ENDOCRINE CELLS IN THE GASTROINTESTINAL TRACT OF SHEEP

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To the memory of my father

Arthur Peter Gurnsey

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"One man in his time plays many parts"

William Shakespeare

ABSTRACT

Previous investigations have shown that the digestive activities of the mammalian GI tract are controlled, in part at least, by biologically active compounds released from endocrine cells in the mucosa of the GI tract itself. Despite this, comparatively few studies have been made of the endocrine cells in the GI tract of sheep. There is also a paucity of information about the suitability and reliability of histochemical and immunohistochemical methods for the identification of GI endocrine cells in sheep.

The aims of this study were to: (a) establish reliable techniques for identifying endocrine cells in the GI tract of sheep, (b) use these techniques to investigate the effects of age on the distribution and densities of various GI endocrine cells, and (c) investigate possible changes in endocrine cell densities due to infection with the helminth parasite Trichostrongylus colubriformis.

Initially, various histochemical and immunohistochemical staining techniques were investigated for their suitability for identifying endocrine cells in mucosal samples from reticulum, rumen, body and antral regions of the abomasum, three duodenal sites, ileum, colon and caecum, as well as the pancreas, of adult animals. As a result, the De Grandi technique was selected to estimate argyrophilic cell densities, EC cells were identified by the fast garnet technique, and ECL cells by their silver staining and morphological characteristics. The PAP immunohistochemical technique was used to identify G, S, and A cells, using antisera to gastrin, secretin, and pancreatic glucagon, respectively.

No endocrine cells of any type were found in the reticulum or rumen. Argyrophilic cell densities were greatest in the abomasal body and proximal duodenum, then decreased distally. EC cell densities were highest in the duodenum, although, like argyrophilic cells, they were found throughout the abomasum and intestines. In contrast, ECL cells were confined to the abomasal body. Greatest densities of G cells occurred in the abomasal antrum and proximal duodenum; they were absent from the abomasal body, ileum and large intestine. S cells were

confined in their distribution to the small intestine.

Pancreatic islets of Langerhans contained A cells, as well as cells with slight argyrophilia; the identity of the latter cells was not determined. A cells were also found in exocrine acini, but these were the only cells in the exocrine portion of the pancreas that were stained by any of the histochemical or immunohistochemical techniques used. No A cells were identified in the mucosa of the GI tract.

These studies also clearly established that ovine G cells are not argyrophilic. This finding is in contrast to those reported for most mammalian species with a simpler form of stomach.

The effects of age on endocrine cell densities were studied using the tissues from 100 - 110 day old foetuses, 2 week and 24 week old lambs, and adult sheep. All endocrine cell types identified in adult sheep were also present at the other ages. However, in the foetuses, endocrine cell densities were lower than in other age groups. The most notable age-related trend was that antral G cell densities increased with increasing age. In contrast, from 2 weeks of age, there was a decrease in intestinal G cell densities with increasing age. It was also clear that D cell densities were much higher in 2 week old lambs than for any other age group. Possible explanations for these age-related changes in endocrine cell densities are discussed.*

The effects on endocrine cell densities of an experimental infection with 40,000 T. colubriformis larvae was investigated in 40 week old lambs. Although the resultant infestation was mild, there was a significant ($P<0.001$) increase in argyrophilic cell densities in the proximal small intestine. Specific identification of the argyrophilic cell type(s) which had increased was not possible, however, the most likely candidates were D_1, X and K cells.

It was concluded from these studies that endocrine cells, similar in morphology and staining characteristics to those of other mammalian species, occur within the mucosa of the abomasum, small and large intestine of sheep. Greatest densities of endocrine cells occur in the

Footnote * D (somatostatin containing) cells were located throughout the abomasum and intestines of all nonadult animals and in pancreatic islets of 2 and 24 week old lambs.

abomasum and proximal duodenum. Cell types identified in the GI tract included EC, ECL, G, S and D cells, while D and A cells were identified in pancreatic islets. It was demonstrated that endocrine cell densities change with age and that significant changes in cell densities can occur in mild trichostrongylosis.

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TABLE OF ABBREVIATIONS

Abbreviation	
Ab	Abomasum
APUD	Amine precursor uptake and decarboxylation
As'74	Antiserum '74
BLI	Bombesin-like immunoreactivity
BSA	Bovine serum albumin
Ca^{2+}	Calcium ions
$CaCl_2$	Calcium chloride
CCK	Cholecystokinin
Chr	Chromaffin
CI	Colour Index
Cl^-	Chloride ions
cm	centimetre
$c.mm^{-2}$	cells per square millimetre
CNS	Central nervous system
CTMC	Connective tissue mast cell
DAB	Diaminobenzidine tetrahydrochloride
DF	Degrees of freedom
DG	De Grandi
Duo	Duodenum
EDTA	Ethylenediaminetetraacetic acid
EM	Electron microscope
emg	electromyogram
epg	eggs per gram
FB	Fast Black
FG	Fast Garnet
Fig.	Figure
g	gram
Gas	Gastrin
GEP	Gastro-entero-pancreatic
GI	Gastrointestinal

GIP	Gastric inhibitory polypeptide
GL	Glucagon-like immunoreactivity
GRP	Gastrin releasing peptide
H and E	Haematoxylin and eosin
HCl	Hydrochloric acid
HCO_3^-	Bicarbonate ions
Histo	Histochemical
hr(s)	hour(s)
5-HT	5-hydroxytryptamine (serotonin)
IgG	Immunoglobulin G
Immuno	Immunohistochemical
IR	Immunoreactive
K^+	Potassium ions
KCl	Potassium chloride
K_2CrO_4	Potassium chromate
$K_2Cr_2O_7$	Potassium dichromate
kg	kilogram
KIU	Kallikrein inhibitor unit
ℓ	litre
LI	Large intestine
m	metre
M	Molar
mg	milligram
Mg^{2+}	Magnesium ions
MH	Masson-Hamperl
min	minute
ml	millilitre
mm	millimetre
MMC	Mucosal (or Migrating) mast cell
mmol	millimole
mol	mole
Mol. wt.	Molecular weight
m.p.	melting point
µl	microlitre
µmol	micromole

n	number/group
N	Normal
Na^+	Sodium ions
No	Animal number
PAP	Peroxidase anti-peroxidase
PBS	Phosphate buffered saline
pers. com.	personal communication
pg	picogram
PHI	Porcine heptacosapeptide
pmol	picomole
PO_4^{3-}	Phosphate ions
PP	Pancreatic polypeptide
%	percent
RIA	Radioimmunoassay
Sec	Secretin
SEM	Standard error of the mean
SI	Small intestine
SM	Sevier-Munger
Som	Somatostatin
Tris	Tris(hydroxymethyl)aminomethane
VFA	Volatile fatty acid
VIP	Vasoactive intestinal polypeptide
vs	versus
wks	weeks
yrs	years

Abbreviations used for specific endocrine cell types are given in Table 1.1.

Abbreviations used for sites from which mucosal samples were taken are given in Tables 2.2 and 7.3.

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