Introduction

The ostrich (Struthio camelus), the only member of the Struthionidae, is the largest living bird. There are four sub-species of ostrich in Africa which differ slightly in size and in the colour of the bare skin of the thighs and neck. Struthio camelus molybdophanes and Struthio camelus massaicus occur in eastern Africa, Struthio camelus camelus in northern and western Africa and Struthio camelus australis in southern Africa [15].

The endocrine system has been widely studied in the avian gastrointestinal tracts, but less often in the oesophagus. Studies using immunocytological methods have been carried out on the digestive tract in the chick [1, 5, 6, 16, 19, 22, 24, 31, 36] more than in other avian species. In contrast to relatively large number of knowledge of the immunohistochecmistry of the digestive tract in the fowl, very little is known about the ostrich digestive tract. BEZUIJ Den HOUD et al. [3] examined peptide-storing endocrine cells in the ostrich gastrointestinal tract but not in the ostrich oesophagus wall. They have reported that the topographical distribution of some endocrine cells in gastrointestinal tract of ostrich differ from that of the chicken [3]. Consequently, the present study investigated the regional distribution and relative frequency of the endocrine cells in the ostrich oesophagus wall by immunohistochecmistry using 8 antisera against serotonin, substance P, calcitonin gene related peptide (CGRP), somatostatin-14, neurotensin, cholecystokinin (CCK), galanin and vaso-active intestinal polypeptide (VIP) to compare the obtained results with data collected on other avian birds.

Material and Methods

ANIMALS AND TISSUE SAMPLES

Five adult male ostriches were used. Birds with body mass of 45-60 kg were anaesthetized by injecting pentobarbitone sodium (50 mg/kg) into pectoral muscles. The left carotid
artery was cannulated at the base of the neck and allowed to
exsanguinations. Tissue samples were taken from oesophagus
and fixed in 4% neutral-buffered formalin for 24 hours. They
were then dehydrated through graded ethanol and embedded
in paraffin. Seven µm-thick sections were obtained and
processed for immunohistochemical staining.

IMMUNOHISTOCHEMISTRY: PAP (PEROXIDASE-ANTI-
PEROXIDASE) METHOD

Immunohistochemical staining was carried out using the
peroxidase-antiperoxidase (PAP) method. The blocking of
endogenous peroxidase was carried out with 0.08% hydrogen
peroxidase (H₂O₂) in methanol for 5 minutes [30]. In order
to block unspecific binding, an incubation with normal goat
serum in 0.1 M phosphate buffered saline (PBS), pH 7.2
(Dilution 1:10) was performed. Sections were incubated for
16-20 hours at 4°C with rabbit IgG antibodies against serotonin
(Zymed Lab., 18.0077), substance P (Chemicon, AB1566),
galanin (Chemicon, AB5909), calcitonin gene-related peptide
(Chemicon, AB5920), somatostatin-14 (Chemicon, AB1976),
neurotensin (Chemicon, AB5496), cholecystokinin (Chemicon,
AB1973) and vaso-active intestinal polypeptide (Chemicon,
AB982). The respective primary antibodies were diluted to
1:200, 1:500, 1:1000, 1:500, 1:200 and 1:1000
in PBS containing 0.25% sodium azide and 2.5% bovine
serum albumin. Sections were then incubated with goat anti-
rabbit IgG (Dako, Z0421, Denmark) followed by rabbit
peroxidase anti-peroxidase complex (Zymed Lab., 61.2003,
San Francisco), both at dilution of 1:50 in PBS, for 1 hour at
room temperature. Sections were washed in PBS for 30 minutes
after each incubation step and finally immersed in glucose
oxidase-DAB (diaminobenzidine)-nickel ammonium sulphate
substrate [26] for 10 minutes. After washing in distilled water
and counterstaining with eosin, sections were dehydrated and
cover slips mounted with aqueous permanent mounting
medium.

The specificity of each immunohistochemical reaction was
determined as recommended by STERNBERGER [29] using
the specific antiserum preincubated with its corresponding
antigen. Sections were examined with Leitz Dialux 20 micro-
scope and photographs were taken. For semi-quantitative ana-
lysis, the average number of positive cells by microscopic field
was determined throughout the identification and the counting
of these cells onto 5 microscopic fields (magnification x 40).

Results

Serotonin, CGRP, somatostatin-14, neurotensin and VIP
immunoreactive cells were not detected along the oesophagus.
Nevertheless, VIP immunoreactivity was only observed
in nerve fibres (Figure 1). By contrast, immunoreactive cells
for the substance P, the CCK and the galanin were detected
in the oesophagus. The substance P positive cells were
spherical to spindle-shaped (Figure 2), whereas CCK positive
cells were spherical to round-shaped (Figure 3) and galanin
positive cells were usually round (Figure 4). The frequencies
of CCK, substance P and galanin immunoreactive cells
ENDOCRINE CELL DISTRIBUTION IN THE OESOPHAGUS OF OSTRICH

were 33.8 ± 3.3, 10.2 ± 1.4 and 4.2 ± 1.3 cells by microscopic field respectively. The frequency of each positive cell type was found constant throughout the whole oesophagus. Moreover, the distribution pattern of each type of immunoreactive cell was roughly identical in the 5 tested male ostriches: the coefficients of variation ranged from 9.0% to 31.0% (Table I).

**Discussion**

Despite intensive immunohistochemical studies on the endocrine function of the gastrointestinal tract in avian species [1, 5-7, 9, 16, 19, 21, 22, 31, 36, 37, 39] very few data on ostrich are available.

Substance P is a decapeptide widely distributed in the brain, spinal cord and peripheral and enteric nervous systems [18, 35]. It has also been identified in the endocrine cells of gastrointestinal tract in some mammals, amphibian, fishes and avian species [8, 10, 19, 34]. In the avian gastrointestinal tracts, substance P immunoreactive cells have been reported in the quail proventriculus [19], in the ostrich duodenum [3] and in the intestine of chick [21, 36, 39] and in the gizzard, antrum, duodenum and colorectum of duck [7, 38], but these immunoreactive cells were not observed in the oesophageal region of the fowl [9]. In the present study, we have also failed to detect serotonin immunoreactivity in the oesophagus of ostrich, whereas serotonin positive cells were observed in chicken oesophagus [24]. These variations were probably not due to the antibody employed because serotonin structure is identical in all animal species but were mainly induced by specie differences in the localisation of these endocrine cells.

Galanin is an original neuropeptide which is not related to other known neuropeptides. Its actions are mediated via Gi-protein-coupled receptors and ion channels, usually producing inhibition of secretion of a transmitter or hormone in the nervous and endocrine system [20, 28]. Although galanin immunoreactivity was only localized in nervous elements of the digestive tract in chicken [24, 25, 32], galanin immunoreactive endocrine cells were found in ostrich esophagus in the present study.

Neurotensin was initially isolated from the hypothalamus and subsequently localized to endocrine type cells in the ileal mucosa [27], and 80 to 90% of neurotensin positive cells are found in the gut, predominantly in the distal jejenum and ileum [11, 17]. Although neurotensin immunoreactivity was recorded in the proventriculus, pylorus, rectum and caecum of the chicken [21], in the proventriculus of the ostrich [3], pigeon, quail and duck [37], no immunoreactive endocrine cells were detected in the esophagus of the ostrich. A similar situation has been observed in the oesophageal region of the chicken, pigeon, and Japanese quail [2].

The straight and cyclic forms of somatostatin, consisting of 14 amino acids were isolated from the hypothalamus of the sheep for the first time [4]. These different forms inhibit

<table>
<thead>
<tr>
<th>Oesophagus endocrine cells</th>
<th>Male 1</th>
<th>Male 2</th>
<th>Male 3</th>
<th>Male 4</th>
<th>Male 5</th>
<th>Mean ±SD</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serotonin</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>CGRP</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>-</td>
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<tr>
<td>Somatostatin 14</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>-</td>
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<tr>
<td>Neurotensin</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>VIP</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>-</td>
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<tr>
<td>Substance P</td>
<td>12</td>
<td>10</td>
<td>11</td>
<td>8</td>
<td>10</td>
<td>10.2 ± 1.4</td>
<td>14.5</td>
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<td>CCK</td>
<td>30</td>
<td>32</td>
<td>34</td>
<td>38</td>
<td>35</td>
<td>33.8 ± 3.3</td>
<td>9.0</td>
</tr>
<tr>
<td>Serotonin</td>
<td>5</td>
<td>3</td>
<td>3</td>
<td>6</td>
<td>4</td>
<td>4.2 ± 1.3</td>
<td>31.0</td>
</tr>
</tbody>
</table>

Table 1: Relative frequencies (cell number / microscopic field x 40) of serotonin, substance P, calcitonin gene-related peptide (CGRP), somatostatin-14, neurotensin, cholecystokinin (CCK), galanin and vaso-active intestinal polypeptide (VIP) immunoreactive endocrine cells in the ostrich oesophagus (n = 5).
with a variable efficiency the secretion of other neuroendocrine hormones [13]. The presence of somatostatin containing cells was observed in the proventriculus and gizzard in duck, bird, chicken [37, 38, 39]. In the oesophagus, these cells were not evidenced whereas they were counted in the small intestine with a relative high frequency [3].

No endocrine cells showing CGRP and VIP immunoreactivity were detected in the oesophagus of the ostrich. On the other hand, VIP immunoreactivity was observed in nerve fibers. The absence of VIP immunoreactive cells suggest that there are no endocrine cells containing these peptides in oesophagus. These results are in agreement with studies reported in the gastrointestinal tract of duck and some avian species [7, 33].

The present study is the first report of localisation and relative frequency of the some endocrine cells in the oesophagus of the ostrich. These results suggest that 1) the distribution of the different endocrine cell type in the oesophagus exhibits some particularities in the ostrich (absence of serotonin positive cells) and 2) it differs in the oesophagus from the other gastrointestinal parts (CCK and galanin positive cells were found in the oesophagus whereas they were absent in duodenum; neurotensin and somatostatin positive cells were not evidenced whereas they have been found in the proventriculus and in the duodenum respectively).

References


29. YAMAN (M.) AND COLLABORATORS.


