum—transverse section: H E × 90

a = epithelium
b = submucosa
c = tunica muscularis
d = melanocytes

FIG. 11 Cecum—transverse section: Masson Fontana × 450

a = mucosa
b = migrating leucocytes
c = entero-endocrine cell
d = crypt

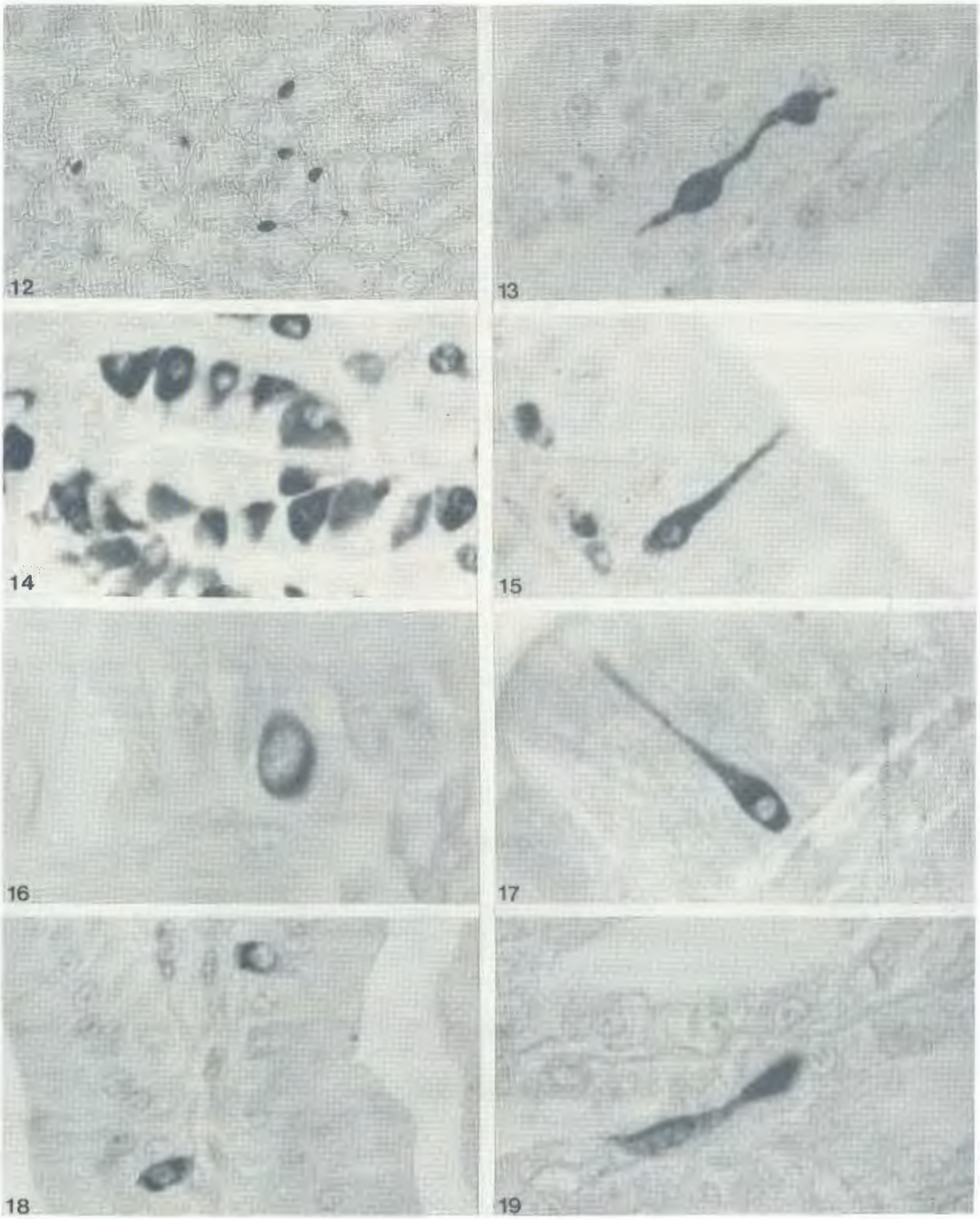


FIG. 12 Somatostatin immunoreactive cells in a deep gland of the proventriculus PAP ×720

FIG. 13 Two cells immunoreactive to glucagon embedded in a proventricular gland and displaying cytoplasmic projections PAP ×1150

FIG. 14 A section through the pyloric region showing numerous cells immunoreactive to gastrin PAP ×1150

FIG. 15 An endocrine cell in the mucosa of the duodenum which was stained by the antiserum to gastrin PAP ×2850

FIG. 16 A cell immunoreactive to bombesin in the glandular tissue of the proventriculus PAP ×1150

FIG. 17 A flask-shaped cell immunoreactive to neurotensin PAP ×1150

FIG. 18 Two cells stained for substance P in the ventriculus PAP ×1150

FIG. 19 A single pancreatic polypeptide cell, showing a short cytoplasmic projection in the proventriculus PAP ×1150

TABLE 2 Distribution of cells showing immono-reactivities for regulatory peptides in the gut of the ostrich

Tissue / Antibody	Proventriculus	Gizzard	Pylorus	Duodenum	Ileum (upper)	Ileum (lower)	Caecum	Rectum
Somatostatin	+++		+++	+++	++	++		
Glucagon	++	+	+			++		
Gastrin			+	+++	++	++		
Bombesin	++	+++						
Neurotensin				+++	++	+		++
Substance P		+	+++	++				
Avian PP	++	++	+++	++	++	++	++	++
Secretin								
Motilin								
GIP								
CCK								

+ = Relative frequency of cells stained
Blank areas indicate no cells stained

large part of the gizzard the serosa was replaced by an adventitia made up of connective tissue and fat.

Immunocytochemistry

Of the antisera listed in Table 1, only those raised to somatostatin, glucagon, gastrin, bombesin, neurotensin, substance P and pancreatic polypeptide (PP), stained endocrine cells in the gastro-intestinal tract of the ostrich (Table 2). All the antisera employed stained cells in known positive tissues and were absorbed by their parent peptides.

Endocrine cells immunoreactive to somatostatin were seen in all the regions of the gastro-intestinal tract except the ceca and rectum (Table 2). In the proventriculus numerous immunoreactive cells, with cytoplasmic projections were embedded deep in the epithelium of the superficial and deep glands. Some of these cells reached the lumens of glands (Fig. 12). In the deep proventricular glands more cells were stained by antiserum 744 and antiserum RPN 1612. Somatostatin immunoreactive cells in the ventriculus were of the closed type, oval to round without cytoplasmic projections and inhabited the basal halfs of the glands. In the small transitional zone between the ventriculus and duodenum the cells immunoreactive to somatostatin were flask shaped, occurred in the epithelium of the villi and crypts but failed to reach the lumen. Towards the distal part of the small intestine these cells became markedly fewer until they disappeard at the junction of the small and large intestine.

Glucagon immunoreactive cells had long cytoplasmic projections and were scattered in the glands of the proventriculus, ventriculus and the pyloric region. Only glucagon immunoreactive cells in the deep proventricular glands had cytoplasmic projections that connected them with neighbouring cells (Fig. 13). Endocrine cells immunoreactive to glucagon were seen in the crypt and villi epithelium of the terminal small intestine only.

Immunoreactivity for gastrin was confined to the pyloric region (Fig. 14) and the proximal small intestine. Immunoreactive cells were mostly round, numerous in the pyloric region and sparsely distributed within the glands. Cells found in the upper intestine were slender flask-shaped with long apices (Fig. 15), and as a rule were observed in the villi only. Although antisera raised to secretin failed to demonstrate cells, an anti-serum to peptide histidine isoleucine known to cross react with secretin stained numerous cells in the duodenum.

Bombesin immunoreactive material was detected in the endocrine cells of the proventriculus and ventriculus. Immunoreactive cells were sparsely distributed in the proventriculus (Fig. 16) and numerous in the ventriculus. The cells were deeply situated in the glandular epithelium and did not reach the lumen. Some of the cells beared cytoplasmic projections.

Most cells immunoreactive to neurotensin were detected in the upper small intestine. In the distal part of the small intestine the cells were sparsely distributed and eventually disappeared at the junction with the large intestine. However, in the terminal part of the rectum a few cells immunoreactive to neurotensin were seen. The cells were slender, broad based with long apices that did not reach the lumen (Fig. 17).

Substance P immunoreactive cells were limited to a very small part of the gastro-intestinal tract, viz. the distal ventriculus and the duodenum. They were of the closed type (Fig. 18) being numerous in the ventriculus but sparse in the duodenum. Immunoreactivity to PP was observed throughout the gastro-intestinal tract, except for the terminal region of the rectum. It was the only antiserum to demonstrate cells in the ceca. In general the cells were sparsely distributed, although numerous in the pyloric region. Immunoreactive cells had typically broad

bases and long slender apices running towards the lumen and only occasionally reaching it. In the proventriculus some cells displayed cytoplasmic projections (Fig. 19).

DISCUSSION

Although the histology of the gastro-intestinal tract of the ostrich corresponded to that of the chicken in general, certain differences were present.

According to Calhoun (1933), Chodnick (1947), and Dawson & Moyer (1948), the surface epithelium of the proventriculus of the chicken is of the regular, simple columnar type. Their findings were supported by those of Magon & Mohan (1976) on the crow (*Corvus splendens*) and sparrow (*Passer domesticus*), and Brock (1925) on the ostrich. The present study confirmed the findings of Brock (1925).

Calhoun (1933) and Chodnick (1947) observed simple tubular glands in the proventriculus of the chicken which correspond with the situation in the crow and sparrow (Magon & Mohan, 1976). Brock (1925) was of the opinion that these glands were laterally compressed, complexly folded and the lumen being narrow and labyrinthine in the ostrich. However, Hodges (1974) regarded these glands to be artifact of fixation. In the present study these glands were found to be of the simple, branched tubular type. According to Calhoun (1933) the deep proventricular glands of birds were distributed throughout the entire organ. This was supported by observations on the crow and sparrow (Magon & Mohan, 1976). However, these findings are in contrast to those of the present study and those of Brock (1925) and Macalister (1964), as the deep proventricular glands of the ostrich are confined to a slipper shaped area along the dorsolateral walls of the proventriculus. There are conflicting opinions as to whether the deep proventricular glands of the chickens are situated in the lamina propria or the submucosa. Some authors (Bradley & Grahame, 1960; Menzies & Fisk, 1963; Toner, 1963) consider them to be submucosal, while Batt (1924) detected them in the lamina propria. However, Calhoun (1933) and Farner (1960) advocate that these glands penetrate the muscularis mucosae, separating it into inner and outer layers. This implies that the glands lie within the muscularis mucosae. According to Brock (1925) bundles from the muscularis mucosae of the proventriculus of the ostrich penetrate into the glands, which therefore fall within the limits of the lamina propria. In the present study, the deep proventricular glands were observed within the muscularis mucosae, which corresponds to the findings of Calhoun (1933) and Farner (1960).

Various investigators are in agreement that elongated, simple tubular glands open into the crypts of the gizzard of the chicken (Calhoun, 1933; Chodnick, 1947; Dawson & Moyer, 1948; Eglitis & Knouff, 1962; Toner, 1964). Eglitis & Knouff (1962) states that the tubular glands extend into the submucosa and that two cell types could be identified, namely columnar surface cells and less tall cells that lined the tubular glands. Calhoun (1933) is in agreement on the cells types, but states that the tubular glands are limited to the lamina propria and that a muscularis mucosae is absent. Chodnick (1947) and Toner (1964a & b) detected four types of cells, namely columnar surface cells, cuboidal or chief gland cells, basal cells and entero-endocrine cells. Magon & Mohan (1976) only demonstrated columnar surface and cuboidal gland cells in the gizzard of the crow and sparrow. In the present study four types of cells were identified which correspond to those detected in the gizzard of the chicken (Chodnick, 1947; Toner, 1964).

Long villi and short crypts of Lieberkuhn are features of the small intestine of the chicken (Calhoun, 1933; Clara, 1925; Chodnick, 1947), turkey (Rosenberg, 1941), crow and sparrow (Magon & Mohan, 1976) and various other avian species (Clara, 1925). In the present study long villi that branched into secondary and tertiary villi to form a labyrinthine surface were observed. In the ostrich the mucosa of the ceca and initial, thick part of the rectum is transformed into circular folds which form distinct compartments in the lumen. Swart, Mackie & Hays (1987), showed that in the ostrich the passage of ingesta through the ceca and rectum is markedly retarded, probably by the action of the circular folds. In the ostrich the intestinal epithelial lining corresponds with those of other birds (Calhoun, 1933; Clara, 1925; Humphrey & Turk, 1970; Kalyanan, Gurumani & Suthanthiran, 1973; Looper & Looper, 1929; Magon & Mohan, 1976; Michael & Hodges, 1973; Rosenberg, 1941). The status of Paneth cells in the avian intestine remains uncertain. They have been detected in the *Turdidae* and *Anas* by Clara (1926) and in the chicken (Bradley & Grahame, 1960; Chodnick, 1947). However, Aitken (1958) and Rosenberg (1941) could not demonstrate Paneth cells in the chicken or turkey. These finds correspond with our results in the ostrich.

Calhoun (1933) states that the intestinal villi of the chicken contain a central lacteal. Subsequently this was not confirmed. In the present study the intestinal villi did not contain a central lacteal.

Ziswiller & Farner (1972) classified the ceca of birds into four groups: the intestinal type which histologically resembles the intestine; the glandular type with a high secretory activity; the lympho-epithelial type which is infiltrated by lymphocytes and the vestigial, non-functional type. The ceca of the ostrich are comparable to the intestinal type, and are known to have an important absorptive function (Swart, Mackie & Hays, 1987). The extent to which lymphoid cells infiltrate the lamina propria of birds seems to vary between species and between various age groups within a species. In the proventriculus of the chicken a lymphocytic infiltration of the lamina propria has been observed (Zietschman, 1911) but Calhoun (1933) could only find lymphoid tissue in the lamina propria of the three oldest chickens examined by him. However, Magon & Mohan (1976) found a considerable amount of lymphoid tissue in the lamina propria of the proventriculus of the crow and sparrow. In the present study the absence of lymphoid tissue in the lamina propria of the proventriculus may be ascribed to the relatively young age of the ostriches examined. In the ostrich lymphocytes sometimes infiltrated the muscularis mucosa and lymphocytic aggregations were also found in the submucosa.

Calhoun (1933) recorded a well developed muscularis mucosae consisting of an outer circular and inner longitudinal layer in the proventriculus and intestine of the chicken. Conversely the muscularis mucosae of the crow and sparrow is weakly developed (Magon & Mahon, 1976). The well developed muscularis mucosae seen in the gastro-intestinal tract of the ostrich is in agreement with Calhoun's (1933) findings. However, the presence of a well

development muscularis mucosae in the gizzard of the ostrich is an unique feature of the species.

In the ostrich the submucosa of the gastro-intestinal tract was poorly developed, except in the ceca and proximal colon. This resembles the situation generally found in birds. The numerous melanocytes seen in the submucosa and tunica muscularis of the cecal tips in the ostrich has not previously been reported. The origin and significance of the melanocytes is unknown.

The muscular tunic of the gastro-intestinal tract of the ostrich parallels the situation in other birds (Calhoun, 1933).

According to Calhoun (1933) the subserosa of the gastro-intestinal tract of the chicken is very thin. In the present study a very well developed subserosa was present throughout the gastro-intestinal tract.

All peptides demonstrated in the gastro-intestinal tract of the ostrich have previously been detected in endocrine cells of the avian gastro-intestinal tract (Rawdon, 1984). Of the peptides not identified in the present study only GIP has not been recognised in the gastro-intestinal tract of birds (Polak, Pearse, Adams & Garaud, 1974a; Rawdon & Andrew, 1981a). Although the topographic distribution of the different types of peptide-storing cells in the ostrich corresponds with the situation in birds in general (Reinecke, Almasan, Carraway, Helmstaedter & Frossmann, 1980; Rawdon & Andrew, 1981a; Seino, Porte & Smith, 1979; Seino, Porte, Yanaihara & Smith, 1979), there are several noteworthy differences.

The distribution of cells immunoreactive to somatostatin in the gastro-intestinal tract of the ostrich is comparable with the situation in other birds (Rawdon, 1984; Seino, Porte, Yanaihara & Smith, 1979). However, the latter cells were present in the gizzard of the ostrich and not in the gizzard of the fowl (Rawdon & Andrew, 1981a). In the ostrich antiserum 744 stained considerably more cells in the deep proventricular glands than antiserum RPN 1612. This shows the heterogeneity of somatostatin, and that one form of the molecule is confined to the proximal gastro-intestinal tract. A similar situation has been observed in the gastro-intestinal tract of the rat (Baskin & Ensink, 1984). The presence of glucagon immunoreactivities in the gizzard, and its absence in the duodenum and upper ileum of the ostrich, is in contrast to the situation in the chicken (Rawdon & Andrew, 1981a). To detect glucagon containing cells in the ostrich an amino-terminal antiserum was employed, known to detect most forms of the peptide in mammalian (Conlon, 1980) and avian (Rawdon & Andrew, 1981a) gastro-intestinal tract. The inability of the antiserum to detect forms of glucagon in the duodenum and upper ileum of the ostrich is not considered. The topographic distribution of glucagon containing cells in the gastro-intestinal tract of the ostrich is distinctly different to those in the chicken.

Larsson, Sundler, Hakanson, Rehfeld & Stadil (1974), Rawdon & Andrew (1981a), and Yamada, Yoshiho, Yamashita, Misu & Yanaihara (1979) reported gastrin immunoreactivities in the pylorus and duodenum of the avian gastro-intestinal tract, and thus support the findings of this study. The presence of gastrin-like immunoreactive cells in the ileum of the ostrich should be regarded with caution. These cells were demonstrated by a carboxyl-terminal antiserum, that also detect mammalian CCK. Should these cells contain CCK it will corroborate the findings of Rawdon & Andrew (1981a) who observed CCK in the ileum of the chicken. Failure of the antiserum specific for CCK to stain cells in the gastro-intestinal tract of the ostrich, is probably due to heterogeneity of the peptide, and thus gives support to the view that the sequence in the 20–25 region of CCK of birds differs from its mammalian counterpart (Rawdon & Andrew, 1981b), or the cells seen in the ileum are indeed gastrin containing cells. However, further investigation is needed to ascertain the exact status of these cells.

The presence of bombesin containing cells in the proventriculus and ventriculus of the ostrich is in line with the findings of various authors on the avian gastro-intestinal tract (Rawdon & Andrew, 1981a; Timson, Polak, Warton, Gatei, Bloom, Usellini, Capella, Solcia, Brown & Pearse, 1979; Vaillant, Dockray & Walsch, 1979). However, the detection of bombesin in the pyloris of the ostrich is an unique finding as it has not been identified in the pyloris of other avian species. Bombesin shares a dipeptide at the carboxyl-terminal with substance P (Mutt, 1982), in the duodenum of the ostrich the distinct population of cells demonstrated by the antiserum raised to substance P was not stained by the antiserum to bombesin. This showed that the antiserum to bombesin does not detect the carboxyl-terminal dipeptide.

The carboxyl-terminal of neurotensin is highly conserved (Carraway, 1981) and antisera orientated towards it should detect the peptide in birds. Neurotensin immunoreactivities recorded in the proventriculus, pylorus, rectum (Rawdon & Andrew, 1981a) and ceca of the chicken (Sundler, Hakanson, Hammer, Alumets, Carraway, Leeman & Zimmerman, 1977), were not seen in the same regions of the gastro-intestinal tract of the ostrich.

The findings of Polak, Pearse, Garaud & Bloom (1974) reported substance P to be present in the mucosa of quail proventriculus. Conversely substance P was not detected in the proventriculus of the chicken (Rawdon & Andrew, 1981a) or the proventriculus of the ostrich by us. No comparable findings to the substance P immunoreactivities in the pylorus of the ostrich have been reported for other avian species (Rawdon, 1984). The presence of substance P containing cells in the duodenum of the ostrich seems to be a common feature of birds as it parallels observations that were made on other avian species (Rawdon, 1984; Vaillant, Dockray & Walsh, 1979).

Pancreatic polyptide (PP) containing cells were observed in the proventriculus, duodenum, ileum and gizzard of the chicken (Rawdon & Andrew, 1981a; Alumets, Hakanson & Sundler, 1978). Our results showed that the distribution of PP containing cells in the gastro-intestinal tract of the ostrich is the same as in the chicken. No pancreatic polypeptide containing cells have been identified in the pylorus and ceca of birds (Rawdon, 1984). However, PP is known to share amino acid sequences with peptide YY(PYY), and therefore cells immunoreactive to PP in the pylorus and ceca of the ostrich may store PYY.

All the antisera employed, failed to stain secretin containing cells in the gastro-intestinal tract of the ostrich. This corresponds with the findings of A. Andrew, University of the Witwatersrand (personal communication) who found that the antisera specific for mammalian secretin are in general not accessible to the avian peptide. However, antiserum 53 which

detects secretin cells in the small intestine of the chicken (Rawdon & Andrew, 1981a) did not stain cells in the ostrich. This suggests that the sequence of the peptide in the gastro-intestinal tract of the ostrich is different to its counterpart in the chicken, or secretin containing cells are absent in the ostrich.

Motilin has been detected in endocrine cells of the avian gastro-intestinal tract (Rawdon, 1984; Rawdon & Andrew, 1981a; Seino, Porte, Yanaihara & Smith, 1979). Our results indicate that motilin is absent in the gastro-intestinal tract of the ostrich. However, antisera orientated to different parts of the peptide will have to be employed before a final conclusion can be made in this regard. Our results corroborate the findings of Polak, Pearse, Adams & Garaud (1974) and Rawdon & Andrew (1981a) that gastrin inhibitory polypeptide does not occur in the gastro-intestinal endocrine cells of the ostrich.

Rawdon & Andrew (1981b) applied several antisera raised to mammalian CCK on the gastro-intestinal tract tissue of the chicken. Their observations indicated that only antisera directed towards the carboxyl-terminal of the peptide are reactive to avian CCK. The antiserum employed in this study was reactive to the mid-portion of CCK 39 (9–20) and therefore failed to recognise the avian CCK.

This study has demonstrated that the topographical distribution of some endocrine cells in the gastro-intestinal tract of the ostrich differs from other avian species. Whether or not this may be attributed to phylogenetic differences is uncertain, because all the observations reported were made on species phylogenetically equally distant from the ostrich. Furthermore, results of this investigation has inferred that at least one of the gastro-intestinal tract peptides of the ostrich (secretin) to be different from its counterparts in the mammal and chicken.

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