ABOMASAL SECRETION
IN
PARASITISED SHEEP

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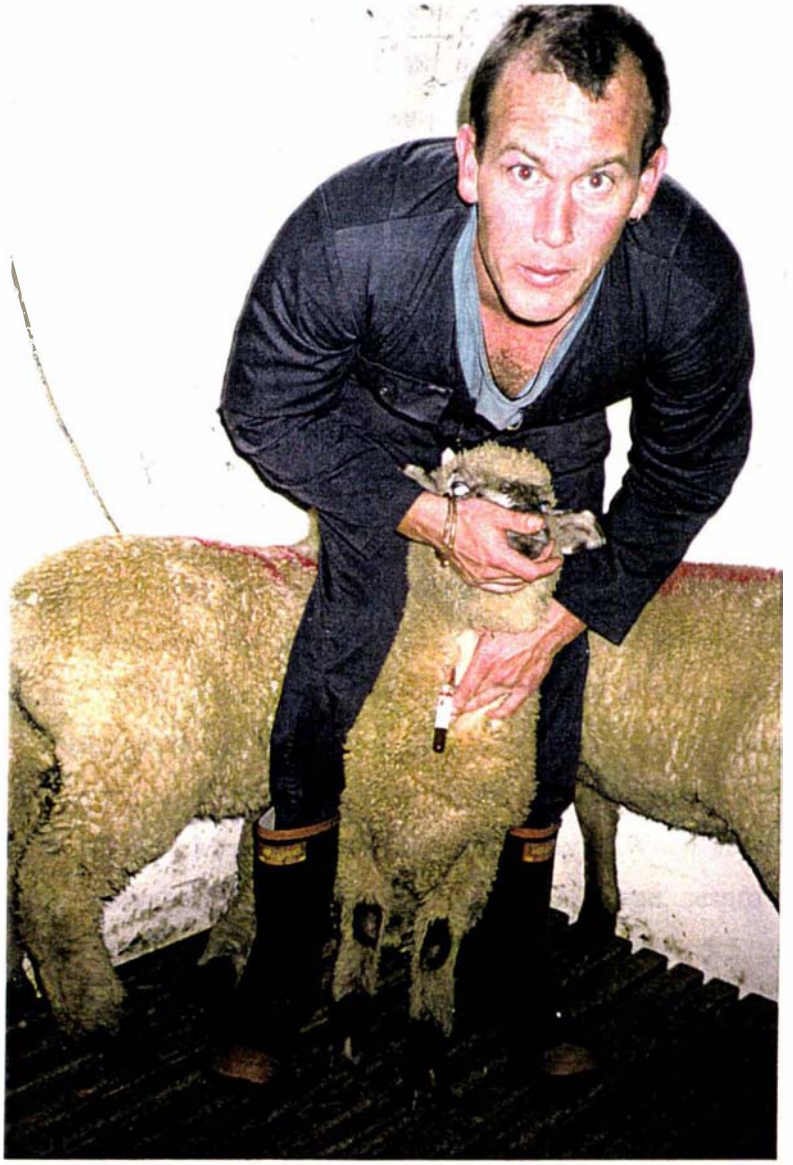

To all the sheep I've loved before

ABSTRACT

The effect of *Ostertagia circumcincta* on the secretory function of the ovine abomasum was studied *in vivo* and *in vitro*. *In vivo*, sheep were infected with larval or adult parasites and the changes in serum pepsinogen, serum gastrin and abomasal pH monitored. *In vitro*, the effect of worm extracts and incubates on the secretion of gastrin, somatostatin and pepsinogen were investigated using segments or dispersed cells of ovine abomasal mucosa. Using these, and a further perifusion technique, the pharmacology of gastrin and somatostatin secretion in the sheep was also investigated.

The *in vivo* study revealed that adult worms transferred directly into the abomasum of parasite-naive sheep initiate immediate changes in serum pepsinogen, gastrin and abomasal pH, showing that larval stages are not essential for the pathophysiological changes. These changes also occurred following infection with larvae but not until about five days post-infection. The increase in abomasal pH and serum gastrin occurred at a similar time, regardless of the dose of larvae or the route of administration. Serum pepsinogen levels increased before gastrin and pH. The normal range for serum pepsinogen, serum gastrin and abomasal pH in the parasite-free sheep were defined (0-500 U tyrosine/litre, 12-64 pM and 2.34-3.26 respectively). When abomasal pH rose and was maintained above pH 5.5 in sheep infected with larvae, serum gastrin levels rapidly returned to normal. When pH subsequently declined below 5.5, gastrin rapidly returned to elevated levels. By three weeks after infection of parasite-naive sheep with larvae, pH had returned to the normal range despite the continued elevation of serum gastrin. Infection with adults and larvae significantly increased the wet weight of the abomasum and this occurred within 8 days of infection with adult worms. Tissue gastrin levels were decreased by infection.

In vitro, solutions prepared with larvae and adult *O. circumcincta* had no effect on, or inhibited, gastrin release. These same solutions had no effect on, or stimulated, somatostatin secretion. Inhibition of gastrin secretion was always accompanied by increased somatostatin secretion although the converse was not true. Worm-derived solutions that inhibited gastrin release were possibly contaminated by microorganisms. Incubation of medium contaminated by an inoculum of abomasal content but without

worms produced solutions that potently stimulated somatostatin and inhibited gastrin release.

The pharmacological study revealed that mechanisms that have been identified in the regulation of gastrin secretion in other animals are present in the sheep. GRP, nicotine and carbachol but not adrenaline stimulated gastrin secretion from segments of antral mucosa in a concentration-dependent manner. Carbachol did not consistently inhibit somatostatin secretion and in most experiments somatostatin and carbachol release were both stimulated. Atropine inhibited basal gastrin release from segments of mucosa indicating a degree of tonic cholinergic discharge. Atropine partially or completely prevented the gastrin response to carbachol. VIP and GIP both stimulated somatostatin secretion but had no effect on gastrin, suggesting that somatostatin either does not restrain gastrin in the sheep or that this is maximal at basal levels. Somatostatin antiserum was not associated with increased gastrin secretion in most experiments.

STATEMENT

This is to certify that the work on which this thesis is based was carried out by the undersigned, and has not been accepted in whole or in part for any other degree or diploma. Assistance received is specifically recorded in the Acknowledgements section bound with this thesis.

David Eric Benjamin LAWTON

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LIST OF ABBREVIATIONS

A cell	glucagon cell
bFGF	fibroblast derived growth factor
BM	basal medium
BSA	bovine serum albumin
CCK	cholecystokinin
CGRP	calcitonin gene-related peptide
cm	centimetres
cpm	counts per minute
CR	control ratio
CR_i	control ratio for individual tissue piece
CV	coefficient of variation
D cell	somatostatin cell
DMPP	1,1-dimethyl-4-phenylpiperazinium
D. viviparus	*Dictyocaulus viviparus*
EC	enterochromaffin
ECL	enterochromaffin-like
EDTA	di-sodium ethylenediaminetetraacetate
EGF	epidermal growth factor
e.p.g.	eggs per gram (faeces)
ES	excretory/secretory
Expt	experiment
Fig.	figure
FR	feeding response
g	grams
G cell	gastrin cell
GABA	gamma amino butyric acid
GIP	gastric inhibitory (poly) peptide
GLP	glucagon-like (poly) peptide
GRP	gastrin releasing (poly) peptide
HBSS	Hank's balanced salt solution
H. contortus/placei	*Haemonchus* spp.
IGF	insulin-like growth factor
IL	interleukin
i.m.	intramuscular
kg	kilogram
L	litre
L_3/L_4	third/fourth stage larvae
M	moles per litre
mg	milligram
ml	millilitre
mm	millimeter
mM	millimolar
mOsm	milliosmoles

mU	milli-international enzyme unit
μ	mean
μ+1s	mean plus one standard deviation
μ+2s	upper limit of the normal range (mean plus two standard deviations)
μl	microlitre
μm	micrometer
μg	microgram
N. brasiliensis	*Nippostrongylus brasiliensis*
O. circumcincta/ostertagi	*Ostertagia* spp.
Oe. radiatum	*Oesophagostomum radiatum*
p	probability statistic
pg	picogram
PHI	peptide histidine isoleucine
pM	picomolar
RIA	radioimmunoassay
rpm	revolutions per minute
Rt	response to treatment
Rt_i	response to treatment by individual tissue piece
SD	standard deviation
SEM	standard error of the mean
SMS	somatostatin
T. axei/colubriformis	*Trichostrongylus* spp.
TGF-α	transforming growth factor-α
Th	T-helper
TNF-α	tumour necrosis factor-α
TR	test ratio
TR_i	test ratio of individual tissue piece
VIP	vasoactive intestinal polypeptide

PREFACE

Nematodes are common parasites of ruminants in New Zealand which cause reduced productivity and serious economic losses to the pastoral industry. Soulsby (1965) cites examples where helminth infections of sheep reduce the growth rate of ewes by 30%, decrease food intake by 50% in six weeks, reduce wool production by 40% and cause a 78% decrease in milk production by lactating ewes. In some studies, it was shown that treating the infection largely restored production, although the losses already accrued were never fully recouped. The control of parasites and the minimization of their effects on animal performance in intensive livestock systems is thus of great importance.

Ostertagia circumcincta is one of the economically important species within the Trichostrongylidae which cause ovine parasitic gastroenteritis. Conventional methods of control of this abomasal parasite rely heavily on the use of anthelmintics to which the worms are progressively developing resistance and consequently are becoming less effective, time-consuming and costly. The levels of infection to which sheep are exposed, particularly at certain times of the year, can be significantly reduced by skilled management techniques that require detailed knowledge of the life cycle of the parasite. The greater incidence of drench resistance has increased the importance of developing new anthelmintic strategies such as vaccination. Another alternative is the exploitation of the physiological effects of the parasite on the abomasum whereby parasite control may be achieved through interfering with these processes and thus cause an environment unfavourable for the establishment of the parasite.

Infection with *O. circumcincta* alters abomasal function: inhibiting acid secretion and causing the hypersecretion of gastrin and increased levels of circulating pepsinogen. It is important to determine which, if any, of these effects are of benefit to either the parasite or the host or whether they may have an adverse effects on either one. There is gathering evidence that the hypergastrinaemia often associated with ostertagiasis contributes to inappetence (Fox *et al.*, 1989a,b). It seems unlikely that the relationship between the parasite and its host has evolved in such a way that both partners cannot initiate changes from which it derives some benefits. It might be expected that the

parasite has developed means to improve its ability to establish in the host, but the host has also evolved mechanisms to restrict adverse effects and even to aid in expulsion of the parasite. One may speculate that the inhibition of acid secretion may be a mechanism developed by the parasite to produce a less harsh environment. On the other hand, undoubtedly the development of immunity by the host allows older animals to restrict their parasite burdens compared with naive animals. It is important to know whether the parasite has developed secretory products capable of actively modifying abomasal function, as interfering with their effects on the stomach may provide a new approach to the control of parasites. Very little is known about the means by which the host and parasite interact but it is an important area of research because of its possible practical implications.

The overall objective of the present experiments was to gain more knowledge of how the parasite and the host tissues communicate with one another and particularly the role of the chemical excretory/secretory worm products on abomasal function. It is possible that these chemicals are not involved in the physiological effects on the host, although they are known to act as antigens. The physical presence of the worms may produce all the necessary stimuli to provoke the physiological inflammatory and immune responses seen in the host. The first aim of the studies reported here was to examine in more detail the changes in abomasal function after experimental infection of sheep by either adult or larval *O. circumcincta* to order to better determine the temporal relationship between acid inhibition and the increases in circulating gastrin and pepsinogen. The second aim was to prepare excretory/secretory products of the parasite, develop suitable *in vitro* techniques for studying ovine abomasal tissue and to use these to investigate whether worm products have physiological effects on the sheep abomasum.

Chapter 1

LITERATURE REVIEW

1.1 INTRODUCTION

The ovine stomach is divided into four chambers, the rumen, reticulum, omasum and abomasum. The most distal of these, the abomasum, forms the developmental and functional homologue of the simpler monogastric stomach and is often parasitised by the trichostrongyloid nematode *Ostertagia circumcincta.*

The proximal three chambers comprise the forestomach. The rumenoreticulum is grossly enlarged to provide a reservoir in which food and bacteria are mixed and cellulose is hydrolysed. Bacterial fermentation of the fibrous diet produces volatile fatty acids such as acetate, propionate and butyrate (Hungate, 1966, 1968) which are absorbed through the chamber walls and provide an important energy source for the host (Engelhardt *et al.*, 1968). The microbes which pass with the digesta into the abomasum are the major source of protein for the ruminant (Hutton *et al.*, 1971).

The main functions of the abomasum are proteolytic digestion and the controlled passage of food. Proteolysis is facilitated by the exocrine secretion of acid and pepsinogen which flow from the gastric glands in a mucus-rich juice. Incoming digesta is acidified by HCl secretion so that it leaves the antrum with a pH of about 2.7 (Harrison & Hill, 1962). At this pH, pepsinogen is instantaneously converted to pepsin (Koelz *et al.*, 1982) and its proteolytic activity is optimal (Hersey, 1989). The mucus serves a protective function (Wallace & Bell, 1992).

Gastric secretion is controlled by neural (central and peripheral), hormonal and paracrine agents (Schubert, 1993, 1994). Gastrin, released by the G (gastrin) cells of the pyloric antrum, is the most important hormonal regulator of acid secretion (Walsh, 1984). In order to maintain the pH of the abomasum within a narrow range, the release of gastrin is sensitive to acid (Schubert, 1993). Acidification of the abomasum below pH 2.7

probably inhibits the release of gastrin in sheep (McLeay & Titchen, 1977b), whereas alkalinization increases the gastrin concentration in the blood (Reynolds *et al.*, 1991).

Infection of the abomasum with *O. circumcincta* interferes with its normal secretory function: there is an increase in pepsinogen and gastrin in the blood and a decrease in acid secretion. Hyperpepsinogenaemia most probably does not represent a state of hypersecretion but rather reflects increased mucosal permeability and failure of pepsinogen to be converted to the active form (pepsin) at raised pH (Jennings *et al.*, 1966).

In order to understand how the parasite may disturb abomasal secretion, it is necessary to understand how the secretion of acid, pepsinogen and gastrin are regulated in healthy sheep, as well as the development of the parasite within the abomasum, the effects it has on the host tissues and the responses it evokes.

1.2 ABOMASUM: ANATOMY AND HISTOLOGY

1.2.1 GROSS ANATOMY

The abomasum consists of three regions that are defined by gland type: (1) a very small cardiac region encircling the omasoabomasal orifice that has cardiac glands (Sommerville, 1956); (2) the body or fundic region that has gastric or fundic glands; (3) the pyloric region that has pyloric or antral glands.

The body region, which is characterised grossly by spiral mucosal folds which protrude into the lumen and run caudally toward the pyloric region, presents about 90% of the surface area of the abomasum (Hill, 1968) and is continuous with the more distal pyloric region. The pylorus includes an antrum, canal and sphincter. The pyloric sphincter is a muscular aperture opening into, and distally continuous with, the descending duodenum. There are no mucosal folds in the pylorus but a few small rugae are present.

1.2.2 MICROANATOMY

The abomasal wall from serosal to luminal surface consists of an outer muscle layer (*tunica muscularis externa*) covered by serosa, a connective tissue layer (*tunica*

submucosa) containing blood vessels and nerve plexuses and an inner glandular mucosa (*tunica mucosa*). The mucosa is divided into three layers: an inner muscle layer (*lamina muscularis mucosae*); a connective tissue layer (*lamina propria mucosae*) containing nerves, lymphatics and capillaries and into which the glands extend; and the outer mucus-secreting columnar epithelium (*lamina epithelialis*) (Getty, 1975).

The histology of the ruminant gastric mucosa has been described (Murray, 1970; Bunnett & Harrison, 1979; Domeneghini & Castaldo, 1981; Rizzotti *et al.*, 1980a,b; Calingasan *et al.*, 1984; Gurnsey, 1985; Kitamura *et al.*, 1985; Wathuta, 1986; Gabella, 1987) and appears to differ little from the typical mammalian pattern. In the body region, the gastric pits occupy about one third of the mucosal thickness and the glands, which are straight and continue from the base of the pits, occupy the remainder, while in the pylorus, the pits are deeper and the glands coiled. Cuboidal to columnar mucus-secreting surface epithelial cells line the abomasum and extend into the pits. A greater variety of cells are found in the glands than in the pits. Other mucus-secreting cells, the mucous neck cells, are located in the middle and basal part of the gastric glands. Acid-producing parietal cells are most common in the neck of the gastric glands. Their density decreases distally toward the antrum in the sheep (Sommerville, 1956). Pepsinogen-producing chief cells are most common at the base of the gastric glands and are intermingled with parietal and mucous neck cells. The pyloric glands are lined by mucus-secreting cells referred to by Murray (1970) as "pyloric gland cells". Isolated endocrine cells are the least numerous and are located among other epithelial cells throughout the abomasum, predominantly at the gland base and near, or in contact with, the basal lamina.

Gurnsey (1985) investigated the endocrine cells of the ovine gastrointestinal tract. In the fundus, he identified many histamine-containing enterochromaffin-like (ECL) cells, serotonin-containing enterochromaffin (EC) cells and somatostatin (D) cells. In the antrum, he identified G, D and ECL cells. Very few EC cells were identified in this region but it was suggested that this may have reflected reduced staining efficiency. 'Open'(communicating with the lumen) and 'closed' EC, ECL and D cells occur. Closed types were found in both the body and antrum, whereas open ECL and D cells were observed only in the antrum. No glucagon (A) cells were found in the abomasum, although Calingasan *et al.* (1984) reported their presence in low numbers. In addition to the cell types Gurnsey observed, Domeneghini & Castaldo (1981) also identified cells they designated D_1 and X in the ox. D cells possess long, nonluminal, cytoplasmic

processes which extend toward, and terminate on, other cell types including parietal and G cells (Larsson *et al.*, 1979). The distribution of endocrine cells differs between species e.g. Rubin (1972) reported few EC cells in the body compared with the pylorus in humans - the opposite to that reported in sheep by Gurnsey (1985). Non-endocrine cells reported by Gurnsey (1985) include connective tissue mast cells (containing serotonin and dopamine), mucosal mast cells (with very little serotonin), fibroblasts and globular leucocytes.

1.2.3 BLOOD AND NERVE SUPPLY

Blood is delivered to the abomasum by the right and left gastric and gastroepiploic arteries along its greater and lesser curvatures. Lesser vessels course throughout the submucosa and capillaries perfuse the *lamina propria*. The venous blood drains to the portal vein and enters the liver.

The abomasal wall is extensively innervated. It receives from the vagus nerves and coeliac ganglion preganglionic cholinergic and postganglionic adrenergic neurons which merge into, and provide extrinsic innervation for, the intrinsic enteric nervous system. Practically all of the cell bodies of the enteric nervous system are found in small ganglia within the two main plexuses, the myenteric and submucous, which lie between the external muscle layers and within the submucosa respectively (Costa & Furness, 1989). Nerve fibres, in turn, form additional plexuses within the muscle layers, around intramural blood vessels and in the mucosa. The plexuses contain nerve cells with processes that originate from receptors in the gut wall and mucosa and others which innervate parietal, chief and endocrine cells. In the abomasum of sheep and goats, small groups of ganglion cells lie in close proximity to the epithelium (Habel, 1956). The intrinsic neurons of the gut include many peptidergic-noncholinergic fibres (Walsh, 1984). Vasoactive intestinal polypeptide (VIP)-like, substance P-like and gastrin releasing peptide (GRP)-like immunoreactive neural elements have been demonstrated throughout the ovine gastrointestinal tract, including the abomasal mucosa (Wathuta, 1986). In the ovine abomasum, nerves showing somatostatin-like immunoreactivity have also been reported (Vergara-Esteras *et al.*, 1990). It was estimated by Gabella (1987) that there were 31 million myenteric neurons in the sheep gastrointestinal tract and that the ganglion neuron density was 2500/cm^2.

1.3 ABOMASAL SECRETION: PEPSINOGEN, ACID AND GASTRIN

1.3.1 GASTRIC SECRETION IN THE SHEEP

The primary stimulus for gastric secretion in sheep is the presence of food in the abomasum (Hill, 1960). As digesta is always present in the abomasum of pasture fed sheep, gastric secretion is relatively continuous (Hill, 1955; Getty, 1975), although it appears that the flow and composition of gastric secretions may vary with feeding (Reynolds & Titchen, 1977). The involvement of the hormone gastrin in the gastric secretory response to feeding has been confirmed (McLeay & Titchen, 1977b; Reynolds *et al.*, 1978).

Gastrin-stimulated acid secretion, in turn, inhibits the parietal cells through a negative feedback loop that becomes effective at and below about pH 2.7 (McLeay & Titchen, 1977b; Reynolds *et al.*, 1991). The involvement of a cholinergic component in the regulation of gastric secretion has also been established through insulin-induced hypoglycaemia (Hill, 1968; Reynolds *et al.*, 1980), vagal stimulation (Bladin *et al.*, 1983), perfusion of antral pouches with acetylcholine (McLeay & Titchen, 1977a) and responses to the administration of atropine (Reynolds *et al.*, 1991). The effect reported by Bladin *et al.* (1983) should be treated with caution as the response they describe (2.5 to 10 pM) approximates the sensitivity of many gastrin assay procedures.

Somatostatin, VIP and GRP have all been identified in the abomasum (Section 1.2.2) and a number of *in vivo* studies have been performed on ruminants to investigate their physiological roles (Bloom *et al.*, 1978; Shulkes & Hardy, 1980, 1982; Bloom *et al.*, 1983; Bladin *et al.*, 1983; Barry *et al.*, 1985; Reid *et al.*, 1988; Shulkes *et al.*, 1994). *In vivo* studies such as these reveal little about the mechanisms involved in the control of gastric secretion. Indeed, little is known of the regulatory mechanisms in sheep, although these are well established for a number of other species (e.g. rat, dog, human). Studies in ruminants have produced results which differ from those in other species: VIP did not inhibit gastrin secretion (Bloom *et al.*, 1978); somatostatin infusion caused an increase in serum gastrin (Barry *et al.*, 1985; Reynolds *et al.*, 1991) - which may have been mediated via the central nervous system or through an effect on the parietal cell;

GRP failed to elicit gastrin release (Bloom *et al.*, 1983; Shulkes & Hardy, 1980; McDonald *et al.*, 1988). These results are discussed below in the context of knowledge of the control of pepsinogen, acid and gastrin secretion in mammals generally.

1.3.2 PEPSINOGEN SECRETION

Pepsinogen is synthesised by the endoplasmic reticulum, stored in secretory granules and released through the apical surface by exocytosis on stimulation of the chief cell (Hersey, 1989). It has also been suggested that pepsinogen may be secreted directly into the blood (Stringfellow & Madden, 1979).

Pepsinogen secretion is regulated by a complex interaction of cholinergic (acetylcholine) and noncholinergic (GRP and VIP) neurons, hormonal (cholecystokinin (CCK) and gastrin) and paracrine (somatostatin and prostaglandin) mechanisms. The basic features appear to be common to all mammalian species although some species differences are apparent.

1.3.2.1 CHOLINERGIC AGENTS

Cholinergic stimulation of pepsinogen secretion appears to be a relatively consistent feature both *in vivo* and *in vitro*. It is inhibited by atropine (Muller *et al.*, 1990) and mediated by M_3-type receptors (Wilkes *et al.*, 1991). Central mechanisms stimulate pepsinogen secretion via cholinergic neurons (White *et al.*, 1991). Pepsinogen secretion provoked by vagal stimulation is atropine-sensitive (Hirschowitz, 1991) and cholinergic secretagogues stimulate secretion from isolated chief cells (Koelz *et al.*, 1982; Sanders *et al.*, 1983). Species differences are apparent e.g. bethanechol has almost no effect in humans while in the dog it is a potent stimulant (Hirschowitz, 1991).

Carbachol stimulated pepsinogen release in a concentration-responsive, atropine-sensitive manner from dispersed bovine and ovine gastric glands (McKellar *et al.*, 1990a). The maximum dose of carbachol used was 3×10^{-6} M, which produced a very small (7%) increase in pepsinogen release (the small increase may reflect the relatively low maximum dose tested). Koelz *et al.* (1982), who tested a wide range of doses on rabbit glands, found that while there was almost no response to 10^{-6} M, there was an increase of about 100% with 10^{-4} M carbachol.

1.3.2.2 GASTRIN

Gastrin has a stimulatory effect on pepsinogen secretion but this appears to vary among species e.g. distinct gastrin receptors have been identified on the guinea pig chief cell (Qian *et al.*, 1993) and numerous studies report a pepsinogen secretory response to this peptide in this species (e.g. Hersey *et al.*, 1983; Raufman *et al.*, 1984). Conversely, Soll *et al.* (1984a) were unable to detect gastrin receptors on canine chief cells and Sanders *et al.* (1983) reported that pentagastrin did not stimulate pepsinogen secretion from isolated cells of this species. No secretory response to pentagastrin was found in the vascularly-perfused stomach of the rat (Kleveland *et al.*, 1986), although gastrin did stimulate pepsinogen secretion from rabbit chief cells directly (Tang *et al.*, 1993). As gastrin-stimulated pepsinogen secretion *in vivo* can be inhibited by atropine (Helander, 1981) and histamine antagonists (Hirschowitz & Gibson, 1978), its physiological effect has been regarded as largely indirect and associated with the secretion of acid locally (Hersey, 1989; Muller *et al.*, 1990; Hirschowitz, 1991).

McLeay & Titchen (1975, 1977b) prepared sheep with separated pouches of the abomasal body and antrum and found that intravenous infusion of pentagastrin stimulated "pepsin" secretion in sheep during antral pouch acidification. However, as acid secretion was stimulated simultaneously, it cannot be concluded that pentagastrin had a direct effect on the chief cells.

1.3.2.3 CHOLECYSTOKININ

CCK appears to have a potent stimulatory effect on pepsinogen secretion both *in vivo* and *in vitro* that is not inhibited by atropine, propranolol or cimetidine (Hersey, 1989). It is currently thought that when gastrin-stimulated pepsinogen secretion occurs *in vitro*, it may be due to interaction with CCK receptors (Hersey, 1989; Tang *et al.*, 1993). However, at least in the guinea pig, distinct gastrin receptors have been identified on the chief cell (Qian *et al.*, 1993). This finding may explain why this species is relatively more responsive to gastrin.

1.3.2.4 ß-ADRENERGIC AGONISTS

ß-adrenergic receptor activation by isoproterenol weakly stimulates pepsinogen secretion

(Hirschowitz, 1991). *In vitro*, propranolol-sensitive, isoproterenol-stimulated pepsinogen secretion has been reported in isolated rabbit (Koelz *et al.*, 1982) and amphibian (Shirakawa & Hirschowitz, 1984) gastric glands. *In vivo* studies, however, have shown both adrenergic stimulation and inhibition (Hersey, 1989). The physiological significance of adrenergically-stimulated pepsinogen secretion is not known.

1.3.2.5 HISTAMINE

Histamine may stimulate pepsinogen secretion *in vivo* (Hersey, 1989), although in the dog it is inhibitory at higher doses (Hirschowitz & Hutchinson, 1977). The response to histamine appears to be an indirect effect mediated via local reflexes initiated in response to stimulated acid secretion (Raufman, 1992a). Exogenous histamine had no effect on pepsin secretion in conscious gastric-fistulated rabbits (Redfern *et al.*, 1991). Since no direct effect of histamine on the chief cell of any species has been observed (Hirschowitz, 1991), it is generally accepted that histamine is an indirect stimulant of pepsinogen secretion.

1.3.2.6 OTHER AGENTS

Other possible stimulants of the chief cell include GRP, gastric inhibitory peptide (GIP), motilin, erythromycin, secretin, VIP and prostaglandins. GRP weakly stimulated guinea pig chief cells (Fiorucci & McArthur, 1990); both secretin and VIP stimulated pepsinogen secretion *in vitro* but may be inhibitory *in vivo* (Schubert, 1993); a stimulatory effect of motilin and erythromycin on isolated guinea pig chief cells has been reported (Fiorucci & Morelli, 1993); gastric inhibitory peptide (GIP) may stimulate pepsinogen secretion (Brown *et al.*, 1989); prostaglandins stimulated pepsinogen secretion (Hersey, 1989; Schubert, 1993). Neither secretin nor prostaglandin E_2 was found to stimulate pepsinogen secretion from isolated rabbit gastric glands by Koelz *et al.* (1982). Schubert (1993) suggested that the inhibitory effect of secretin or VIP *in vivo* may be due to concomitant stimulation of somatostatin release, which he found mediated VIP-inhibition of gastric acid secretion by the perfused mouse stomach (Schubert, 1991).

1.3.2.7 SOMATOSTATIN

Inhibition of pepsinogen secretion by somatostatin is direct (Raufman, 1992a). Somatostatin release may similarly account for the lack of stimulation by secretin or prostaglandins reported by Koelz *et al.* (1982) as D cells would be normally associated with chief cells in the gland preparation. Glucagon has been reported to inhibit pepsinogen secretion *in vivo* (Konturek *et al.*, 1975). The mechanisms by which somatostatin and glucagon affect the chief cell are unknown, although recent work by Felley *et al.* (1994) suggests that chief cells may possess an additional mediator for agents stimulating cyclic AMP (cAMP). Peptide YY and neuropeptide Y both inhibited *in vitro* pepsinogen secretion stimulated by VIP, secretin and prostaglandin-E_2 but not by carbachol or CCK (Raufman & Singh, 1991).

1.3.2.8 INTRACELLULAR PATHWAYS

Mediation of secretagogue-stimulated pepsinogen secretion is by at least two pathways. One, which is coupled to prostaglandin, secretin, VIP and ß-adrenergic receptor activation, involves activation of adenylate cyclase by receptor binding through stimulatory guanyl-nucleotide-binding protein (G_s) and subsequent increase in intracellular cAMP; the other, also coupled via G protein to cholinergic, CCK, gastrin and GRP receptors, involves hydrolysis of membrane lipids to inositol phosphate by phosphoinositidase and a subsequent increase in mobilized intracellular calcium. The receptors and signal-transduction mechanisms regulating pepsinogen secretion and the methods and advances that have aided in their elucidation are reviewed by Raufman (1992b). Inhibition of only one transduction pathway (cAMP) explains why neuropeptide Y and peptide YY did not inhibit inositol phosphate-mediated stimulation. When two stimulatory agents are used, one increasing cAMP and the other calcium, then a more than additive effect results, while two agents that both act by the same mechanism give less than an additive response (Raufman *et al.*, 1983; Matsumoto *et al.*, 1987).

1.3.2.9 SUMMARY

1.. pepsinogen secretion *in vivo* is associated with acid secretion and many of the observed pepsinogen responses may be indirect;

2.. cholinergic agonists stimulate pepsinogen secretion from isolated chief cells

directly;

3.. CCK is a potent stimulus to pepsinogen secretion. Gastrin receptors have been reported on guinea pig chief cells. In most animals, however, gastrin-stimulated pepsinogen secretion is considered to be indirect;

4.. ß-adrenergic agonists appear to stimulate pepsinogen secretion *in vitro*;

5.. histamine does not have a direct effect on chief cells;

6.. somatostatin inhibits pepsinogen secretion directly via paracrine connections.

In sheep, carbachol (3×10^{-6} M) weakly stimulated pepsinogen secretion from dispersed bovine and ovine gastric glands. Pentagastrin stimulated pepsin secretion by the sheep stomach *in vivo*, although this may be an indirect response.

1.3.3 ACID SECRETION

1.3.3.1 BIOCHEMICAL AND FUNCTIONAL MORPHOLOGY OF THE PARIETAL CELL

Acid is secreted by the parietal cells. Protons are pumped into the lumen by H^+-K^+-ATPases while HCO_3^- is secreted at the basolateral membrane and carried away in the portal circulation (Sachs *et al.*, 1989). Central to the function of the cell is its polar orientation which is normally maintained *in vivo* by the seal provided by the *zonular occludens*. *In vitro*, H^+ secreted by the proton pump is immediately neutralized by concomitant HCO_3^- secretion. For this reason, indirect methods such as increased oxygen consumption and accumulation of the weak base aminopyrine are used to estimate indirectly the acid secretory activity of isolated parietal cells (Chew, 1989).

The biochemistry of gastric acid secretion has been reviewed by Sachs *et al.* (1989). The parietal cell biology, which involves all aspects from cell stimulation to secretion of the effluent product, has been reviewed by Forte & Soll (1989). Following stimulation and as part of secretion, profound morphological, biochemical and functional changes occur that significantly alter the ultrastructural appearance of parietal cells. In the unstimulated cell, the apical surface and the canaliculi contain short stubby microvilli. There is an abundance of tubulo- and micro-vesicles and numerous large mitochondria, which together occupy the majority of the cytoplasmic volume. The appearance of the unstimulated parietal cell is similar to that described by McLeay *et*

al. (1973) in the ovine abomasum infected by *O. circumcincta.* When stimulated, there are enlarged canalicular spaces, a greatly expanded apical membrane surface with elongated microvillar projections and a reduction in cytoplasmic tubulovesicles. Parietal cells have this appearance in actively secreting fundic abomasal pouches in sheep (McLeay *et al.*, 1973).

Karam & Forte (1994) treated rabbits with omeprazole (an H^+-K^+-ATPase inhibitor) for five days and found that the appearance of the parietal cells changed. On the basis of their ultrastructure, parietal cells were classified as appearing normal, altered or degenerated. In control tissues, altered and degenerated cells accounted for 6% of the population, while in omeprazole-treated tissues they accounted for 64%. After three days recovery from the omeprazole treatment, the parietal cells and the gastric mucosa appeared to recover their normal morphology. Karam & Forte (1994) point out that omeprazole binding is irreversible and, therefore, for recovery either cells regenerate or are replaced. The latter is consistent with the increased number of mitotic figures and preparietal cells they observed in the treated rabbits.

Parietal cell HCl secretion involves activation of at least two pathways. As for the chief cell, one involves activation of adenylate cyclase and the other hydrolysis of membrane phospholipid. Evidence for these pathways was extensively reviewed by Chew (1989). Stimulatory agents act via either of these pathways. Inhibitory agents act by inhibiting these pathways.

1.3.3.2 PHYSIOLOGY OF ACID SECRETION

1.3.3.2.1 Cephalic Phase

The cephalic phase of acid secretion has been reviewed by Feldman & Richardson (1986) and the central mechanisms involved by Taché (1991) and Schubert (1994). It is triggered by smell, sight, thought and taste of food and is easily conditioned. A cephalic phase has been demonstrated in the sheep (McLeay & Titchen, 1970). Cephalic phase stimulation is relayed via the vagus nerve to postganglionic cholinergic and noncholinergic (GRP and VIP) neurons located in the submucosal plexus of the stomach (Konturek *et al.*, 1990; Schubert, 1994). The acid secretory response to cephalic stimulation is mediated both directly on the parietal cell and indirectly through the release of gastrin.

1.3.3.2.2 Peptone

Peptone solutions stimulate acid secretion by the combined effect of induced gastrin secretion and the activation of fundic cholinergic and noncholinergic neurons responding to receptors in the mucosa (Schubert *et al.*, 1990, 1992; Schubert, 1994). From the receptors, neurons extend to, and synapse on, postganglionic parasympathetic fibres which innervate parietal or histamine-containing cells, thereby stimulating acid secretion. The postganglionic fibres are also innervated by the descending vagal preganglionic neurons that mediate the cephalic phase of secretion. Thus, systemic capsaicin, vagotomy, or coeliac ganglionectomy reduced, but did not eliminate, the acid response to peptone (Ramos *et al.*, 1992).

1.3.3.2.3 Distension

Distension of the body of the stomach with liquid or an air filled balloon elicits an increase in acid secretion. As with chemical stimuli, receptors in the stomach wall influence postganglionic parasympathetic neurons via local reflex arcs. Consequently, atropine markedly inhibits the acid response to distension, although it enhances the gastrin response. Substantial distension of the antrum stimulates acid secretion by increasing gastrin and decreasing somatostatin secretion, while low grade distension has the opposite effect (Schubert & Makhlouf, 1993). The distension-induced release of gastrin may be prevented by pretreatment with the ß-adrenergic antagonist propranolol or the instillation of an acid solution. Adrenergic receptors have not been identified on parietal cells. Activation of sympathetic neurons, however, has been shown to inhibit acid secretion (Schubert, 1993). Mårdh *et al.* (1987) reported that adrenaline increased histamine-stimulated aminopyrine accumulation by porcine parietal cells but that it was ineffectual on its own.

1.3.3.2.4 Luminal pH

Luminal pH regulates the secretion of acid, primarily through the modulation of gastrin release. Particularly in ruminants, incoming digesta is only slightly acid and raises the gastric pH, resulting in increased gastrin and acid secretion. Consequent acidification of digesta inhibits gastrin release via increased somatostatin secretion, thus attenuating acid secretion directly and without neural involvement (Schubert & Makhlouf, 1992). There is some debate over whether alkalinization *per se* stimulates the release of gastrin

secretion or merely facilitates it (Penston & Wormsley, 1987) and this is discussed in Section 1.3.4.1.2.

1.3.3.2.5 Enterogastrones

Enterogastrones (also 1.3.3.4.2) are substances presumed to be released in response to the presence of fats, carbohydrates and acid in the duodenum which inhibit gastric acid secretion (Gregory, 1962; McLeay & Fitzgerald, 1982; Rhee *et al.*, 1991).

1.3.3.3 STIMULATORS OF ACID SECRETION

Soll & Walsh (1979) concluded that the three stimulants likely to have physiological roles in the regulation of gastric acid secretion are histamine, acetylcholine and gastrin. In addition to cholinergic neurons, bombesin/GRP- and VIP-containing neurons are also important modulators of acid secretion. Collectively, the neurons are activated locally by chemicals in the lumen and distension as well as by the central nervous system (Section 1.3.3.2.1). Ultimately, intramural neurons and acidification of the gastric contents regulate acid secretion.

1.3.3.3.1 Histamine

Histamine is released from mucosal mast and ECL cells into the interstitial fluid (Schubert, 1993) where it binds to H_2-type receptors on the parietal cell (DelValle *et al.*, 1991). Much debate surrounds the histamine-containing cells and their relative importance in different species. The substantial work of Håkanson *et al.* (1986) suggests that ECL cells are the predominant histamine-containing cell in rodents. In the hedgehog, rabbit and pig, ECL and mast cells are both present in moderate numbers, while mast cells predominate in the cat, dog and humans. Despite the relative abundance of ECL cells in the rodent, mast cell-deficient mice had 50% less total gastric histamine than normal animals (Stechschulte *et al.*, 1990). It appears that mast cells are also important physiological regulators of acid secretion, as the mast cell stabilizer DS-4574 significantly inhibited carbachol- and pentagastrin-stimulated acid secretion by 85% in the rat (Tabuchi & Furuhama, 1994) and pig (Yamashika *et al.*, 1994), although it did not affect histamine-stimulated acid secretion. Both gastrin and acetylcholine stimulate the release of histamine from mucosal stores (Schubert, 1994).

Histamine stimulates aminopyrine accumulation in isolated parietal cell preparations from the dog (Soll & Wollin, 1979), rat (Dial *et al.*, 1981), humans (Mårdh *et al.*, 1985), pig (Mårdh *et al.*, 1987) and rabbit (Chew *et al.*, 1989). No response was found in isolated bovine glands by McKellar *et al.* (1990a) to doses that caused at least 12-fold increases in other *in vitro* preparations (Mårdh *et al.*, 1985) as well as in comparable gland preparations from other species (Berglindh *et al.*, 1976).

Histamine-mediated parietal cell activation is by both increased cyclic AMP (Chew, 1991) and increased intracellular calcium (DelValle *et al.*, 1992). Elevated intracellular calcium levels, however, appear to be of little importance in histamine-stimulated HCl secretion as chelation of calcium had little effect on the secretory response (Ljungström & Chew, 1991). Histamine is believed to potentiate the effects of acetylcholine and gastrin, which probably stimulate the parietal cell directly through increased intracellular calcium (Ljungström & Chew, 1991; Sandvik *et al.*, 1993). H_1-, H_2- and H_3-type receptors have been identified. H_1- and H_2-types are coupled to the intracellular calcium- and adenylate cyclase-mediated pathways respectively. H_3-type receptors have been implicated in the autocrine regulation of histamine synthesis and release (Schubert, 1994).

1.3.3.3.2 Acetylcholine

Acetylcholine or carbachol stimulates aminopyrine accumulation in parietal cell cultures from dogs (Soll, 1980), humans (Mårdh *et al.*, 1985), pigs (Mårdh *et al.*, 1987), rabbits (Chew *et al.*, 1989) and rats (Pfeiffer *et al.*, 1990). Schubert (1992) argued that a small amount of histamine appeared to be necessary to potentiate the acid response to acetylcholine. To what extent this is permissive is unclear, as current evidence, reviewed by Schubert (1993, 1994), clearly demonstrates through the use of H_2-receptor antagonists that acetylcholine acts directly on the parietal cell which is equipped with M_3-type receptors (Wilkes *et al.*, 1991). Acetylcholine is probably released from postganglionic fibres on preganglionic vagal stimulation (Chew, 1991). Not all is of vagal origin, however, as atropine inhibits gastric acid secretion more than does vagotomy (84 and 50% respectively) in gastric fistulated conscious rabbits (Redfern *et al.*, 1991), indicating the importance of local reflex arcs and the stimulation of postganglionic fibres.

Hollande *et al.* (1993, 1994) found that carbachol stimulated histidine decarboxylase activity (a measure of histamine synthesis) and histamine release from isolated rabbit fundic mucosal cells, and that the effect was mediated via M_1-type receptors. Indirect stimulation via histamine release therefore also appears likely in this species.

1.3.3.3.3 Gastrin

Gastrin is very important in the regulation of acid secretion. The postprandial release of gastrin accounted for approximately 90% of the meal-induced release of gastric acid in humans (Blair *et al.*, 1987); distension-induced acid secretion in dogs was totally attributable to endogenous gastrin (Kovacs *et al.*, 1989); gastrin is the principal mediator of meal-stimulated acid secretion in rats (Lloyd *et al.*, 1992a). Intravenous pentagastrin stimulated acid secretion in sheep (McLeay & Titchen, 1977b). Although gastrin appears to be responsible for most of the meal-stimulated acid response, it is likely that some is due to synergism with cholinergic stimulation (Hirschowitz, 1989).

Some degree of direct gastrin stimulation of the parietal cell appears likely in all species, although Schubert (1994) considers this unequivocal only in the dog. Gastrin receptors have been demonstrated on the parietal cells of dogs (Soll *et al.*, 1984a) and rabbits (Roche *et al.*, 1991) and pentagastrin stimulated isolated parietal cells from rabbits (Chew & Hersey, 1982), humans (Mårdh *et al.*, 1985), pigs (Mårdh *et al.*, 1987; Cabero *et al.*, 1993) and dogs (Park *et al.*, 1987). In the rat, it did not stimulate on its own but directly potentiated the effect of histamine (Cabero *et al.*, 1991). Certainly, gastrin stimulates histamine release: Gerber & Payne (1992) observed that infusion of dogs with pentagastrin resulted in a large increase in histamine release; gastrin stimulated histamine release from enriched canine ECL cells *in vitro* (Chuang *et al.*, 1992); a putative gastrin receptor has been cloned from an ECL cell tumour (Nakata *et al.*, 1992) and Hollande *et al.* (1993, 1994) found that gastrin stimulated histidine decarboxylase activity and histamine release by isolated rabbit fundic cells. Debate continues over whether gastrin stimulates acid secretion directly or via histamine release, or both. Although Chew & Hersey (1982) observed direct pentagastrin stimulation of rabbit parietal cells, the response was very small, which may explain why other investigators (Berglindh *et al.*, 1976) failed to detect a response. Schubert (1993, 1994), considers indirect gastrin-stimulated histamine release to be the more important. Although usually effective on parietal cells, the efficacy of gastrin on these cells is considerably less than

that of acetylcholine and histamine (Soll & Wollin, 1979; Forte & Soll, 1989). Soll (1980) found that cimetidine had no effect on gastrin-stimulated acid secretion by isolated canine mucosal cells (suggesting a direct action), while Grossman & Konturek (1974) reported that metiamide (another H_2-receptor antagonist) inhibited pentagastrin-stimulated acid secretion by the canine stomach (suggestive of an indirect action). CCK stimulated acid secretion *in vitro* by a direct effect on the rabbit parietal cell (Chew & Brown, 1986) by interacting with the same receptor as gastrin (Roche *et al.*, 1991).

1.3.3.4 INHIBITORS OF ACID SECRETION

1.3.3.4.1 Somatostatin

Somatostatin is the main physiological inhibitor of acid secretion. It has a direct paracrine effect on the parietal cell and an indirect effect via G cells in the antrum that afford it an important regulatory role in the control of acid secretion over which it exerts tonic restraint (Schubert, 1994). In the dog, it also appears that somatostatin exerts a tonic inhibitory influence on histamine secretion and thus indirectly further restrains acid secretion (Payne & Gerber, 1992; Chuang *et al.*, 1993). It was noted above that CCK stimulates isolated parietal cells to secrete acid but *in vivo* has a net inhibitory effect. Findings such as these may be explained by the effect of substances such as CCK on somatostatin secretion (Lloyd *et al.*, 1992b). CCK is a far more potent stimulator of somatostatin secretion than is gastrin, while, conversely, gastrin is a far more potent stimulator of acid secretion (DelValle *et al.*, 1993). DelValle *et al.* (1993) were able to show that the relative potencies with which gastrin and CCK stimulated somatostatin secretion was related to their relative affinities for different CCK-type receptors on the canine D cell (the so-called selective type-A (CCK-A) and CCK-B receptors). Yamada *et al.* (1984) found that adrenaline stimulated these cells while carbachol inhibited them. It appears that bombesin (Schubert *et al.*, 1988b), VIP (Schubert, 1991) and secretin (Chey *et al.*, 1981) also exert their inhibitory effects on acid secretion by releasing somatostatin.

The role of GRP neurons on acid secretion has not been clarified, although the high density in the fundic mucosa (Dockray *et al.*, 1979) and the presence of GRP binding sites on both D and parietal cells (Nakamura *et al.*, 1988) suggests it may modulate acid secretion via somatostatin. High doses of GRP and bombesin inhibit acid secretion in

dogs (Hirschowitz & Molina, 1983) and humans (Varner *et al.*, 1981), although lower doses stimulate acid secretion through stimulation of gastrin (Walsh *et al.*, 1981). In contrast, in rats and mice, all doses inhibit acid despite stimulation of gastrin. Both bombesin and GRP have another indirect inhibitory action on gastric acid by acting on the central nervous system with the efferent pathway via the sympathetic nervous system (Taché *et al.*, 1986). The possible different effects of GRP on fundic and antral D cells is discussed in Section 1.3.4.2.4.

Somatostatin receptors have been identified on parietal cells of the rat (Reyl *et al.*, 1979) and dog (Park *et al.*, 1987). As the inhibition induced by somatostatin is pertussis toxin-sensitive in these and other species (Schmidtler *et al.*, 1992; Park *et al.*, 1987; Schubert *et al.*, 1988b), somatostatin-receptor binding is believed to activate G_i, an inhibitory subunit of the receptor-G_s-adenylate cyclase complex (Schepp *et al.*, 1992). An additional activation pathway, perhaps through protein kinase C, has also been suggested (Sugano *et al.*, 1986).

Luminal perfusion of the isolated mouse stomach with acid caused an increase in somatostatin secretion in proportion to the increase in luminal acidity, while alkalinization of the lumen reduced somatostatin release (Schubert *et al.*, 1988a). These findings have been confirmed in conscious dogs (Greenberg *et al.*, 1992) in which the secretion of both acid and somatostatin could be evoked by insulin-induced hypoglycaemia. When the acid response was blocked by omeprazole or ranitidine, so was the secretion of somatostatin. Both of these studies were interpreted as suggesting that paracrine somatostatin release serves as a negative feedback mechanism restraining acid secretion, although in neither was it demonstrated that the somatostatin was not of antral origin.

1.3.3.4.2 Enterogastrones

Enterogastrones may include secretin, CCK, GIP, neuropeptide Y, peptide YY, oxyntomodulin and glucagon-like peptide-1 (GLP-1). In the rat, pentagastrin-stimulated acid output was profoundly inhibited by the placement of oleic acid in the duodenum, which coincided with a significant increase in secretin (Rhee *et al.*, 1991). This inhibitory effect was completely reversed by intravenous injection of rabbit antisecretin serum but not by normal rabbit serum. Schubert (1993, 1994) considers the most likely

physiological enterogastrones to be CCK and secretin and that a neural component, partly a vagal afferent reflex, also exists. Although CCK stimulated acid secretion *in vitro* by a direct effect on the rabbit parietal cell (Chew & Brown, 1986), *in vivo*, however, CCK inhibits acid secretion in humans (Brooks & Grossman, 1970), dogs (Mayer *et al.*, 1982) and rats (Lloyd *et al.*, 1992b), an effect the latter authors showed to be mediated by somatostatin. McLeay & Bell (1980) report CCK-stimulated acid secretion in milk-fed calves, a result at variance with findings in other species. The authors suggest that this may arise as the stimulus to secretion they provide, and on top of which the CCK effect was superimposed, "might be regarded as more physiologic" and that, consequently, low-affinity inhibitory "sites" on the receptors were not activated (i.e. these are activated at high doses). It is perhaps more likely that the relative binding affinities of CCK receptors (selective type-A versus non-selective type-B) on bovine D and parietal cells differs from those in other species. Alternatively, the observed CCK-induced decrease in abomasal motility and emptying of the saline meal may have stimulated distension-induced acid secretion. Unfortunately no indication is given of the meal volume.

GIP, which is produced by endocrine cells of the duodenum and jejunum, is capable of inhibiting acid secretion (probably through somatostatin) and may well be a physiological enterogastrone (Brown *et al.*, 1989). Neuropeptide Y and peptide YY are both capable of inhibiting acid secretion (Schubert, 1993) and may possibly be further enterogastrones (Schubert, 1992). Secretion of neuropeptide Y by the isolated vascularly-perfused rat stomach is stimulated by acetylcholine (McIntosh *et al.*, 1992). As this secretion was sensitive to hexamethonium and not atropine, it appears that nicotinic sites, presumably synapses within the mucosa, were activated. Peptide YY increased the secretion of somatostatin from the fistulated cat stomach (Bado *et al.*, 1993).

1.3.3.4.3 Epidermal Growth Factor (EGF) and Transforming Growth Factor-α (TGF-α)

TGF-α receptors are present on parietal cells (Chew, 1991). EGF inhibits histamine-stimulated aminopyrine accumulation in isolated parietal cells from rats (Shaw *et al.*, 1987), pigs (Sjodin *et al.*, 1992) and rabbits (Wang *et al.*, 1993). TGF-α also inhibited rabbit parietal cells (Goldenring *et al.*, 1993). In these studies, EGF did not inhibit

carbachol-stimulated acid secretion, although this has been previously reported (Konturek *et al.*, 1984). Sachs (1990) discusses how this anti-secretory function may contribute toward the creation of a "healing environment" in which the "growth and repair" functions of these substances would be enhanced.

1.3.3.4.4 Prostaglandins

Prostaglandins, particularly Pg-E_2, are potent inhibitors of histamine-stimulated acid secretion (Whittle & Vane, 1987; Hirst, 1989). Their effect, like that of somatostatin, is pertussis toxin-sensitive, indicating activation of G_i (Chew *et al.*, 1989) and is mediated by specific receptors coupled to this protein (Seidler *et al.*, 1989). This action explains why prostaglandin potently inhibited acid secretion stimulated by histamine but not by gastrin or carbachol in parietal cells of the rat (Baird *et al.*, 1980) and dog (Soll *et al.*, 1986). Prostaglandins did not inhibit histamine secretion *per se* in the dog (Payne & Gerber, 1992) although they did in the rat (Sandvik & Waldum, 1988). In addition to their peripheral effect, prostaglandins also inhibit centrally-derived cholinergic stimulation (Taché, 1991). The opposite effect of prostaglandins on the parietal and chief cells is interesting, particularly as the latter is believed to be via increased adenyl cyclase activity (Schubert, 1992) i.e. it stimulates the adenyl cyclase pathway in one cell type while inhibiting it in another. This suggests that the pathways themselves may well differ in the two cell types.

1.3.3.4.5 Interleukin-1 (IL-1)

IL-1, a potent mediator of inflammation, has been shown to inhibit acid secretion. Robert *et al.* (1991) go as far as to describe it as the most potent inhibitor of gastric acid known, although almost all of the work with this peptide has been done in rats. It has been suggested that its action may be mediated by prostaglandins (Robert *et al.*, 1991), by modification of vagal efferent activity, also through prostaglandin-dependent pathways (Saperas *et al.*, 1990) or by inhibition of gastrin-stimulated histamine release (Wallace *et al.*, 1991). The latter suggestion was made as Wallace *et al.* found that IL-1 inhibited only acid secretion stimulated by exogenous pentagastrin and not that by histamine dihydrochloride or bethanechol, although the pentagastrin-stimulated acid secretion could be completely blocked by cimetidine (an H_2-receptor antagonist). The inhibition of pentagastrin-stimulated acid secretion was also unaffected by indomethacin

(an anti-prostaglandin) or bilateral vagotomy in this study.

1.3.3.4.6 Other Inhibitors

Other substances which may inhibit acid secretion include nitric oxide (Martinez-Cuesta *et al.*, 1992); adenosine (Gerber *et al.*, 1984, 1988); pituitary adenylate cyclase-activating polypeptide (Mungan *et al.*, 1992), cytokines other than IL-1 (Saperas *et al.*, 1992), calcitonin gene-related peptide (CGRP) (Schubert, 1994) and opioids (Esplugues *et al.*, 1992). Further substances may be both inhibitory and stimulatory depending on the level of action e.g. GLP-1 is inhibitory *in vivo* (O'Halloran *et al.*, 1990; Jarrousse *et al.*, 1993) but stimulatory *in vitro* (Schmidtler *et al.*, 1991, 1994).

1.3.3.5 SUMMARY

1.. acid secretion is affected by physiological changes associated with a meal. These include a cephalic phase, distension, chemical stimuli (peptone) and the pH of the luminal contents;

2.. histamine released from ECL and mast cells stimulates parietal cells directly and potentiates the stimulatory effect of gastrin and acetylcholine;

3.. cholinergic agonists stimulate acid secretion directly by stimulation of parietal cells and indirectly by inhibiting somatostatin secretion and stimulating histamine release;

4.. gastrin stimulates acid directly through stimulation of the parietal cells and indirectly through histamine release. CCK stimulates acid secretion *in vitro* but inhibits it *in vivo* through somatostatin release;

5.. somatostatin tonically restrains acid secretion directly by an effect on the parietal cell and indirectly by its effect on G and histamine-containing cells;

6.. a number of other substances such as VIP and GRP may influence acid secretion through their effect on somatostatin release;

7.. IL-1, prostaglandins, EGF, TGF-α and enterogastrones inhibit acid secretion directly or by stimulating somatostatin release.

In sheep, a cephalic phase of acid secretion has been observed. Pentagastrin stimulates acid secretion in sheep. CCK was found to stimulate acid secretion in calves. Histamine did not stimulate aminopyrine accumulation in parietal cells of dispersed bovine glands.

1.3.4 GASTRIN SECRETION

1.3.4.1 PHYSIOLOGY OF GASTRIN SECRETION

In the sheep, as in other species, the major source of gastrin is the mucosa of the pyloric antrum (Reynolds *et al.*, 1991). In mammals generally, its release into the circulation is controlled by neural elements (cholinergic, VIP and GRP neurons) and the paracrine mediator somatostatin (Schubert, 1993, 1994). These regulators of G cell secretory activity are, in turn, sensitive to input from the central nervous system (cephalic phase), the degree of gastric distension and the chemical composition of the luminal contents. The normal stimulus for gastrin secretion is, therefore, the ingestion of a meal as this excites the senses with smell and taste, distends the stomach, raises the pH of the contents and presents a variety of food derived chemicals.

1.3.4.1.1 Chemical Composition of Ingesta

The chemical composition of the food appears to be important in the gastrin response. Fat and carbohydrates appear to be poor stimulants of gastrin secretion, whereas partly digested protein (peptone) is a potent stimulant (Blair *et al.*, 1975). A number of other substances such as caffeine and calcium are also stimulatory (Carr & McGuigan, 1973; Walsh, 1984). Graded concentrations of peptone produce corresponding increases in serum gastrin, except at low intragastric pH (Eysselein *et al.*, 1992).

Uncertainty surrounds the mechanisms by which proteins and their breakdown products influence gastrin secretion. Somatostatin infusion inhibited the acid response to a peptone meal by 50% in gastric fistulated dogs (Seal *et al.*, 1982). When added to the lumen of the vascularly-perfused rat stomach or to the medium bathing antral mucosal segments, peptone stimulated gastrin and inhibited somatostatin secretion (Saffouri *et al.*, 1984b). Tetrodotoxin abolished the responses, while atropine reduced the increase in gastrin and converted the decrease in somatostatin to an increase. Collectively, these results suggest that the effect of peptone is mediated via neurons and has effects similar to vagal stimulation. To investigate this similarity further, Schubert *et al.* (1992) repeated the experiment with the additional combination of (1) peptone and a GRP antagonist and (2) peptone, atropine and the antagonist. They concluded from their results that: (a) the secretory response to peptone was mediated entirely neurally and

involved both cholinergic and noncholinergic-GRP neurons; (b) inhibition of somatostatin secretion by cholinergic neurons predominated, permitting cholinergic and GRP neurons to elicit an "optimal" response from G cells.

In contrast, other studies in the rat and in other species suggest that protein products have a direct effect on the G cell and not through neural pathways (Lichtenberger *et al.*, 1980). Both amino acids, and more particularly their corresponding amines (metabolites derived from amino acids), directly stimulated gastrin release from isolated canine G cells (DelValle & Yamada, 1990). Although not pure, these cells were certainly without neural elements. The results of this study (sensitivity to inhibition by somatostatin) suggest that amino acids and amines interact with the G cell via separate mechanisms: amino acids appeared to stimulate G cells in a manner similar to other receptor-mediated secretagogues (sensitive to somatostatin); amines appeared to act independently of any known receptor-mediated action (insensitive to somatostatin). It was suggested that amines may diffuse into the G cell and destabilize gastrin granules by neutralization. Subsequently, Dial *et al.* (1991) reported that amines could directly stimulate the release of gastrin from isolated gastrin granules. As had been proposed by DelValle & Yamada (1990), it was shown that amines tended to alkalinize the gastrin granule interior. These findings contradict the suggestion of Schubert *et al.* (1992) that the response to peptone is mediated entirely neurally.

Amino acids vary in their potency as stimulants of gastrin secretion. Dockray & Gregory (1989) reported that the most potent amino acid stimulant is tryptophan. Cysteine, phenylalanine and hydroxyproline are also believed to be particularly effective (Strunz *et al.*, 1978; Taylor *et al.*, 1982) while glycine and alanine are no longer believed to be so (Van Bruchem, 1977).

Protein (or its digestion products) is also stimulatory to acid secretion in the sheep (van Bruchem & van 'T Klooster, 1980). Volatile fatty acids were considered to be minor stimulants to acid secretion in this species, although van Bruchem & van 'T Klooster attributed this primarily to their buffering capacity.

1.3.4.1.2 Gastric Acidity

Acidification of the gastric contents inhibits the secretion of gastrin in all species studied. Becker *et al.* (1973) followed the release of gastrin by the canine antrum

exposed to solutions of varying pH: as the pH was lowered, the amount of gastrin released declined. Conversely, in this study, exposure to neutral pH was accompanied by increased gastrin release. As the mucosa was not otherwise stimulated, it would appear that alkalinization *per se* stimulated gastrin secretion. Baker *et al.* (1993) did not find any consistent relationship between plasma gastrin concentration and the median gastric pH in fasted ponies experiencing periods of spontaneous alkalinization. They suggested that either the sampling frequency may have been insufficient to detect gastrin fluctuations or the episodes of spontaneous alkalinization may have been too short to stimulate gastrin hypersecretion. The results may also indicate that alkalinization *per se*, in the absence of additional stimuli, may be inadequate to stimulate gastrin secretion. If a stimulus had been present, presumably spontaneous alkalinization would not have occurred. It would have been interesting if some form of stimulus to gastrin secretion had been provided (such as a $CaCO_3$ pellet placed in the stomach) prior to the recording periods and if spontaneous alkalinization had then still occurred. Whether alkalinization on its own is an effective stimulus to gastrin secretion remains contentious (reviewed by Penston & Wormsley, 1987).

Acid-inhibition of gastrin secretion appears to be mediated through somatostatin release as endogenous or exogenous acidification of the gastric lumen increases somatostatin secretion in rats (Schusdziarra *et al.*, 1978), dogs (Seal *et al.*, 1982), humans (Colturi *et al.*, 1984) and mice (Schubert *et al.*, 1988a). When the acid secretory response to insulin-induced hypoglycaemia in dogs was blocked by omeprazole or ranitidine, then so was the otherwise concurrent increase in somatostatin (Greenberg *et al.*, 1992). Similarly, alkalinization of the mouse stomach by infusion of buffered saline or of the rat antrum by fundectomy led to decreased somatostatin and increased gastrin secretion (Schubert *et al.*, 1988a; Ryberg *et al.*, 1990). In the mouse stomach, acid-stimulated somatostatin release was resistant to tetrodotoxin, suggesting that neural activity is not essential for the response (Schubert *et al.*, 1988a). Hypergastrinaemia (three times basal levels) developed in sheep within 24 hours of omeprazole treatment, although no change in plasma somatostatin was detected (Read *et al.*, 1992). Since somatostatin is a paracrine regulator, the absence of a change in plasma concentration did not preclude a local effect on the G cell of decreased somatostatin secretion. In humans, omeprazole-induced hypergastrinaemia can be inhibited by the long-acting somatostatin analogue SMS 201-995 (Meijer *et al.*, 1993).

Sheep do not appear to be fundamentally different from other mammals. Ash (1961a,b) concluded that the acidity of the ovine abomasum is of major importance in the control of acid secretion. Hill (1968) suggested that acidification of the abomasal contents reduced the secretion of acid by an effect on the antrum, as was later confirmed by McLeay & Titchen (1977a,b) who found that resting acid and "pepsin" secretion were reduced by acidification of antral pouches to pH≤2.7. Perfusion of the antral pouch with acetylcholine, whilst normally stimulatory, had no effect at pH 2.5. Intravenous pentagastrin, however, restored gastric exocrine secretion despite antral pouch acidification. The authors suggested, therefore, that the inhibitory effect was mediated through reduced gastrin secretion. Earlier work (McLeay & Titchen, 1974, 1975) demonstrated that antrectomy resulted in reduced exocrine secretion, suggesting a stimulatory function for this region. The infusion of rumen fluid into the abomasum markedly increased both the pH of the contents and gastrin secretion (Reynolds *et al.*, 1991), but when the pH of the rumen fluid was reduced to 3.0 prior to infusion, both abomasal pH and serum gastrin concentration remained close to control levels. The influence of distension was unlikely, since the infusion of a similar volume of mannitol solution was without effect. This suggests that alkalinization of the antrum, if not directly causal, at least permitted the increase in gastrin secretion. Whether or not alkalinization *per se* stimulates gastrin secretion is perhaps of little consequence in the ruminant as the delivery and presence of digesta is relatively continuous.

1.3.4.1.3 Gastric Distension

Gastric distension contributes to meal-stimulated gastrin secretion through the activation of both cholinergic and noncholinergic neurons (Schubert *et al.*, 1982a,b). Neural involvement is evidenced by the sensitivity of the response to tetrodotoxin and atropine. Activation of VIP neurons and VIP release is believed to be part of the physiological response to distension: the VIP antagonist 10-28 abolished the gastrin and somatostatin responses to low-grade distension; secretin (a VIP homolog) also stimulated somatostatin secretion, through which it can inhibit carbachol-stimulated gastrin secretion (Wolfe *et al.*, 1983). VIP, like GRP, is located throughout the mammalian gut within neurons, most of which appear to be intrinsic (Fahrenkrug, 1989). Fibres extend throughout the gut wall and form a dense network in the *lamina propria* and around fundic and pyloric glands. Vagal stimulation causes the release of VIP in the calf (Bloom *et al.*, 1978), pig (Holst *et al.*, 1992b), cat (Fahrenkrug *et al.*, 1978) and lamb (Reid *et al.*, 1988). Like GRP release, the release of VIP appears to be atropine-resistant and hexamethonium-

sensitive (Fahrenkrug, 1989). The physiology of distension-induced gastrin secretion and the role of VIP have been discussed by Schubert & Hightower (1989b). They summarise the distension-induced response as follows: (1) low-grade distension activates preferentially VIP neurons that stimulate somatostatin and thus inhibit gastrin secretion; (2) increasing distension leads to progressive recruitment of cholinergic neurons that cause a reversal of the response to a stimulation of gastrin and inhibition of somatostatin; (3) elimination of the cholinergic influence with atropine returns the response to that observed with low-grade distension.

1.3.4.2 REGULATION OF GASTRIN SECRETION

Under physiological conditions, gastrin is released by a meal through a combination of neural stimulation, principally via vagal pathways, breakdown products of proteins and increasing gastric distension. The resulting acid secretion exerts a negative feedback on gastrin release. The principal neural stimulatory pathways are cholinergic or via GRP neurons. The importance of the continuous restraint exerted by somatostatin is well recognised and many effects on gastrin release are not directly on the G cell but by regulating somatostatin secretion e.g. acid feedback and gastric distension. The ways in which these and other modulators of gastrin release act on the G and D cells have been investigated in a variety of *in vivo* and *in vitro* preparations and have revealed the complexity of the neural and paracrine regulatory mechanisms.

1.3.4.2.1 Cholinergic Agonists

Cholinergic agonists, such as methacholine and carbachol, dose-dependently inhibit somatostatin and stimulate gastrin secretion in a range of species (Saffouri *et al.*, 1980; DuVal *et al.*, 1981; Richelsen *et al.*, 1983). Inclusion of somatostatin antibodies in some of these preparations has shown that, for the most part, cholinergically-stimulated gastrin secretion is mediated through the concomitant inhibition of somatostatin synthesis and release. Similarly, a maximal gastrin response to stimulants such as GRP occurs only when the inhibitory influence of somatostatin is withdrawn either by antiserum or inhibition by cholinergic agonists. Bombesin/GRP stimulated gastrin and somatostatin secretion in the rat, but the gastrin response was considerably less than that stimulated by methacholine, however, when somatostatin antiserum was added to the perfusate, the bombesin-stimulated gastrin response surpassed the maximal response to methacholine (DuVal *et al.*, 1981). The ability of somatostatin to inhibit bombesin- or

GRP-stimulated gastrin secretion has been demonstrated in dogs (Sugano *et al.*, 1987) and humans (Campos *et al.*, 1990). Atropine, on the other hand, converts a cholinergically-induced decrease in somatostatin secretion to an increase above basal levels (Makhlouf *et al.*, 1989). Collectively, these results indicate that suppression of endogenous somatostatin accounts for only part of the gastrin response to cholinergic agonists, and that noncholinergic neurons, perhaps activated via nicotinic receptors (note the effect of 1,1-dimethyl-4-phenylpiperazinium (DMPP); Section 1.3.4.2.3), may participate through the release of GRP.

1.3.4.2.2 Gastrin Releasing Peptide

GRP is produced and released within the mammalian gut only by intrinsic enteric neurons (Dockray *et al.*, 1979). The cell bodies of these neurons are in the myenteric plexus and fibres extend throughout the submucosa and mucosa of the stomach (Walsh, 1989a). In the antrum, these nerves terminate near the base of the pyloric glands, where there is an intense concentration of GRP receptors in the region of the endocrine cells (Vigna *et al.*, 1987). The infusion of GRP or bombesin into dogs (Bertaccini *et al.*, 1974), rats (DuVal *et al.*, 1981), humans (De Jong *et al.*, 1987) and pigs (Holst *et al.*, 1987a) induced significant increases in plasma gastrin. The direct action of GRP on the G cell has been demonstrated *in vitro*: bombesin dose-dependently stimulated gastrin release from isolated rat and human antropyloric glands (Richelsen *et al.*, 1983) or enriched antral G cells (Campos *et al.*, 1990) and GRP and bombesin stimulated gastrin release from primary canine G cell cultures through a calcium-dependent mechanism (Sugano *et al.*, 1987; Giraud *et al.*, 1987). Repeated injections of GRP caused G cell hyperplasia (Lehy *et al.*, 1983). GRP infusion had no effect on gastrin release in conscious calves (Bloom *et al.*, 1983) or sheep (Shulkes & Hardy, 1980; McDonald *et al.*, 1988). McDonald *et al.* consider it "most unlikely" that GRP is a mediator of vagally-induced gastrin release in ruminants as it is in other mammals (see below). In another investigation (McLeay *et al.*, 1989), infusion of bombesin into sheep was reported to "variably" stimulate gastrin release. In this latter study, the response did not occur in all animals, it was only apparent at the conclusion of the 30 minute infusion period and was associated with the infusion only of 15 pMoles/kg and not 7.5 or 30 pMoles/kg bombesin.

1.3.4.2.3 Vagal or Electrical Field Stimulation

Electrical field or vagal stimulated gastrin secretion is predominantly mediated by GRP neurons. Both caused the release of GRP and increased gastrin secretion from the stomach of rats (Nishi *et al.*, 1985) and pigs (Knuhtsen *et al.*, 1984), as did the nicotinic stimulant DMPP (Schubert *et al.*, 1985). Many similar studies have confirmed the effect of electrical field or vagal stimulation on gastrin secretion while not monitoring GRP release in humans (Feldman *et al.*, 1979), sheep (Bladin *et al.*, 1983) (although in this species the effect was very small) and cats (Uvnäs-Moberg *et al.*, 1984). Where tested, in all such studies resistance to atropine and sensitivity to hexamethonium were consistent features of the electrical field- or vagally-induced GRP and gastrin response. This implicates the involvement of noncholinergic neurons (both stimulatory GRP and inhibitory VIP) and perhaps explains why in many studies the serum gastrin response to vagal excitation was found to be marginal. The involvement of GRP has been clearly demonstrated by specific GRP-antibodies which substantially or completely abolish the gastrin-response to electrical stimulation. GRP antiserum removed 60% of the gastrin response to DMPP or electrical stimulation (Schubert *et al.*, 1985). The remainder was largely removed by atropine, which had an additive effect with GRP antiserum-induced inhibition. Atropine also abolished the decrease in somatostatin release that these treatments induced. In an almost identical earlier study, Schubert *et al.* (1982a) abolished the gastrin response with the axonal conductance blocker tetrodotoxin, thus confirming its neural dependence.

1.3.4.2.4 Adenosine

Adenosine is a nucleoside that is involved in the regulation of number of physiological functions including the inhibition of acid secretion *in vivo* (Gerber *et al.*, 1984) and parietal cell function *in vitro* (Gerber *et al.*, 1988). Schepp *et al.* (1990) demonstrated that adenosine also modulates gastrin release from canine G cells in primary culture. The response to preferential agonists and antagonists, they interpreted as showing that adenosine had a dual inhibitory and stimulatory actions on the G cells that was mediated by A_1- and A_2-type receptors respectively. G cells formed only 12% of their cultures and they acknowledge that the receptors are not, therefore, necessarily on the G cells themselves.

1.3.4.2.5 Somatostatin

Somatostatin exerts continuous tonic inhibition on the G cell. The close functional relationship between gastrin and somatostatin was appreciated through their reciprocal release patterns in rodents, pigs, dogs and humans (Bolman *et al.*, 1978; Saffouri *et al.*, 1980; Price *et al.*, 1985; Befrits *et al.*, 1990). Studies such as that of Holst *et al.* (1983) found that stimuli (e.g. acidification of the antrum) which affected the secretion of one peptide had the opposite effect on the secretion of the other. The continuous nature of the restraint somatostatin exerts on the G cell was first demonstrated by the vascular perfusion of the rat stomach with excess somatostatin antiserum (Saffouri *et al.*, 1979) which resulted in significant increases in gastrin secretion throughout the period of infusion. These results have been reconfirmed many times in the rat (Martin *et al.*, 1994) and other species (Holst *et al.*, 1992a). Saffouri *et al.* (1984a) have further suggested that the restraint exerted by basal somatostatin is optimal since VIP did not depress gastrin secretion despite increasing somatostatin secretion. Contrary to this, Shulkes & Hardy (1982) reported that infusion of somatostatin into non-pregnant adult sheep decreased the basal plasma gastrin level from 14 ±2 pM to 9 ±1 pM, a difference which is barely detectable by most radioimmunoassay (RIA) methods. In contrast to its minimal effect on basal gastrin secretion, somatostatin clearly inhibits the secretagogue-stimulated gastrin secretion described above.

Somatostatin receptors have been identified on human G cells (Gable *et al.*, 1989) and on canine G and D cells (Giraud *et al.*, 1987; Park *et al.*, 1989). This evidence further supports a paracrine action of somatostatin on the G cell and indicates that somatostatin may autoregulate D cell activity by an autocrine mechanism, at least in the dog. It is also *possible* that somatostatin has some hormonal effects on gastrointestinal cells as its level in the plasma increases in response to a meal (Lewin, 1992). However, as this probably represents effluent from the interstitial fluid, it is likely that the concentration locally where it is targeted is far greater. The plasma somatostatin may also have originated from other tissues where it is synthesised, including the pancreas and other parts of the gastrointestinal tract. A further difficulty in correlating gastrin and somatostatin release from intact animal and whole stomach preparations arises from the possible different effects on fundic and antral D cells since the greater mass of fundic tissue would cause effects on that tissue to mask effects on the antrum.

Gastrin stimulates somatostatin release from the fundic D cell (Section 1.3.3.4.1) by low

affinity binding to the less specific CCK-B receptors (DelValle *et al.*, 1993). Its direct action on the antral D cell was demonstrated by Schubert *et al.* (1991b) who abolished the GRP-stimulated somatostatin response by rat antral mucosa with the gastrin antagonist L365260. Such a reciprocal action may confuse interpretation of the effect of GRP on somatostatin secretion in intact preparations. Buchan *et al.* (1990) reported significant CCK-stimulated somatostatin secretion from human antral D cells, although gastrin was without apparent effect. Infusion of gastrin into dogs significantly increased somatostatin independently of an effect on acid (Guzman *et al.*, 1980). Similarly, intravenous pentagastrin or G-17 infusion increased serum somatostatin levels in conscious sheep after pretreatment with omeprazole (Shulkes & Read, 1991). This response was related to dose, which may explain why in a subsequent study, omeprazole did not increase plasma somatostatin although endogenous serum gastrin levels trebled (Read *et al.*, 1992). Bochna *et al.* (1987) found that, in addition to a GRP-induced neural acetylcholine release, gastrin also inhibited acetylcholine release from rat antral mucosal neurons. This, through lessening of tonic restraint on the D cell, may effectively increase somatostatin release. Collectively, the potential influences which interplay and regulate net D cell secretion may account for the sometimes variable findings in otherwise very similar studies. Since the method of preparation of the rat and human antropyloric glands and the perifusion technique described by Richelsen *et al.* (1983) eliminates local neural reflexes and the accumulation of secreted gastrin, the lack of a bombesin-induced somatostatin response in this work, as compared with others, may reflect features of an experimental system which is without neural connections.

GRP-stimulated somatostatin release, unlike GRP-stimulated gastrin secretion, is equivocal and conflicting observations have been reported. GRP (or bombesin) has been found to stimulate (rat isolated stomach, DuVal *et al.*, 1981; pig perfused antrum, Holst *et al.*, 1987a, 1993; rat isolated stomach, Guo *et al.*, 1988) to have no effect on (rat and human antropyloric glands, Richelsen *et al.*, 1983) or even to inhibit (rat *in vitro*, Nishi *et al.*, 1985) somatostatin release. These discrepancies may be related to the effects on D cells in the two parts of the stomach or the nature of the preparation. Immunization of sheep against somatostatin by Shulkes *et al.* (1994) resulted in a gastrin response to GRP infusion that was not apparent in control sheep, or in earlier work. Concurrent somatostatin release may explain the lack of a gastrin response observed by Bloom *et al.* (1983), Shulkes & Hardy (1980) and McDonald *et al.* (1988). McDonald *et al.* suggested that ruminants may differ from other mammals in their sensitivity to GRP.

It is possible that, rather than a lack of sensitivity to GRP, ruminants differ in that GRP usually stimulates somatostatin release. Some experimental findings may be confused by GRP concomitantly stimulating cholinergic neurons, which would itself inhibit somatostatin. Bochna *et al.* (1987) reported "potent" bombesin-stimulated acetylcholine release from rat antral mucosal neurons. This is consistent with decreased somatostatin secretion, although Guo *et al.* (1990) who report bombesin-induced somatostatin release by the isolated perfused rat stomach, found that the response was inhibited by atropine, implying that cholinergic pathways may have been stimulatory.

It is uncertain whether the D cells of the fundus and antrum are regulated similarly. Holst *et al.* (1993) concluded that control of porcine D cells in the two regions by GRP differed although both are stimulated by VIP and inhibited by cholinergic agonists. Fundic D cells responded to perifusion with GRP with decreased somatostatin release. Schubert *et al.* (1988b, 1991b) reported two studies in which they examined separately the secretory behaviour of segments of rat antral and fundic mucosa. In both studies, GRP stimulated somatostatin secretion from segments of either region. As DMPP stimulated and methacholine inhibited somatostatin secretion by segments from either region in a very similar fashion in the earlier study, the authors concluded that results obtained from the whole stomach would accurately reflect the behaviour of D cells in the antrum. However, in the later study, tetrodotoxin inhibited GRP-stimulated somatostatin secretion from the fundus but augmented it in the antrum. GRP-induced responses were both augmented by atropine. The secretion of somatostatin by antral segments was also inhibited by the gastrin receptor antagonist L365,260, which, in turn, augmented the gastrin response. This, the authors suggest, indicates that GRP stimulates somatostatin secretion by distinct mechanisms in both regions. In the fundus, it appears that GRP acts indirectly on D cells by activating stimulatory noncholinergic neurons, and to a lesser extent by inhibitory cholinergic neurons. In the antrum, GRP acts indirectly through gastrin and to a lesser extent by activating inhibitory neurons. These authors did not consider what effect gastrin may have had on the fundic tissue had it been present in the medium. Similarly, the discrepancy reported by Holst *et al.* (1993) may reflect gastrin-stimulated somatostatin secretion. It is also possible that gastrin may be inhibitory to the stimulatory noncholinergic neurons described by Schubert *et al.* (1991b), particularly if, as Bochna *et al.* (1987) found for cholinergic neurons, GRP is stimulatory and gastrin is inhibitory.

1.3.4.2.6 Catecholamines

Catecholamines have long been known to affect the secretion of gastrin *in vivo*: the administration of adrenaline to rats (Hsu & Cooper, 1977), dogs (Hayes *et al.*, 1972) and humans (Stadil & Rehfeld, 1973; Brandsborg *et al.*, 1975) caused an increase in serum gastrin concentration, while propranolol blocked the response to adrenaline and depressed basal gastrin levels (Kronberg *et al.*, 1974; Fournet *et al.*, 1977; Hsu & Cooper, 1977). The effect of catecholamines is largely considered to be indirect and mediated through interaction with enteric neurons: splanchnic nerve stimulation caused the release of GRP in calves (Bloom & Edwards, 1982) and GRP neurons also appear to be stimulated by isoproterenol (Short *et al.*, 1985b). Isoproterenol-stimulated gastrin and somatostatin secretion from the vascularly-perfused rat stomach were abolished by the inclusion of GRP antibodies in the perfusate (Short *et al.*, 1985b). Stimulation of the vagus increased somatostatin secretion in *pigs* with intact adrenals while it inhibited somatostatin release in adrenalectomized animals (Olesen *et al.*, 1987).

In vitro work, however, suggests that catecholamines may also have direct effects on both the G and D cell: ß-agonists (noradrenaline and/or isoproterenol) but not α-agonists (clonidine and/or phenylephrine) stimulated gastrin secretion from isolated pieces of rat antrum (Hayes *et al.*, 1978; Harty *et al.*, 1988); isoproterenol, adrenaline and noradrenaline stimulated somatostatin secretion from the vascularly perfused rat stomach (Koop *et al.*, 1980; adrenaline and isoproterenol stimulated somatostatin secretin from enriched canine fundic D cells (Yamada *et al.*, 1984); both adrenaline and terbutaline (a $ß_2$-agonist) concentration-dependently stimulated propranolol-sensitive gastrin secretion from isolated canine G cells (Buchan, 1991). A significant increase was obtained only with 10^{-6} M and greater adrenaline concentrations (1000 times circulatory levels) by Buchan (1991). Of more significance, perhaps, was the synergistic effect that adrenaline had on bombesin-stimulated gastrin secretion (Buchan, 1991).

1.3.4.2.7 Peptide Histidine Isoleucine (PHI)

PHI is a structural homologue of VIP which is found in high concentration in neuronal elements throughout the mammalian gastrointestinal tract (Yiango *et al.*, 1985). PHI inhibits gastrin secretion and synthesis by cultured rat antral cells by a mechanism that, unlike VIP, may not be via somatostatin. In the antrum, PHI neurons can be activated

experimentally by nicotinic agonists or by electrical field stimulation, or physiologically by chemical (digested protein or peptone) and mechanical (distension) stimuli (Schubert & Makhlouf, 1992).

1.3.4.2.8 Prostaglandins

Prostaglandin-E_2 stimulated somatostatin secretion and inhibited gastrin secretion by the vascularly-perfused rat stomach (Saffouri *et al.*, 1980). Schepp *et al.* (1994) consider that the effect of prostaglandins (stimulatory or inhibitory) depends on prostanoid type and experimental model and is consistent with two distinct prostaglandin receptors: enprostil (a synthetic prostaglandin analogue) selectively activated inhibitory mechanisms, while PGE_2 appeared to interact with both inhibitory and stimulatory mechanisms.

1.3.4.2.9 Gastric Inhibitory Peptide

GIP inhibited carbachol-stimulated gastrin secretion from cultured rat antral mucosa (Wolfe & Reel, 1986). As somatostatin antibodies abolished this inhibitory capacity, its mechanism of action in this study appeared to be via somatostatin. Similarly, GIP increased somatostatin and inhibited gastrin secretion by the isolated pig antrum (Holst *et al.*, 1983). Brown *et al.* (1989) reviewed the effects of GIP and concluded that it may inhibit gastrin secretion under some circumstances.

1.3.4.2.10 Gamma Amino Butyric Acid (GABA)

GABA has been identified in the enteric nervous system (Jessen, 1981). The addition of GABA to rat antral mucosal fragments stimulated gastrin and inhibited somatostatin secretion (Harty & Franklin, 1986). These effects, which were blocked by the GABA receptor antagonist bicuculline, were abolished by tetrodotoxin, were atropine-sensitive, hexamethonium-resistant and augmented by physostigmine, indicating that GABA affects antral gastrin and somatostatin release through stimulation of antral postganglionic neurons.

1.3.4.3 SUMMARY

1.. gastrin secretion is affected by physiological conditions in the stomach,

particularly those that are associated with ingestion of a meal. These include a cephalic phase, changes in pH, distension and chemical substances (particularly peptone);

2.. luminal acidification is associated with decreased gastrin secretion that appears to be mediated via somatostatin release;

3.. alkalinization results in increased gastrin secretion, probably through the removal of somatostatin restraint;

4.. somatostatin exerts continual tonic inhibition on the G cell. The tonic inhibition may be optimal;

5.. optimal stimulation of gastrin secretion by an agonist requires suppression of ambient somatostatin;

6.. vagal control of somatostatin secretion is multifactorial and involves cholinergic, adrenergic and peptidergic components, both intrinsic and extrinsic to the enteric nervous system;

7.. cholinergic agonists stimulate gastrin secretion by inhibition of somatostatin secretion. There is no conclusive evidence that they stimulate the G cell directly;

8.. nicotinic agonists stimulate noncholinergic neurons which may release GRP or VIP;

9.. GRP stimulates the G cell directly to release gastrin. Intravenous infusion of GRP increases plasma gastrin in most animals. GRP may also stimulate the D cell to secrete somatostatin. GRP appears to be the transmitter involved in vagally-stimulated gastrin secretion;

10.. VIP stimulates the D cell to secrete somatostatin;

11.. adrenergic neurons appear to influence the neural connections to the G and D cell and may also stimulate both cell types directly;

12.. gastrin appears to stimulate the D cell to secrete somatostatin, possibly by inhibiting cholinergic neurons;

13.. somatostatin may exert autocrine restraint on the D cell;

14.. a range of other physiological substances influence G cell activity: prostaglandins may stimulate or inhibit depending on prostanoid type, GIP may restrain gastrin secretion by stimulating somatostatin secretion, adenosine may have a dual action.

In sheep: abomasal pH is of major importance in the control of gastrin secretion. Gastrin secretion appears to be inhibited below pH 2.7. Alkalinization effectively

stimulates gastrin secretion. Infusion of GRP/bombesin did not increase plasma gastrin in calves or lambs, probably due to simultaneous somatostatin release as, in sheep immunised against somatostatin, GRP infusion did increase gastrin levels. Pentagastrin increased serum somatostatin levels after pretreatment with omeprazole.

1.3.4.4 TROPHIC AND OTHER EFFECTS OF GASTRIN

An acute trophic effect of gastrin on the rat gastric mucosa was clearly demonstrated by the incorporation of radioactive leucine over a 15 minute incubation period (Johnson *et al.*, 1969). A chronic trophic effect has also been demonstrated (changes in mucosal thickness and cell number) after 21 days of pentagastrin administration (Crean *et al.*, 1969). Histamine, on the other hand, in both of these studies did not cause hyperplasia. In another study, it was found that, although pentagastrin-stimulated cell proliferation was evident in the fundus and duodenum after 16 hours, a decrease had occurred in the antrum (Casteleyn *et al.*, 1977). In dogs, cell proliferation was clearly evident after 20 hours (Willems *et al.*, 1972). Endogenous hypergastrinaemia, associated with drug-induced achlorhydria, appears equally capable of stimulating ECL and other cell lines (Ryberg *et al.*, 1990; Eissele *et al.*, 1991). Hypergastrinaemia induced by acid blockade caused ECL hyperplasia in the chicken, hamster and guinea pig stomach (Axelson *et al.*, 1988). Other neuropeptides such as bombesin/GRP, VIP and substance P also have mitogenic properties. Their potential role in the control of cell proliferation has been reviewed by Zachary *et al.* (1987).

Other effects of gastrin in the sheep may include reduced rumenoreticular motility (Carr *et al.*, 1970; Ruckebusch, 1971; Nicholson, 1982), slowed omasal emptying (Onapito *et al.*, 1978) and abomasal emptying (Ruckebusch, 1971; Bell *et al.*, 1975, 1977), affects which may decrease food intake (Fox *et al.*, 1989a,b). Gastrin may increase the net secretion of water and ions in the small intestine and inhibit the absorption of electrolytes at high doses (Kimberg, 1974).

1.4 GASTRIC MUCOSAL DEFENSE

The nature of gastric function requires the gastric mucosa to maintain its integrity, while the environment the stomach creates within itself continually challenges this integrity. The mucosa is, therefore, well equipped to resist and recover from injury. Under normal

conditions, the secretion of mucus and bicarbonate, the flow of blood, the production of antioxidants and the generation of new cells adapt to protect the mucosa. Nishizaki *et al.* (1994) found that these defence mechanisms were enhanced during acid secretion. More severe challenges, such as provided by abomasal parasites, may evoke a full inflammatory reaction, cellular proliferation and an immune response (Wallace & Bell, 1992). After injury, mucosal repair may involve migration of cells into a gap and is, in part, a function of cell replacement.

1.4.1 GASTRIC MUCOSAL BARRIER

This term refers to a concept. It relates to the ability of the stomach wall to maintain a huge H^+ concentration gradient and to resist degradation by pepsin. It consists of an anatomical and histological component (**intrinsic**) and various physiological processes (**extrinsic**). The intrinsic barrier is the epithelium *per se*, the pathways that cross it, the cellular junctions and membranes, and the mechanisms for controlling intracellular pH in the epithelial cells (Powell, 1984). Tight junctions (*zonular occludens*) between cells form a continuous belt around the cell apex and are less conductive than the cells themselves. Consequently, most conductance across the epithelium is transcellular (Hirst, 1989). The *zonular occludens* is considered to form a seal between interstitium and lumen and is important for certain absorptive and secretory processes. A further property of the intrinsic barrier is the ability of the damaged epithelium to repair itself. The extrinsic barrier has pre- and post-epithelial components (Powell, 1984). The former relates to mucus and HCO_3^- secretion, the latter to blood flow and tissue acid-base balance.

1.4.1.1 MUCUS

Mucus adheres to the mucosal surface and provides a stable unstirred layer. The mucus acts as a permeability barrier to pepsin (Allen, 1981) and maintains a pH gradient between the lumen and the surface of the mucosal cells (Williams & Turnberg, 1980). In spite of not being intrinsically resistant to H^+ diffusion, it effectively provides a barrier by retaining secreted HCO_3^- (Allen, 1989). Pentagastrin infusion increased the mucus gel thickness (Nishizaki *et al.*, 1994). Whether or not the mucus layer is continuous or contains transient or permanent pores remains controversial (see Allen, 1989).

1.4.1.2 BICARBONATE SECRETION

The secretion of HCO_3^- with the mucus by the surface epithelial cells sustains the pH gradient from lumen to cell surface and thus protects the cells. Damage to the epithelium allows leakage of HCO_3^- into the lumen and results in increased HCO_3^- secretion (Flemström & Garner, 1989). On the other hand, damage may itself be incurred through the inhibition of HCO_3^- secretion. Prostaglandins are important in the stimulation of the alkaline secretion (Flemström & Garner, 1989) which, coupled with their inhibition of acid secretion, facilitates functional recovery (Takeuchi *et al.*, 1994). These authors also demonstrated the involvement of nitric oxide in the alkaline response in the rat.

1.4.1.3 MUCOSAL BLOOD FLOW

The mucosal blood flow increases rapidly after exposure of the gastric epithelium to acid or irritants, the so-called reactive hyperaemic response, which Wallace & Bell (1992) suggest removes any back-diffusing acid or toxins before they can accumulate to concentrations that are cytotoxic. These authors present evidence which suggests that injury caused by platelet-activating factor and nonsteroidal anti-inflammatory drugs is ischaemia-induced through their tendency to induce leucocyte adherence to the vascular endothelium. Two substances present in gastric mucosal neurons which increase mucosal blood flow are nitric oxide (Pique *et al.*, 1992) and CGRP (Li *et al.*, 1992). Antagonism of CGRP completely inhibited the hyperaemic response of the rat stomach to ethanol-acid-induced injury. Pentagastrin infusion has been shown to increase mucosal blood flow in rats through a histamine-dependent mechanism (Nishizaki *et al.*, 1994).

1.4.1.4 ADAPTIVE CYTOPROTECTION

This is the ability of the mucosa to increase its resistance to damage. Prostaglandins have been ascribed a cytoprotective role (Hirst, 1989) since the severity of gastric lesions after injury is greater after indomethacin administration (Lugea *et al.*, 1992; Yoneda & Taché, 1992). Gastric ulceration is correlated with low prostaglandin levels in the elderly and is greater in patients given nonsteroidal anti-inflammatory treatment (Cryer *et al.*, 1992; Shorrock & Rees, 1992).

1.4.2 CELL REPLACEMENT

The replacement of cells is continuous and normally the entire surface epithelium is renewed within three to six days in most adult mammals (Willems, 1991). Replacement of the parietal cell population, however, is relatively slow (Lipkin, 1973; Willems, 1991). Parietal cell turnover time in the mouse averages 54 days (Karam, 1993) but may be accelerated when circumstances demand. Karam & Forte (1994) found in rabbits that concomitant to increased parietal cell degeneration after five days omeprazole treatment there were more mitotic figures and preparietal cells indicating parietal cell production. After only three days recovery, the parietal cell population appeared completely normal, although it is questionable whether this could be achieved in this time solely by cell replacement. Lehy & Williams (1976) deduced that the turnover time of antral G cells in the mouse is between two and three months.

Growth factors which regulate normal mucosal growth and initiate adaptive responses to injury include EGF, TGF-α, fibroblast derived growth factor (bFGF), platelet-derived growth factor and insulin-like growth factors (IGF-1, IGF-2, insulin) (Harty & Ren, 1992). The signals these provide are transduced to the nucleus of stem cells and promote genetic processes that induce their division, multiplication and differentiation. The role of polyamines as the putative 'signal' has been reviewed by McCormack & Johnson (1991) who suggest that trophic hormones, such as gastrin (see also Johnson, 1987), and growth factors exert their influence by regulating polyamine synthesis. It is interesting, therefore, that Johnson & Gutherie (1980) found that secretin inhibited the effect of gastrin but not the effect of EGF on mucosal growth.

Following natural or experimental injury to the gastric mucosa of humans or rats, an increase in EGF, TGF-α and their receptors have been reported (Wright *et al.*, 1990; Tarnawski *et al.*, 1992; Polk *et al.*, 1992). bFGF is believed to be released from inflammatory cells, such as mast cells and macrophages, which migrate into sites of injury following release of chemotactic substances by dying cells (Karam & Forte, 1994). bFGF promotes fibroblast proliferation and the formation of new connective tissue. Tryptase, another mast cell granule constituent, is also a fibroblast mitogen (Ruoss *et al.*, 1991). It is not clear whether prostaglandins promote cell proliferation or rather appear to do so through decreased cell loss (Goodlad *et al.*, 1991).

1.4.3 INFLAMMATION

It is now widely accepted that inflammatory and immune processes affect the physiological function of the gastrointestinal tract and that the separation of physiology and immunology is entirely artificial. Fiocchi *et al.* (1994) describe the mucosa of the normal gut as being in a permanent state of "physiologic intestinal inflammation". It has been claimed that inflammation and the immune response to gastrointestinal nematodes may account for much of the productivity losses associated with these parasites (Cobon & Woodrow, 1991).

Inflammation is the most common response of organs to insults. It has three main components: haemodynamic changes, increased capillary permeability and leucocytic exudation. The mediators involved in an inflammatory reaction are derived from a variety of cell types including resident cells, such as mast cells and fibroblasts, and recruited cells such as eosinophils, neutrophils and macrophages and include cytokine (interleukin series) and noncytokine (prostaglandins, leukotrienes, platelet-activating factor, growth factors) products.

1.4.3.1 CYTOKINES

Cytokines are immunoregulatory and proinflammatory products secreted by a variety of cell types that are important mediators of gut inflammation and the local and systemic manifestations associated with gut injury (Fiocchi *et al.*, 1994; Herfarth & Sartor, 1994). Some, such as IL-1, clearly interfere with normal physiological functions including gastric acid secretion (Section 1.3.3.4.5). IL-1 is produced by monocytes, macrophages, platelets, fibroblasts, endothelial cells and enterocytes, and elicits local inflammatory reactions such as oedema, T and B cell activation, neutrophil infiltration and further cytokine production (Wershil, 1992; Herfarth & Sartor, 1994). It may also affect gastrointestinal motility and food intake: IL-1ß suppressed acetylcholine and adrenaline release in *in vitro* preparations of rat jejunal longitudinal muscle-myenteric plexuses (Main *et al.*, 1993; Hurst & Collins, 1993) and mediated anorexia and weight loss in rats with experimentally-induced colitis (McHugh *et al.*, 1992, 1993).

A number of cytokines are produced by activated T-helper (Th) cells. These lymphokines include IL-2, -3, -4, -5, -6, etc. and interferon-Y, each produced variously

by either the Th1 or Th2 subsets (Herfarth & Sartor, 1994). Activation of the Th2 subset appears to be associated with gastrointestinal helminths (Svetic *et al.*, 1993; Finkelmann *et al.*, 1994). There is some evidence that IL-6 (pro-inflammatory) and IL-8 (leucocyte attraction) are also produced by epithelial cells (Fiocchi *et al.*, 1994). Mast cells, although not necessarily derived from the mucosa, appear to produce tumour necrosis factor-α (TNF-α), IL-3, -4, -5 and -6, and four members of the macrophage inflammatory protein-1 gene family. Similarly, eosinophils from a number of species have been found to contain a variety of multifunctional cytokines, and may be a potential source of IL-3, IL-5, IL-1α, IL-6, TGF-α, TGF-ß and TNF-α (Wershil, 1992). Lamas *et al.* (1991) found that TNF-α could enhance the production of nitric oxide by bovine endothelial cells, some of the possible actions of which have been mentioned above.

MacDonald and Spencer (1988) found that activation of mucosal T cells in human small intestine *in vitro* produces rapid and profound crypt epithelial cell hyperplasia.

1.4.3.2 EOSINOPHILS

Eosinophils are effector cells of immunity and hypersensitivity reactions and are a predominant feature of gastrointestinal inflammation, particularly that associated with helminth infection (Butterworth, 1984). Eosinophils, like neutrophils, have secretory and phagocytic properties and possess receptors for immunoglobulins and complement components (Kay, 1985). They are filled with granules which contain a variety of substances such as major basic protein, eosinophil cationic protein, peroxidase, histaminase and eosinophil neurotoxin. On the one hand these substances are believed to be destructive to helminth larvae, but they are also capable of considerable tissue damage (Kay, 1985). It has been suggested that eosinophil-derived products may moderate or regulate mast cell-induced inflammatory change (Butterworth, 1984) although some of these products actually increase mast cell activity (Rothwell, 1989). Without complement or antibody to promote the release through degranulation of these substances, eosinophils are ineffective (Glauert *et al.*, 1978).

1.4.3.3 MAST CELLS

Mast cells play an important role in the pathogenesis of the inflammatory process: agents that can cause mast cell degranulation (e.g. sodium taurocholate) can induce

gastric mucosal injury (Rees *et al.*, 1978); mast cell stabilizers (e.g. sodium cromoglycate) can reduce the severity of gastric injury (Goossens *et al.*, 1987) and mast cell-deficient mice are significantly more resistant to gastric injury induced by ethanol than normal litter mates (Galli *et al.*, 1988). Similarly, in rats with a mastocytosis induced by *Nippostrongylus brasiliensis* infection, gastric mucosal injury to ethanol was augmented by the addition of parasite-derived antigen in sensitised rats (Rioux & Wallace, 1994). This effect was abolished by pretreatment with either dexamethasone (an anti-inflammatory) or a leukotriene D_4-receptor antagonist, suggesting that it is mast cell activation through a leukotriene-dependent mechanism that increases susceptibility to injury. Mast cells, however, are also capable of liberating many substances with protective effects such as IL-1 and nitric oxide. Fibroblast proliferation is promoted by tryptase, a mast cell protease (Ruoss *et al.*, 1991). Thus, mast cells release mediators that can increase the susceptibility of the mucosa to injury, they may participate in the regulation of gastric acid secretion (Stanovnik & Logonder-Mlinsek, 1993; also see Section 1.3.3.3.1) and they may regulate various protective functions such as adaptive mucosal blood flow responses when the mucosa is exposed to an irritant.

1.5 OSTERTAGIASIS

Clinical signs of ostertagiasis in sheep include anorexia, diarrhoea and possible weight loss. Most infections are subclinical and cause production losses: wool growth, weight gain and lambing percentages may all be reduced (Soulsby, 1965). Contributing to these effects are reduced appetite, disturbed protein metabolism and impaired protein utilization (Sykes & Coop, 1977; Parkins *et al.*, 1982a,b). In turn, these metabolic disturbances may be associated with secretory lesions, manifest as hyperpepsinogenaemia, hypergastrinaemia and hypochlorhydria. At elevated abomasal pH, pepsinogen is not converted to pepsin (Jennings *et al.*, 1966), which reduces the ability of the animal to digest protein and allows bacteria to proliferate (Nicholls *et al.*, 1987). Hypergastrinaemia may contribute to inappetence (Fox *et al.*, 1989a,b) and processes causing diarrhoea (Kimberg, 1974).

Histopathological changes develop in the abomasal mucosa. An inflammatory reaction occurs, immune processes are activated and physical damage is caused by the developing larvae. Associated with these, there is disruption of the intrinsic mucosal barrier and increased permeability. Consequently, Na^+ levels increase in the lumen

(McLeay *et al.*, 1973), plasma is lost (Holmes & MacLean, 1971) and the unactivated pepsinogen can diffuse back into the blood (Jennings *et al.*, 1966).

A number of studies have followed the development of the secretory lesions after experimental infection of sheep with *O. circumcincta* or cattle with *O. ostertagi*. The initial development of the secretory lesions occurs while the larvae develop into adult worms, a process accompanied by morphological damage. This correlation has resulted in a causal association being widely accepted between larval development and *Ostertagia*-associated lesions. The transfer of adult *Ostertagia* directly into the abomasa of sheep (Anderson *et al.*, 1985) and cattle (McKellar *et al.*, 1986, 1987) has shown that they too can produce these disturbances, indicating that larval development can only partially account for the lesions and leading to the suggestion that parasite-derived factors may be important in the disease (McKellar *et al.*, 1987).

1.5.1 LIFE CYCLE OF *O. CIRCUMCINCTA*

The life cycle of *O. circumcincta* is direct. Fertilized eggs are passed in the faeces and develop to infective (ensheathed) third stage larvae in the environment (Keith *et al.*, 1990). After oral infection, the larvae are stimulated by conditions within the rumen (Eh, pH and the CO_2/HCO_3^- equilibrium) to complete the second ecdysis and exsheath (Sommerville, 1957; Rogers & Sommerville, 1960, 1963, 1968).

On arrival in the abomasum, the larvae invade the gastric glands and pits. Four days after infection, most larvae are found within the glands and have completed the third ecdysis (Armour *et al.*, 1966; Denham, 1969). Subsequently, larvae may either emerge from the glands as early fourth stage larvae (from Day 4 to 5), continue to grow in the mucosa to late fourth stage or immature adults before emerging, or enter a hypobiotic state (Sommerville, 1954, 1963). After a single infection, most larvae have developed into immature adults and emerged from the glands on to the surface of the abomasal mucosa by about Day 12. In cattle, development of *O. ostertagi* to a similar stage usually takes 21 days (Armour & Ogbourne, 1982). The earliest that immature *O. circumcincta* adults occur in the "lumen" appears to be on Day 8 (Denham, 1969). Gravid females have been observed on Day 12 (Denham, 1969) and patency on Day 13 (McLeay *et al.*, 1973).

The rate and path of larval development appears to be influenced by strain (Armour *et al.*, 1967a,b), infective dose (Dunsmore, 1960; Elliott, 1974a,b; Durham & Elliott, 1976) and immune status of the sheep (Dunsmore, 1961). Thus, Dunsmore (1960) found that infection of sheep with 1,000 *O. circumcincta* produced only 1-2% larval inhibition while infection with 100,000 produced 20% inhibition (Armour *et al.*, 1966). Elliott (1974), who infected four groups of four parasite-naive sheep with 2, 8, 32 or 128 thousand larvae, found only adults in animals dosed with 2,000 while immatures (< 5 mm) predominated after 28 days in those given the largest dose, and further, the percentage recovery decreased with increasing dose. This, and other studies, indicate that development is slowed and either fewer worms establish or there is greater elimination with increasing dose. A substantial loss of worms was reported by Armour *et al.* (1966) 16 days after infection of sheep with 100,000 larvae. Density-dependent population dynamics such as these suggest that an increased infective dose will not necessarily produce a greater change in pathology, although it may alter the pathogenesis.

Density-dependent population dynamics as shown by *Ostertagia* suggest that parasites are in some way provided with information about the current population size. Removal of adult *O. circumcincta* from sheep resulted in the resumed development of inhibited larvae (Dunsmore, 1963). Egg production in this species is also affected by density and, therefore, does not accurately reflect the number of worms present (Anderson *et al.*, 1965; Barger, 1986). The signals by which such information is provided are not known. Nematode population biology, expulsion and hypobiosis have been reviewed by Barger (1986), Rothwell (1989) and Michel (1974) respectively.

1.5.2 ABOMASAL MORPHOLOGY

The morphological changes in the abomasum of sheep infected with *O. circumcincta* are well documented (Sommerville, 1954, 1956; Armour *et al.*, 1966; Durham & Elliott, 1976; Elliott & Durham, 1976; Coop *et al.*, 1977; Anderson *et al.*, 1988). Similar changes have been reported in cattle following infection with the closely related species *O. ostertagi* (Anderson *et al.*, 1965; Jennings *et al.*, 1966; Ritchie *et al.*, 1966; Murray *et al.*, 1970), in sheep with *Haemonchus contortus* (Charleston, 1965; Hunter & McKenzie, 1982) and in pigs with *Hyostrongylus rubidus* (Davidson *et al.*, 1968).

The histopathological effects of a single experimental dose of larvae on parasite-naive sheep have been most comprehensively described by Armour *et al.* (1966). In this, as in a number of other studies, infected animals were serially slaughtered and the chronological development of the changes observed. Armour *et al.* considered most of the structural and histopathological changes to be associated with larval activity, and in particular their invasion of, and emergence from, the mucosa. Armour (1970) also considered parasite emergence to be the major pathological event in bovine ostertagiasis. Consistent with this, the larval infection of calves caused marked cellular changes in the mucosa (Anderson *et al.*, 1965; Murray *et al.*, 1970) while the direct abomasal transfer of adult *O. ostertagi* provoked minimal histopathological effects (McKellar *et al.*, 1987). Armour *et al.* (1966) observed that the morphological lesions regressed gradually from 35 days after infection. Regression also occurs rapidly after anthelmintic treatment of infected, naive or immune sheep (Elliott & Durham, 1976).

Armour *et al.* (1966) observed lesions in and around infected glands four days after infection of sheep with 100,000 *O. circumcincta* larvae, but since the sheep were first killed on Day 4, changes may have developed prior to this. The invaded glands were characterised by small epithelial protrusions (nodules) and local loss of distinction between the epithelium and *lamina propria.* Effects evident in adjacent glands, which were elongated (stretched), had abnormally-appearing parietal cells, frequent mitotic figures and apparent basophilia were considered by Armour *et al.* to indicate hyperplasia and a lack of differentiation. Neutrophils and eosinophils were seen migrating from the *lamina propria* toward the infected gland suggesting an inflammatory response, whereas there are almost no eosinophils in the parasite-free abomasum (Charleston, 1965). By Day 8, inflammation had become more apparent (increased numbers of granular leucocytes and oedema) and an immune response had begun (lympoblast and plasma cell activity was present in all three zones of the mucosa). There was variation in gland disturbance: some appeared much as they had on Day 4, others were markedly distended, while from others the larvae had emerged. The spectrum of lesions observed reflects the variable development of larvae (Section 1.5.1).

The glands from which larvae had emerged by Day 8 were full of eosinophils and neutrophils and their epithelia were characterised by taller, clear mucus-secreting cells (Armour *et al.*, 1966). Surrounding glands also showed further cellular changes described as hyperplasia and metaplasia. The proliferative changes around the infected

gland produced nodules of umbilicated appearance. The early development of these nodules was apparent from Day 4. Nodules are generally considered to develop where there are developing larvae, although McLeay *et al.* (1973) reported the presence of 5-10 discrete white mucosal plaques (presumably nodules) in isolated and uninfected fundic pouches prepared prior to infection of parasite-naive sheep. This suggests that either nodules may be present without the presence of larvae, the sheep were contaminated, or larvae migrated via the circulation to the isolated pouch. Occasional larvae have been seen penetrating the *lamina propria* (Armour *et al.*, 1966) and the intravenous inoculation with *O. ostertagi* has produced patent infections in calves (Williams *et al.*, 1974). It is unfortunate that McLeay *et al.* did not examine the plaques for parasites. Where larvae had crossed the mucosa and penetrated the *lamina propria*, mesenchymal giant cells and eosinophil infiltration were apparent (Armour *et al.*, 1966).

After 16 days, many nodules were still present while cytolysis was evident where parasites had emerged, resulting in a slight mucosal depression. After 21 days, more larvae had emerged and more glands were undergoing or had undergone some cytolysis and the mucosa continued grossly to resemble 'Morocco-leather' (a term used to describe the appearance of lesions that have coalesced). Surface epithelium was sloughing off at this time (Day 21), there was an abundance of eosinophils, apparent hyperplasia and a lack of cellular differentiation. The immune response was reflected by functional plasma cells, developing germinal centres, infiltration of globular leucocytes and lymphocyte activity in local lymph nodes.

On Day 35, the mucosa had began to return to normal and all three layers of the epithelium were easily differentiated. As more and more parasites emerged, the lesions progressively regressed and the state of mucosal repair reflected the number of worms still in the mucosa. The mucosa was most severely disturbed from Day 8 to 16, which Armour *et al.* (1966) attributed to maximal emergence at this time. In cattle, the lesions described by Ritchie *et al.* (1966) and Murray *et al.* (1970) were essentially the same, except that in this species development occurs more slowly.

The distribution of lesions within the mucosa reflects the distribution of developing larvae, however, the distribution does not appear to be consistent. In one study, some sheep developed lesions primarily in the body region while other sheep developed lesions primarily in the pylorus (Durham & Elliot, 1975). Armour *et al.* (1966) found

lesions in the fundus developed earlier and were prominent before those in the pyloric region, while Sommerville (1954) reported the opposite.

The stimuli that evoke the cellular changes described above are unknown. Murray *et al.* (1970) suggested that stretching of the glands by growing larvae may stimulate cell division to maintain epithelial continuity. Factors such as EGF, bFGF and prostaglandins (Section 1.4) that are implicated in the normal and abnormal rejuvenation and recovery of gastric mucosa must be considered likely candidates. Increased levels of many inflammatory mediators have been identified in parasitised tissues. After primary infection of the rat with *N. brasiliensis*, prostaglandin E_2 increased 10-fold in intestinal tissues (Dineen & Kelly, 1976). Marked mastocytosis followed *Ostertagia* infection (Murray *et al.*, 1970). In addition to tryptase and bFGF, degranulation of mast cells releases histamine which is chemotactic to eosinophils (Clark *et al.*, 1975). Besides having an anti-parasitic function (Butterworth, 1984), eosinophils produce enzymes that inactivate the mediators of inflammation released by mast cells, such as histaminases, which are present in both eosinophils and neutrophils (Zeiger *et al.*, 1976).

Morphological changes in the gastrointestinal system of rats and mice infected with *N. brasiliensis* or *Trichinella spiralis* appear to be T-cell dependent (Ferguson & Jarrett, 1975; Manson-Smith *et al.*, 1979). Villus atrophy and crypt hyperplasia were delayed, reduced or absent from thymectomized rats or mice infected with these nematodes, while the changes were enhanced by adoptive transfer of immune mesenteric lymph node cells. Chemicals released from lymphoreticular cells may influence the replication and/or functioning of various gastric cell lines in a manner similar to their effects on lymphocytic and haemopoietic cells. The possibility that cytokinetic mediators released locally in the mucosa as part of the inflammatory and immune responses are capable of influencing epithelial cell proliferation and development during ostertagiasis, either directly or via interaction with mesenchymal cells in the *lamina propria*, justifies consideration and further investigation.

1.5.3 ABOMASAL FUNCTION

The presence of *Ostertagia* in the abomasum of sheep and cattle inhibits acid secretion (Armour *et al.*, 1966; Jennings *et al.*, 1966; McLeay *et al.*, 1973; Anderson *et al.*, 1976a,b, 1981, 1985; Titchen & Anderson, 1977) and causes the loss of albumin into

the gut (Holmes & McLean, 1971) and increased luminal sodium ions (McLeay *et al.*, 1973). Associated haematological and biochemical changes in the blood which reflect gastric dysfunction are well documented in sheep infected with *O. circumcincta* (Todd *et al.*, 1951; Horak & Clark, 1964; Holmes & McLean, 1971; McLeay *et al.*, 1973; Anderson *et al.*, 1976a,b; Titchen & Anderson, 1977; Anderson *et al.*, 1981, 1988). These include hypoalbuminaemia, greatly increased protein turnover, hypergastrinaemia, hyperpepsinogenaemia, haemoconcentration and hypophosphataemia. Similar changes occur in cattle infected with *O. ostertagi* (Anderson *et al.*, 1965; Fox *et al.*, 1987; Fox, 1993).

1.5.3.1 HYPERPEPSINOGENAEMIA

Hyperpepsinogenaemia is a characteristic feature of ostertagiasis. It has been reported in sheep infected with *O. circumcincta* (McLeay *et al.*, 1973; Coop *et al.*, 1977), in cattle with *O. ostertagi* (Anderson *et al.*, 1965; Murray *et al.*, 1970; Fox *et al.*, 1987; Taylor *et al.*, 1989; Conner *et al.*, 1989) and in deer with mixed ostertagid infections (Connan, 1991). Indeed, plasma pepsinogen is widely used and recommended as an aid to diagnosis of ostertagiasis in cattle (Selman *et al.*, 1977), although some workers doubt its usefulness (Mylrea & Hotson, 1969). Elevated plasma pepsinogen levels have also been reported in sheep infected with *H. contortus* (Mapes & Coop, 1970). Whereas the "normal" plasma concentration in parasite-free sheep is below 500 mU/L (Holmes & McLean, 1971; Anderson, 1972; Coop *et al.*, 1977), animals in the field had values ranging from 100 to 2500 mU/L (Anderson, 1973). The values reported for parasite-naive sheep by Anderson *et al.* (1988) (>1500 mU/ml) are outside the generally accepted range. In artificially-infected, young, worm-free calves, the changes in serum pepsinogen concentration correlated well with the number of *O. ostertagi* larvae given (Mylrea & Hotson, 1969). Preinfection levels were 300 mU tyrosine/L and these rose to 5000 mU/L by Day 28 in some cases.

The maximum increase in plasma pepsinogen in experimentally-infected cattle occurs between about Day 19 and Day 27, when larvae would be expected to emerge from the glands (Jennings *et al.*, 1966; Taylor *et al.*, 1989). In sheep, Armour *et al.* (1966) found plasma pepsinogen had increased by the time the first samples were taken seven days after infection. McLeay *et al.* (1973) determined the plasma pepsinogen every four days and found that levels were raised on Day 4 and that these, as also found by Armour *et*

al., reached a maximum on Day 16 before declining. During the infection, pepsinogen levels increased up to ten times pre-infection concentrations.

The increase in plasma pepsinogen has generally been attributed to a "leak lesion": the back-diffusion of pepsinogen across the impaired gastric mucosal barrier that is exacerbated by pepsinogen levels that are increased by the failure of conversion to pepsin in the abomasum at elevated pH (Jennings *et al.*, 1966; Murray *et al.*, 1970). Pepsinogen increased by two- to three-fold in the blood of calves treated with omeprazole (Fox *et al.*, 1989a). Although they suggest that this effect may be mediated through gastrin and the direct release of pepsinogen into the blood, this remains purely speculative. The "leaking" of pepsinogen into the blood coincides with loss of albumin into the gut (Holmes & McLean, 1971) and increased luminal sodium ions (McLeay *et al.*, 1973). The concept of a local permeability-reabsorptive lesion is supported by the observation of McLeay *et al.* that increased luminal sodium was not apparent in isolated worm-free pouches.

The lesion occurs also in subsequent infections since the pattern of plasma pepsinogen levels in cattle during the grazing season closely follow the availability of infective larvae on the pasture (Armour *et al.*, 1979). In the first season, the lesion was attributed to mucosal damage, while in the second season, when the cattle had developed some immunity (low egg counts etc.), the rise was attributed to an allergic-type hypersensitivity response, itself associated with increased mucosal permeability. A far more immediate response was found by Pitt *et al.* (1988) in cows infected with *O. ostertagi* than in a similar study on calves (Fox *et al.*, 1987). Similarly, Reid & Armour (1975) found that serum pepsinogen levels were consistently higher in sheep than in lambs and they suggest that this may reflect greater mucosal damage in older animals due to a hypersensitive state. McKellar & Bogan (1987) infected sheep with *O. circumcincta*, some of which were also given the mast cell stabiliser sodium cromoglycate. While the stabiliser had no effect on uninfected controls, its administration to infected sheep was associated with an increased and more immediate pepsinogen response. While speculative, this work does raise interesting questions about the hypersensitive reaction as the basis of elevated plasma pepsinogen in immune sheep and the acceptance of the leak lesion.

The transfer of adult *O. circumcincta* directly into the abomasum of sheep by Anderson

et al. (1985) increased plasma pepsinogen levels within 28 to 48 hours of infection. They suggest that, as plasma pepsinogen increased without the introduction of larvae, extensive invasion of the mucosa by parasites is not the only explanation for the lesion, a suggestion supported by McKellar *et al.* (1986, 1987). However, those sheep given a more purely adult worm population took twice as long to reach maximum pepsinogen levels and these levels were much less than in those animals given the "mixed" population. Indeed, relative to the preinfection values of the "mixed" group it is difficult to uphold their conclusion that any rise occurred in the "mainly adult" group. McKellar *et al.* (1986, 1987) reported an immediate increase in plasma pepsinogen after the transfer of adult *O. ostertagi* into calves. Plasma pepsinogen levels doubled within the first 24 hours after transfer and remained elevated until slaughter 4 to 21 days later. The authors suggest that the sudden onset of hyperpepsinogenaemia may indicate that: (a) adult parasites damage the mucosa more than is generally realised; (b) adult parasites (or their products) may stimulate the production and secretion of pepsinogen by chief cells; (c) pepsinogen may be secreted directly into the blood; or (d) gastrin may somehow be involved.

Stringfellow and Madden (1979) suggested that pepsinogen may be secreted directly into the blood as they failed to demonstrate an increase in mucosal permeability in calves infected with *O. ostertagi* using horse radish peroxidase. Secretions prepared from *Ostertagia* species by McKellar *et al.* (1990a) stimulated the secretion of pepsinogen from dispersed bovine and ovine gastric glands and atropine reduced the response of the secretagogues. Although the increase in pepsinogen secretion from the ovine glands was statistically significant, it was only 5% in magnitude, so that the conclusion that this response indicates "that the secretions are extremely potent", is questionable. Also, the lack of a pepsinogen response to "extracts" prepared from the same worm pool and to living adults or larvae is noteworthy. McKellar considers that, as the response was partially blocked by atropine (5% reduced to 2%), the active principal in the parasite secretions is likely to be a parasympathomimetic agent. The same group had previously reported (Mostofa & McKellar, 1989) that atropine lowered the plasma pepsinogen values of sheep infected with larval and adult *O. circumcincta*, although most of these decreases were statistically insignificant. This they considered to demonstrate the production by the parasites of excretory/secretory products which have potent muscarinic actions and which directly stimulate the production of pepsinogen by the abomasum and its secretion into the plasma. However, the effect of atropine on plasma pepsinogen they reported appears to correlate more with absolute plasma levels than any particular

infection regime.

1.5.3.2 HYPOCHLORHYDRIA

Primary infection of sheep with *O. circumcincta* is accompanied by a marked rise in abomasal pH after about one week (Armour *et al.*, 1966; McLeay *et al.*, 1973; Anderson *et al.*, 1976a,b, 1981, 1985; Titchen & Anderson, 1977). Post-infective hypochlorhydria has also been observed following experimental infection of sheep with *H. contortus* (Anderson *et al.*, 1965; Christie, 1970; Nicholls *et al.*, 1987, 1988) and cattle with *O. ostertagi* (Jennings *et al.*, 1966). pH increases sharply after about 20 days of infection of cattle by *O. ostertagi*. The later development of hypochlorhydria in cattle compared with sheep is considered to reflect the longer development time of *O. ostertagi* (Armour, 1970) and is consistent with the later development of morphological and other lesions. Following infection of sheep with *H. contortus*, hypochlorhydria developed within two to four days (Dakkak *et al.*, 1982).

After infection, abomasal pH may rise relatively rapidly (to levels as high as pH 7.0) after which it falls gradually over the course of infection. The pH may remain disturbed for some time. Sykes & Coop (1977) found that after daily dosing with *O. circumcincta* the abomasal pH in five of eight sheep was still above 4.7 after 14 weeks. Reacidification of the abomasum occurs relatively rapidly, however, if the worms are removed. Normal abomasal pH values were re-established 54 to 172 hours after anthelmintic purging of *O. circumcincta* from sheep (Anderson *et al.*, 1976b). Such results suggest that the presence of the parasite *per se* is essential for the maintenance of hypochlorhydria. A similarly quick, although naturally occurring, return to normal pH has been observed in cattle following the loss of *O. ostertagi* (Jennings *et al.*, 1966). On reinfection of the purged sheep, the pH never rose to the same level and in two of four animals did not rise above 3.5 (Anderson *et al.*, 1976b).

Precisely when the abomasal pH rises after infection is unclear. This uncertainty is due to inadequate sampling frequency in some studies and to an imprecise definition of a raised pH. Armour *et al.* (1966) found the abomasal pH to have risen by Day 8, but no measurements were taken between Days 4 and 8. McLeay *et al.* (1973), who monitored post-infection pH changes more closely (before and after feeding for 24 days), determined that levels were raised from Day 11 to 13 onward. It remained elevated

(above 5.0) over this period in two of the sheep but had declined to preinfection levels by Day 15 in a third. There was only a 3.6% recovery of worms from the third sheep, which may account for the less prolonged period of hypochlorhydria. Anderson *et al.* (1976b), using the same collection regime, reported marked hypochlorhydria from Day 12 after primary infection. It is not, however, possible from either the studies of McLeay *et al.* or Anderson *et al.* to determine precisely when the lesion developed and neither defines what constitutes a raised pH. Certainly, Anderson *et al.* (1976b) consider a pH of 3.5 to be within the normal range. From their data, it appears that the pH in sheep 1 rose steadily from, and was raised by, Day 4. Indirect inference is made by Titchen and other authors that pH values below 4.0 are not raised, based on the relationship between stomach pH and basal plasma gastrin reported in dogs by Becker *et al.* (1973), which may not be identical to that in sheep.

Sheep infected with adult *O. circumcincta* by direct transfer into their abomasa developed hypochlorhydria five to seven days later (Anderson *et al.*, 1985). This timing is again subject to the interpretation of a normal pH. At least two of the three animals in one group had an abomasal pH above 4.0 on the third to fourth day, values usually considered well outside the normal range for sheep. McKellar *et al.* (1986, 1987) similarly transferred *O. ostertagi* into calves but reported no change in pH which they suggest may indicate that the host/parasite relationship may differ in cattle and sheep or that Anderson *et al.* unwittingly transferred larvae with the adult worms. The coincidence between marked morphological changes and a significant elevation of pH in the abomasum and the emergence of larvae have been causally linked. The later development of hypochlorhydria in cattle infected with *O. ostertagi* is consistent with the slower development of this parasite in cattle compared with *O. circumcincta* in sheep (Jennings *et al.*, 1966; Ritchie *et al.*, 1966; Armour, 1970). The transfer of adult worms by Anderson *et al.* (1985) suggests that it may not be the emergence of larvae but, at least in part, the appearance of adults that is important for the elevation in pH.

The histological appearance of the parietal cells changes with infection and this has traditionally been considered to reflect the loss of function (Ross, 1963; Jennings *et al.*, 1966; Armour *et al.*, 1966; McLeay *et al.*, 1973; Coop *et al.*, 1977; Soulsby, 1982). While the appearance of the parietal cells may reflect their inhibition, Murray *et al.* (1970) suggested the opposite, that the hypochlorhydria reflects the change in the mucosal cell population to an undifferentiated non-functional one. McLeay *et al.* (1973)

described the appearance of abnormal parietal cells in the infected abomasum as "similar to cells of gastric mucosa subjected to agents suppressing secretion". Certainly the loss of function is temporary and is restored quickly following the removal of the worm population (Anderson *et al.*, 1976b), which may indicate that it is not entirely dependent on the production of new cells.

The process or processes involved in the development of post-infective hypochlorhydria are unknown. McLeay *et al.* (1973) demonstrated that the reduced secretion of acid during infection with *O. circumcincta* was not due to a lack of parietal cell stimulation: while the pH of the parasitised abomasum was raised, separated pouches, themselves free of parasites, were hypersecreting acid. The hypersecretion of acid by the pouches was attributed to concomitant hypergastrinaemia as it was ablated following antrectomy (Anderson *et al.*, 1981) and occurred despite a reduction in food intake (McLeay *et al.*, 1973) which in normal sheep reduces secretion from such pouches (McLeay & Titchen, 1970). The inhibition of acid secretion was localised to those areas in contact with the parasites or their secretions (McLeay *et al.*, 1973), which ruled out the direct action of a systemically-based mechanism involving circulating factors. If a systemic response were involved, it presumably must be effected locally, perhaps by inflammatory and immune processes stimulated by mucosal mast cell degranulation (Rothwell, 1989).

The parasites may provoke the local response in the tissues by their physical presence, by producing chemical substances which act directly on the host cells or by stimulating the host's inflammatory and immune processes. The integrity of the gastric mucosal barrier is essential for maintaining the polar orientation of the parietal cell and is critical to its function. If the parasite affects acid secretion by disrupting the gastric mucosal barrier locally, this may partially explain the localised nature of the lesion. Reduced establishment of the parasite on subsequent reinfection may compromise less the mucosal integrity so that sufficient function is retained to acidify the abomasal contents.

Despite its local nature, the inhibition or reduction of acid secretion may reflect processes initiated by the host in response to the parasite. Substances such as prostaglandins, IL-1, EGF and TGF-α have the potential to inhibit acid secretion although this may not be their primary function (Section 1.3.3.4). Injury to the mucosa associated with larval development is likely to promote the release of such substances. The importance of other mediators, primarily associated with the immune system, is doubtful as Anderson *et al.* (1976b) found that the pH disturbance was reduced on

reinfection of sheep with *O. circumcincta*. This may, in part, reflect the efficiency of processes that develop to prevent the establishment of further parasites.

Acid secretion may be inhibited by parasite-derived factors, as suggested by McLeay *et al.*(1973) and Titchen & Anderson, (1977). Such an aetiology would be consistent with a lesser response after previous exposure if the host developed immunity to these secretions. Klesius (1993) suggested that immunity to *Ostertagia* may be dependent on the development of immunity to immunomodulatory substances produced by the parasite. The antigenic properties of trichostrongylid excretory-secretory products are the subject of much current research (McGillivery *et al.*, 1989, 1990; Savin *et al.*, 1990; Dopheide *et al.*, 1991; Frenkel *et al.*, 1992). Eiler *et al.* (1981) reported that extracts prepared from *O. ostertagi* when administered intramuscularly to rats reduced the secretion of gastric acid. Although equivalent administration of saline was without effect, the response does not indicate that the extract need have done more than stimulate an increase in systemic levels of IL-1 or other inflammatory mediators. McKellar *et al.* (1990b), using the same methods which gave a 5% stimulation of pepsinogen secretion from isolated bovine abomasal glands, were unable to stimulate acid secretion (as determined by aminopyrine accumulation) with presumed secretory/excretory products of *O. ostertagi*. The lack of a response is not entirely unexpected as freshly prepared glands were used.

The production of a specific acid inhibitory substance by the parasite suggests that elevation of the abomasal pH may be of direct benefit to the parasite. There are several indications that this may be the case. The egg laying performance of *H. contortus* is optimal between pH 4.0 and 4.5. Eiler *et al.* (1981) studied the *in vitro* survival of *O. ostertagi* and found that worm survival increased as pH was raised toward neutrality. Blanchard & Wescott (1985) suggested that *O. circumcincta* may elevate abomasal pH so as to make conditions less favourable for the establishment of *H. contortus* but, since *H. contortus* infection itself raises pH, this seems unlikely. Mapes & Coop (1973) found that the percentage of worms that were fourth stage larvae was positively related to abomasal pH and its disturbance may, therefore, influence population dynamics. Cimetidine administration to sheep raises the abomasal pH and reduces the adult worm population (Hall & Oddy, 1984), although these may not necessarily be causally related.

1.5.3.3 HYPERGASTRINAEMIA

Hypergastrinaemia occurs in sheep experimentally infected with *O. circumcincta* (Anderson *et al.*, 1976a,b, 1981, 1985, 1988; Titchen & Anderson, 1977; Blanchard & Wescott, 1985) or with *H. contortus* (Blanchard & Wescott, 1985; Nicholls *et al.*, 1985, 1988) and in cattle infected with *O. ostertagi* (Fox *et al.*, 1987, 1988a,b, 1993; Snider *et al.*, 1988a). Plasma gastrin also increases in rats infected with *T. spiralis* (Castro *et al.*, 1976) and pigs infected with *Strongyloides ransoni* (Enigk & Dey-Hazra, 1978). Hypergastrinaemia associated with small intestinal parasites may be of small intestinal not gastric origin. Whereas "normal" plasma gastrin levels are below 100 pM in healthy sheep (Nicholls *et al.*, 1988; Anderson *et al.*, 1988), after infection with *O. circumcincta*, the plasma or serum gastrin may be substantially elevated: Anderson *et al.* (1981) recorded maximum levels from 155 to 677 pg/ml between 11 and 20 days after infection and Titchen (1982) described 20-fold increases, although most reported increases are more modest. After anthelmintic treatment, gastrin levels, like abomasal pH, returned to normal (Anderson *et al.*, 1981), indicating the importance of the continued presence of the parasites for the disturbance.

Precisely when plasma gastrin levels rise after infection is unclear. As with the hypochlorhydria, this is partly due to inadequate sampling frequency in some studies. Of particular interest is the relationship between the increase in circulating gastrin and the increase in abomasal pH, since a rise in gastrin before pH would suggest that factors other than removal of acid inhibition are responsible for the hypergastrinaemia. The answer to this question depends upon the subjective and clearly ill-defined criterion of *what is an elevated pH*? In addition, the time of elevation of plasma gastrin is important. Anderson *et al.* (1981) collected blood samples from sheep every fourth day after infection and found that serum gastrin increased within eight days of the first dose of larvae. This increase may, however, actually have occurred on any of the fifth, sixth or seventh days. It occurred both in naive and previously-exposed sheep, although in the latter the lesion developed more slowly and was less profound.

Nicholls *et al.* (1988) cite the work of Anderson *et al.* (1981, 1985), Titchen (1982) and Nicholls *et al.* (1985) as evidence that plasma gastrin may increase before a major change in pH occurs. However, although Anderson *et al.* (1976a) stated that hypochlorhydria did not appear to initiate the hypergastrinaemia as it occurred in

advance of the elevation of abomasal pH, there is no indication of when pH rose or how this was assessed. Subsequently, Anderson *et al.*, (1976b) revisited this question and refer to one of the suggestions made by McLeay *et al.* (1973) that the inhibition of parietal cells may be by a factor released by the parasites. The gastrin data associated with the 1976b work was presented in Anderson *et al.*, (1981) and revealed that the plasma gastrin was elevated by the eighth day after the first dose of larvae, which they determined to precede the rise in abomasal pH. However, they did not consider a pH below 4.0 to be raised. The emphasis they place on the contribution of parasites *per se* toward the development of hypergastrinaemia may thus not be warranted. There was a later qualification by Titchen (1982) in stating that "gastrin increased before major changes in pH". A further increase in plasma gastrin occurred in all or these studies with the major increase in abomasal pH.

Other reports are less supportive of hypergastrinaemia preceding a rise in abomasal pH. The increase in abomasal pH reported by Nicholls *et al.* (1985) was "closely associated" with the rise in plasma gastrin that occurred after two to four days in sheep experimentally infected with *H. contortus*, although these authors clearly did not consider pH raised unless it was above 4.0. Fox *et al.* (1993), with reference to their and other previous work, suggest that the rise in abomasal pH is the main stimulus for the hypergastrinaemia seen in susceptible cattle following infection by *O. ostertagi* as the timing of the rise in gastrin does not differ noticeably from that for pH, although these variables were not measured simultaneously.

The transfer of mixed and mainly adult worm populations directly into the abomasum of sheep resulted in increased plasma gastrin concentrations within 24 to 48 hours in five of six animals (Anderson *et al.*, 1985). In these same five animals, pH was above the pre-infection mean at this time although still within the defined preinfection range, leading to the conclusion that gastrin rose before pH. It is not so certain that this enables a clear separation of hypergastrinaemia from the elevated pH in the early stages of infection. The hypergastrinaemia persisted when the abomasal pH was returning toward normal towards the end of the infection in two sheep. McKellar *et al.* (1987) reported increased plasma gastrin concentrations in some calves directly infected with adult *O. ostertagi* that were not accompanied by a significant pH change, which they believed confirmed the suggestion of Anderson *et al.* (1985) that stimuli to G cells during infection with *Ostertagia* are not pH dependent. Further investigation into the

possible effect of parasites directly on the G cell when removed from its intimate relationship with luminal pH is necessary before the role of parasite-derived substances can be clarified.

Fox *et al.* (1993) suggest that the rise in plasma gastrin concentration in *Ostertagia*-infected calves may be due to an increase in peptide synthesis, a reduction in gastrin turnover and the release of stored peptide into the blood. Their study indicates that there was a significant release of stored gastrin as maximal plasma concentrations were accompanied by depleted tissue stores which they attribute to a reduction in gastrin per G cell and in apparent G cell numbers. The latter may simply reflect failure of "depleted" cells to stain effectively. These results are in contrast with those of Anderson *et al.* (1988) who detected more gastrin in the abomasa of infected sheep than in uninfected control animals. This increase was more pronounced in adult animals than lambs. In the lambs there was actually a decrease in the gastrin content per g mucosa. The discrepancy between the Fox and Anderson studies may reflect the length of the relative infections and be unrelated to species. Fox *et al.* (1993) slaughtered all animals on or before Day 28 while the lambs and sheep in the study by Anderson *et al.* were killed after seven and 20 weeks respectively. The relative length of the infections is of even greater significance given the slower development of the parasite and accompanying lesions in the bovine. After 20 weeks the G cell population may well have adapted to the parasite-induced secretory regime through both an increase in G cell number and gastrin production per cell.

1.5.4 SECRETORY AND EXCRETORY PRODUCTS OF *O. CIRCUMCINCTA*

It has been suggested that excretory/secretory (ES) mediators of *Ostertagia* may be involved in the physiological disturbances associated with the parasitism of sheep and cattle (McLeay *et al.*, 1973; Titchen, 1982; McKellar *et al.*, 1987). They have variously suggested that worm products may stimulate pepsinogen and/or gastrin secretion or inhibit parietal cell activity. In contrast to the lack of success in demonstrating that these products have any physiological action, their antigenic properties are well documented.

Helminth parasites characteristically secrete/excrete lactate, succinate, acetate,

proprionate and other fatty acids (Bryant, 1993). These fermentation products are produced despite the frequent availability of oxygen. Many other components including enzymes have also been identified in the ES mix as workers search for worm-derived substances with antigenic properties. ES products released during *in vitro* maintenance of *Trichostrongylus colubriformis* induced a high level of protection against challenge with the parasite (Rothwell & Love, 1974). Subsequently, an 11-kDa protein and a 20-kDa globin-like protein, both of which prime the immune system of the guinea pig, have been identified in ES products of this nematode (Dopheide *et al.*, 1991; Frenkel *et al.*, 1992). Similarly, at least 15 peptides from molecular weights ranging from 10 to 100 thousand that stimulate lymphocyte proliferation have been identified in ES products of *H. contortus* (Schallig *et al.*, 1994).

Acetylcholinesterase has been identified in the ES products of many nematode species including *T. colubriformis*, *T. axei*, *Oesophagostomum venulosum*, *Oe. radiatum*, *N. brasiliensis*, *O. circumcincta*, *Chabertia ovina*, *H. contortus*, *H. placei*, *Cooperia pectinata* and *Dictyocaulus viviparus* (Ogilvie *et al.*, 1973; Griffiths & Pritchard, 1994; McKeand *et al.*, 1994a). The importance of acetylcholinesterases to the proper functioning of nematode cholinergic pathways is evidenced by the use of acetylcholinesterase inhibitors in many anthelmintics (Opperman & Chang, 1992). The role of those secreted is less clear and all putative roles are the result of speculation. Some suggestions include: a biochemical holdfast (Ogilvie & Jones, 1971); inhibition of mucus secretion (Philipp, 1984); modification of mediator release and other immune reactions against the parasite (Rhoads, 1984). Comparison of third stage, fourth stage and adult worm extracts of *D. viviparus* indicated that acetylcholinesterases were only produced by later developmental stages of this parasite (McKeand *et al.*, 1994b).

Proteinase release by helminth parasites is also well documented (von Brand, 1973; Knox & Jones, 1990). Many functions have been ascribed to these including penetration of host tissue by *Nector americanus* (Matthews, 1982); proteolytic anticoagulation by *Ancylostoma caninum* (Hotez & Cerami, 1983); inactivation of complement and cytotoxic mediators released by leucocytes (Leid, 1987); proteolysis of pepsin by *Ascaris suum* (Martzen *et al.*, 1990). At least four proteases are present in the ES products of *H. contortus* (Karanu *et al.*, 1993).

ES substances produced by parasites are now also accorded a regulatory or

neuromodulatory role (Lightowlers & Rickard, 1988; Pearce & Appleton, 1992; Pritchard *et al.*, 1994). Soluble extract of *O. ostertagi* was found to be chemotactic to eosinophils (Klesius *et al.*, 1985). The chemotaxin has since been suggested to be a lectin through its inhibition by various sugars (Klesius, 1991). Eosinophil infiltration during the early stages of *Ostertagia*-induced inflammation was recorded in sheep (Armour *et al.*, 1966; 1.5.2) and cattle (Snider *et al.*, 1988b). The attraction of these by the parasite and subsequent release of cytotoxic substances by the eosinophils may play an important role in the inflammatory process associated with ostertagiasis. Klesius (1993) reviews the possibility that antigen-independent substances within the ES products of *O. ostertagi* may regulate immune cell function, citing four sets of evidence: (1) parasite-mediated suppression of lymphocyte reactivity; (2) suppression of antibody production; (3) eosinophil chemotaxis; (4) lymphocyte proliferation. He hypothesises that regulation of host cellular responses is not confined to the immune system. A protective antigen from *T. colubriformis* has been cloned and shown to have considerable homology with immune interferon induced protein (Dopheide *et al.*, 1991). If this protein has actions comparable to those of the protein normally synthesised in response to the lymphokine, it may participate in the regulation of T cells and subsequently IgE production (Pritchard, 1993).

1.5.5 IMMUNITY TO *O. CIRCUMCINCTA*

Immunity and the immune response to *Ostertagia* embrace a wide variety of host-parasite interactions as well as an enormous number of interactions amongst host cells. After exposure to *O. circumcincta*, healthy adult sheep are able to mount a strong active immunity (Elliot & Durham, 1976; Douch *et al.*, 1984; Smith *et al.*, 1985b). This normally develops while the sheep is continuously exposed to low larval intake from pasture. Consequently, older sheep are usually more resistant than younger sheep. Protective immunity against *O. circumcincta* began to develop after 4 to 8 weeks of exposure in five-month-old lambs infected daily with 1000 larvae (Seaton *et al.*, 1989). By 12 weeks, the animals were almost completely immune to incoming worms. The development of resistance to subsequent challenge correlated with a rise in serum antibody titre and an increase in the number of intraepithelial globule leucocytes in the gastric mucosa.

Immunity to helminths appears to be generally similar to the immune response to other

antigenic determinants. As worms present large, non-phagocytosable surfaces to the host's defences they are most vulnerable to cells capable of mediating extracellular events. Nematode infections are characterised by high IgE responses, eosinophilia and mastocytosis and the interaction of these (Armour *et al.*, 1966; Smith *et al.*, 1986; Wakelin, 1992; Miller, 1993; Pritchard, 1993). The eosinophil/IgE/mast cell axis is thus of particular importance in defense against these parasites (Butterworth, 1984). IgA is the dominant immunoglobulin in gastrointestinal secretions (Wakelin, 1978). A host that is producing IgE in response to *O. circumcincta* antigens may be considered sensitized or allergic to the parasite. In immune sheep, there are large numbers of mast cells and globule leucocytes in the gastric mucosa (Smith, 1988). Contact between *Ostertagia* and mast cells sensitized with specific IgE that was produced in response to current or previous infections leads to degranulation of the mast cell and the release of many inflammatory mediators (Kay, 1985). Some comment as to the nature and actions of such substances has been made (Section 1.4). Mast cells could also interact with larvae coated with complement (Sher & Glover, 1976) which may non-specifically induce their degranulation (Nawa, 1979). This may be of particular importance in primary infections prior to the production of specific IgE and other antibodies. On reinfection of immune sheep, there is an increase in mast cell protease in the lymph, demonstrating that mast cell degranulation has occurred (Smith, 1988). Simultaneous release of histamine and other components would affect mucosal permeability and afford a temporary increase in plasma pepsinogen (Yakoob *et al.*, 1983).

Infection with *O. circumcincta* resulted in increased cell and IgA traffic within gastric lymph (Smith *et al.*, 1983a,b, 1984). In immune sheep, the cellular reaction followed reinfection by two to four days and preceded the IgA response by a few days. A parasite specific antibody response occurred within seven days of infection of immune sheep (Gill *et al.*, 1994). In previously-unexposed sheep, the cellular (lymphoblast) response began five to eight days after infection and was more sustained than in previously-infected sheep, but there was little or no response in the IgA concentrations (Smith *et al.*, 1987; Smith, 1988). These sheep were all over 10 months of age and younger animals were less immunologically responsive (Duncan *et al.*, 1978; Smith *et al.*, 1985a, 1985b). Immunization of lambs under five months of age with *H. contortus* antigens was completely ineffective (Duncan *et al.*, 1978). Armour *et al.* (1966; Section 1.5.2) reported lymphoblast and plasma cell activity in all three zones of the mucosa by Day 8 in parasite-naive, six-month-old sheep infected with *O. circumcincta*, changes

which suggest that an immune response and antibody production had begun by this stage. The series of investigations by Smith *et al.* indicate that the host's immune response differs in naive and exposed animals.

Essentially, parasite-naive sheep are unable to mount an immune response when challenged for the first time. The immune system begins to react within the first week although it is some time (at least four weeks) before it affords any measurable protection against infection. In part, this may be due to the release of immunomodulatory substance by the parasite (Section 1.5.4). The immunological responsiveness of lambs is generally recognised (Smith, 1988) as being less than in sheep although the reason for this is unknown.

1.6 RETROSPECTIVE

Although the morphological and physiological lesions associated with ostertagiasis have been well defined from many experimental infections of ruminants, little progress has been made in identifying how the lesions are produced in the host. The most noted physiological effects on the sheep abomasum of infection with *O. circumcincta* are an increase in the pH of its contents, the hypersecretion of gastrin and increased *leakage* of pepsinogen into the circulation. These lesions predominantly develop at the time of emergence of fourth stage larval or adult worms, although it is also clear that the adult stage is capable of producing these physiological changes in the stomach without development from one stage to the next. In contrast, the presence of developing larvae in the mucosa has marked effects on the morphology but this alone causes relatively little disturbance to its function. There is no consensus on when the three functional lesions develop and their relationship to one another from studies on intact animals after experimental infection. Many questions remain unanswered, particularly whether or not the hypergastrinaemia is independent of the hypochlorhydria. The experiments described in Chapter 2, in which sheep were infected with larval or adult *O. circumcincta*, address these questions. Firstly, normal values for the three parameters in parasite-naive sheep were more clearly defined. In turn, this allowed the timing of the rises for each parameter after administration of the parasites, and hence their association, to be determined more precisely with regard to each other and with the development of the parasite.

Examination of the mechanisms involved in physiological processes is often more easily achieved using *in vitro* systems than in the whole animal, hence appropriate ovine abomasal *in vitro* preparations were developed to study parasite ES products (Chapter 3). Whereas gastric function in other mammalian species, especially rodents, has been extensively studied, comparatively little is known about the control mechanisms in ruminants. Pharmacological investigations of gastrin and somatostatin secretion from ovine antral tissue *in vitro* are reported in Chapter 4. Larval and adult worms were incubated under a variety of conditions and the effects of the incubates on gastrin and somatostatin secretion by the ovine gastric antrum were examined (Chapter 5). As the results of these studies suggested that the abomasal microflora could be involved, brief examination of microbial products on gastrin secretion is reported in Chapter 6.

Chapter 2

EFFECT OF ADULT OR LARVAL *OSTERTAGIA CIRCUMCINCTA* ON ABOMASAL pH AND SERUM GASTRIN AND PEPSINOGEN

2.1 INTRODUCTION

Infection of the sheep abomasum by *O. circumcincta* is often associated with symptoms reflecting gastrointestinal malfunction: anorexia, diarrhoea and possible weight loss. Protein metabolism may be disturbed and protein utilization impaired (Sykes & Coop, 1977; Parkins *et al.*, 1982a,b), acid secretion may be inhibited (Armour *et al.*, 1966; Jennings *et al.*, 1966; McLeay *et al.*, 1973; Anderson *et al.*, 1976a,b, 1981, 1985; Titchen & Anderson, 1977), plasma pepsinogen may increase (Armour *et al.*, 1966; McLeay *et al.*, 1973; Coop *et al.*, 1977) as may plasma gastrin (Anderson *et al.*, 1976a,b, 1981, 1985, 1988; Titchen & Anderson, 1977; Blanchard & Wescott, 1985) (see Chapter 1, 1.5.3). At an elevated abomasal pH, pepsinogen is not converted to pepsin (Jennings *et al.*, 1966), which reduces the ability of the animal to digest protein. The elevated pH also allows bacteria to proliferate (Nicholls *et al.*, 1987). Hypergastrinaemia reduces rumenoreticular motility (Carr *et al.*, 1970) and gastric motility (Bell *et al.*, 1977) and may contribute to inappetence (Fox *et al.*, 1989a,b) and processes causing diarrhoea (Kimberg, 1974).

The aetiology of hypochlorhydria and hypergastrinaemia is unknown and it is also unclear precisely when the abomasal pH and plasma gastrin rise after infection. This uncertainty is due to low sampling frequency in some studies and to an imprecise definition of normal and raised values (see Chapter 1, 1.5.3). Plasma pepsinogen increases before either plasma gastrin or abomasal pH and is generally attributed to a "leak lesion" (Chapter 1, 1.5.3.1). The relationship between the increase in circulating gastrin and the increase in abomasal pH is of particular interest, since a rise in gastrin

before pH as reported by Anderson *et al.* (1981) would suggest that factors other than removal of acid inhibition are responsible for the hypergastrinaemia. Whereas some workers (Anderson *et al.* 1981; Nicholls *et al.*, 1985) suggest there is a pre-hypochlorhydric rise in circulating gastrin, others (e.g. Fox, 1993) consider that the increase in abomasal pH to be the *main* stimulus for hypergastrinaemia seen in sheep and cattle.

The direct transfer of adult *Ostertagia* into the abomasa of sheep (Anderson *et al.*, 1985) and cattle (McKellar *et al.*,1986, 1987) also produced these disturbances, indicating that the mucosal damage associated with the progression of larvae to adult stages can only partially account for the changes and has led to the suggestion that parasite-derived factors may be important in the disease (McKellar *et al.*, 1987). In sheep, the plasma pepsinogen and gastrin levels increased within 28 to 48 hours of transfer. Although the abomasal pH was above the pre-infection mean at this time, it was still within the defined preinfection range, leading to the conclusion that gastrin rose before pH (Anderson *et al.*, 1985). In their study, hypochlorhydria was recognised five to seven days after infection, but this is subject to the interpretation of a normal pH: at least two of the three animals in one group had an abomasal pH above 4.0 on the third to fourth day. In the calves, McKellar *et al.* reported increased plasma gastrin concentrations that were not accompanied by a significant pH change, which they believed confirmed the suggestion of Anderson *et al.* (1985) that stimuli to G cells during infection with *Ostertagia* are not necessarily pH dependent.

Because interpretation of the association between increased serum pepsinogen, serum gastrin and abomasal pH in previous studies was limited by low sampling frequency and the poorly defined normal range for these parameters, a more precise definition of normality is essential to clarify when the disturbances occur after larval infection or the transfer of adult worms into the abomasum, the relationship of these changes to one another and their relationship to parasite development. In the present study, data for serum pepsinogen, serum gastrin and abomasal pH were collected from control, parasite-naive sheep and in sheep experimentally-infected with adult *O. circumcincta* or with larvae either given intraruminally or directly into the abomasum.

2.2 MATERIALS AND METHODS

2.2.1 EXPERIMENTAL DESIGN

Five groups of sheep were experimentally infected with *O. circumcincta*:

(A) a pilot study in which 2 previously-parasitised sheep were infected with 30,000 larvae (experiment 1 (Expt 1));

(B) 4 parasite-naive sheep were infected with 50,000 larvae (Expt 2);

(C) 4 parasite-naive sheep were infected with 150,000 larvae followed by a trickle infection of 10,000 larvae thrice weekly from Day 21 to Day 45 (Expt 3);

(D) 4 parasite-naive sheep were infected with 150,000 exsheathed larvae via an abomasal cannula (Expt 3);

(E) 4 parasite-naive sheep were infected with 15,000 adult worms via an abomasal cannula (Expt 3).

Control animals were included in each Expt.

Blood and abomasal fluid samples were collected at least twice daily after infection and for 3 days in Expt 1, 4 days in Expt 2 and 7-8 days in Expt 3 prior to infection.

2.2.2 ANIMALS

Expt 1. Four 32 week old male Romney cross sheep (34 to 38 kg) that had been raised on pasture and exposed to field parasitism were treated with anthelmintics (Appendix 2) and either infected with 30,000 *O. circumcincta* larvae intraruminally by tube (Group A, Sheep #1-2) or maintained as parasite-free controls (Sheep #3-4).

Expt 2. Ten 20 week old Romney cross sheep (20 to 40 kg) raised to be parasite-naive were either infected with 50,000 *O. circumcincta* larvae intraruminally by tube (Group B, 2 male, 2 female, Sheep #5-8) or maintained as parasite-naive controls (3 male, 3 female, Sheep #9-14).

Expt 3. Eighteen 20 week old poll Dorset sheep (16 to 25 kg) raised to be parasite-naive were either infected with: (i) 150,000 larvae intraruminally by tube (Group C, 4 male, Sheep #15-18), (ii) 150,000 exsheathed L_3 larvae through an abomasal cannula (Group D, 2 male, 2 female, Sheep #19-22), (iii) 15,000 adult *O. circumcincta*

transferred directly to their abomasa via a cannula (Group E, 4 male, Sheep #23-26) or maintained as parasite-naive controls (6 male, Sheep #27-32). Group C were subsequently given 10,000 larvae thrice weekly from 21 to 45 days after the initial infection.

The sheep were housed in individual metabolism crates, provided with water *ad libitum* and fed once daily with 900 g lucerne chaff (Expt 1), 200 g lucerne chaff plus 600 g lucerne nuts (Expt 2) or 800 g lucerne nuts plus 200 g aged hay (Expt 3). Some infected sheep, particularly in the two groups given 150,000 larvae, did not consume all the feed offered on some days and a number of these animals also developed diarrhoea at various times. Faecal samples were collected on arrival (followed by prophylactic anthelmintic dosing (Appendix 2)), at the time of surgery and again two days later. After one week of habituation, each sheep was surgically fitted with an abomasal cannula while under general anaesthesia and sampling commenced after one week of postoperative recovery. At the end of the Expt, the sheep were weighed and then killed with Pentobarb 500 (Chemstock Animal Health). Group A were killed on Day 33, Group B on Day 25, Group C on Day 55, Group D on Day 30 and Group E on Day 8 (Day 0 was the time of infection).

2.2.2.1 SURGERY

Abomasal cannulae were inserted aseptically under general anaesthesia into the greater curvature at the junction between body and pyloric regions and exteriorized through the ventral right flank. Anaesthesia was induced by intravenous administration of Saffan (Pitman-Moore, NZ) and maintained by Flurothane (ICI Pharmaceuticals) inhalation. Prophylactic antibiotic treatment (Streptopen, Pitman-Moore, 5mls i.m. daily) was given for three days post-operatively. All the surgical preparations were kindly performed by Dr G W Reynolds.

2.2.3 BLOOD AND ABOMASAL FLUID SAMPLES

2.2.3.1 SAMPLE COLLECTION

Jugular blood was collected by venepuncture into plain Venoject tubes, allowed to clot at room temperature and centrifuged at 1500 revolutions per minute (rpm) for 20

minutes. The serum was stored at -20°C for subsequent determination of serum pepsinogen and serum gastrin concentrations. Abomasal contents were sampled via the cannulae. A lavage syringe was fitted into the cannula and about 3 mls of water was pushed into the cannula to flush out any consolidated digesta. Contents were drawn into the syringe and pushed back into the abomasum a number of times to ensure the sample was representative. About 1-2 ml of abomasal contents were taken for determination of abomasal pH.

Jugular blood and abomasal contents were sampled 30 minutes prior to, and either 3 hours (Expt 1) or 2 hours (Expt 2 and 3) after, feeding. In Expt 3, additional samples were collected from the adult transfer group (Group E) every two hours from 0 to 24 hours after infection, every four hours from 24 to 72 hours and then every six hours until euthanasia on Day 8; from Group C (larvae via stomach tube) every four hours from Day 4 to 6 and every six hours from Day 7 to 10; from Group D (exsheathed larvae) every two hours from 0 to 36 hours, every four hours from 36 to 72 hours, and every six hours to Day 9.

The abomasal cannula was lost and not replaced from sheep #27 on Day 35 and from sheep #22 on Day 23. No further abomasal samples were collected from these sheep. The latter sheep was given antibiotics (Streptopen, Pitman-Moore, 5mls i.m. daily) for the remainder of the Expt and since the abomasum was severely scarred and there was substantial adhesion to the abdominal wall at post-mortem, no abomasal weight was recorded. The cannula of sheep #15 opened overnight on three occasions (Days 28, 40, 41) and abomasal contents were lost. After each incident, there was a marked increase in abomasal pH which was accompanied by a simultaneous rise in serum gastrin concentration. For this reason, the serum gastrin and abomasal pH values at these times were excluded from the group mean. This same sheep died of accidental causes unrelated to the parasite infection or to the cannula on Day 44 and no abomasal weight or estimated worm count was obtained.

2.2.3.2 ABOMASAL pH

The abomasal pH was measured within 15 minutes of sample collection with a PHM82 Standard pH Meter (Radiometer, Copenhagen).

2.2.3.3 SERUM PEPSINOGEN

Serum pepsinogen concentration was estimated using a modification of the method described by Uete *et al.*, (1969) and is described in detail in Appendix 1.1. Briefly, the serum was acidified with HCl to activate the pepsinogen which releases tyrosine from serum proteins by proteolytic activity. The tyrosine liberated was estimated colorimetrically after oxidation by Folin-Ciocalteau reagent under alkaline conditions. The difference between incubated and unincubated tubes was expressed as mU tyrosine/L. The repeatability of the assay was assessed from 30 assays of two control samples of deer serum, one with a 'high' (>400mU/L) and the other 'low' (<400mU/L) pepsinogen concentration. The mean and SD were 594 ± 65 and 206 ± 80mU/L for the 'high' and 'low' pools giving coefficients of variation of 11% and 39% respectively.

2.2.3.4 SERUM GASTRIN

Serum gastrin was determined by RIA using the method of Simpson *et al.* (1993) which is a modification of that of Hansky & Cain (1969) and is described in detail in Appendix 1.2. The antiserum used was Hansky's Ab74 which was a generous gift of Dr Hansky. Synthetic human nsG17 (Research Plus, Bayanne, N.J., U.S.A.) was used to prepare radioactive label and standards. All samples were assayed in triplicate. The mean sensitivity of the assay was 3.8 ± 0.4 pM. Within assay variation was 15% (±17) and between assay variation was 9.6% ± 6 (n = 9) for all assays.

2.2.4 ABOMASAL TISSUE SAMPLES

For sheep in Expt 3, the abomasum was opened, emptied of contents, blotted dry and weighed at post-mortem. The internal surface of the abomasum of sheep in Groups C and D was photographed. Gastrointestinal tissues were collected from sheep in Expt 2 and 3 for an associated but completely independent morphological study. The histological procedures involved in that study required the rapid collection of tissue samples with as little disturbance as possible. The priority given to this at post-mortem compromised or prevented post-mortem worm counts although worms were visible in all infected sheep.

2.2.4.1 TISSUE GASTRIN

A tissue sample was collected from the lesser curvature of the abomasum about 2 cm above the pylorus from infected sheep of Groups C and Group D (#16-21) and from control sheep of Expt 3 (#27-32). Approximately one gram of antral mucosa was scraped from underlying tissue, placed in a microfuge tube, snap frozen in liquid nitrogen and stored at -70^{0}C for extraction of tissue gastrin. The frozen tissue was weighed and the gastrin extracted by boiling in 10 ml distilled water in a boiling water bath for 30 minutes. The tissue fragment was removed, the tube was centrifuged at 1500 rpm for 20 minutes and the supernatant frozen and stored at -20^{0}C until the gastrin concentration was estimated by RIA.

2.2.5 PARASITOLOGY

Details of all aspects of the parasitology are described in Appendix 2.

A pure strain of *O. circumcincta* was maintained. Larvae used to infect sheep in these Expts had been recently cycled through donor sheep, were stored at 10^{0}C and had a viability greater than 98%. Exsheathed larvae used in Expt 3 were induced to complete the second ecdysis with 0.2% sodium hypochlorite one hour before abomasal infection. The adult worms used in Expt 3 were raised in eight donor sheep killed exactly four weeks after their infection. These worms were examined and counted prior to their transfer to recipient animals four hours after the slaughter of donor animals.

Faecal floats were performed on fresh faeces collected per rectum. At least two slides were prepared for each faecal sample. A post-mortem parasitological examination of the digestive tract of some sheep was performed. When positive faecal floats were returned, the number of eggs per gram (e.p.g) were estimated using the method described by Stafford *et al.* (1994) in which each egg counted represents 50 e.p.g. Faeces were sampled weekly in Expt 1 and 2 and daily in Expt 3. The abomasal samples taken from infected sheep in Expt 3 for pH determination were examined for the presence of parasites in order to determine whether this constituted a significant loss of worms. Where female worms were found in the sample collected from those infected intraruminally with larvae, the number of eggs per worm were counted. At post-mortem, the number of worm in the abomasal contents was estimated in larval-infected

groups of Expt 2 and 3.

2.2.6 STATISTICS

All values for serum pepsinogen, serum gastrin and abomasal pH from samples collected from parasite-naive sheep were used to calculate the mean (μ) and sample standard deviation (s) for each parameter for each sheep and for each group. In each case, $\mu + 2s$ was calculated to define the upper limits of the normal range. For abomasal pH, $\mu+1s$ was also calculated because of the close association between pH and gastrin secretion (see Chapter 1, 1.3.4.1.2). Group mean responses were also calculated for all 3 parameters. The mean feeding response (FR) for each control group was calculated from the average daily post-feeding value minus the pre-feeding value.

Wilk-Shapiro/rankit plot values were determined using the software package STATISTIX (Statistix Analytical Software, USA). Measurements of abomasal weight and tissue gastrin content at post-mortem in Expt 3 were compared by one way Analysis of Variance using the software package MINITAB (Minitab Inc., U.S.A.).

2.2.7 INTERPRETATION OF RESULTS

From values obtained from uninfected sheep, the upper limit of the normal ranges for serum pepsinogen, serum gastrin and abomasal pH were calculated for each sheep and for each group. Any value above the defined upper limit ($\mu+2s$) was considered to be abnormal. If two or more successive sample values were greater than this level (as normally occurs 0.0625% of the time), the parameter was determined to be significantly raised from the earlier of these. The time instant from which each parameter was considered raised was determined graphically.

2.3 RESULTS

2.3.1 DEFINITION OF NORMAL VALUES

The abomasal pH, serum gastrin and serum pepsinogen values of uninfected sheep (before and after feeding) all formed mound shaped distributions and had Wilk-Shapiro values approaching 1.0, indicating that the data collected for each parameter

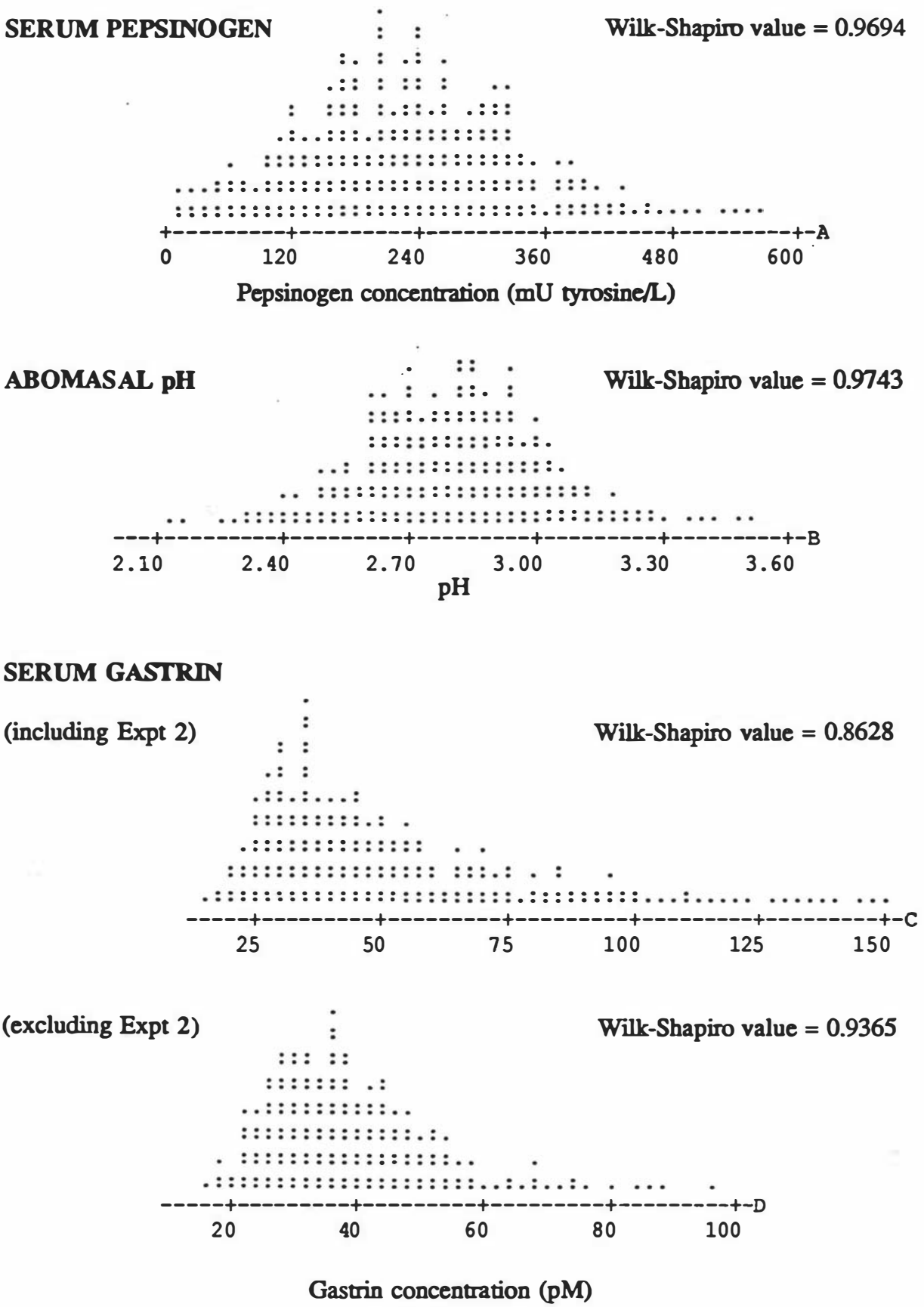

Figure 2.1 Distribution of serum pepsinogen, abomasal pH and serum gastrin values determined from samples collected from parasite-free sheep. Each dot represents four data points for serum pepsinogen and serum gastrin (excluding Expt 2), five data points for pH and six data points for serum gastrin (including Expt 2). The Wilk-Shapiro/rankit plot values were calculated using the software package STATISTIX, where 1 is a normal distribution and 0 is a random distribution.

Table 2.1. Calculated mean and upper limit of the normal range for serum pepsinogen, serum gastrin and abomasal pH of individual sheep prior to infection and of control groups. The upper limit of the normal range was defined as the mean plus two standard deviations (μ+2s).

Experiment and Treatment	Sheep number	Serum Pepsinogen (mU tyrosine/L)		Serum Gastrin (pM)		Abomasal pH	
		μ	μ+2s	μ	μ+2s	μ	μ+2
30,000 L_3 GROUP A	1	158	188	51	61	2.74	3.45
	2	118	203	33	36	2.73	2.98
Control group Expt 1	3 & 4	215	417	39	139	2.79	3.26
50,000 L_3 GROUP B	5	182	315	51	73	2.55	2.98
	6	193	464	55	71	2.74	3.35
	7	264	485	69	103	2.71	3.35
	8	221	368	65	109	2.65	3.11
Control group Expt 2	9-14	256	503	81	139	2.81	3.29
150,000 L_3 and trickle infection GROUP C	15	295	392	24	36	2.89	3.16
	16	206	378	41	64	2.82	3.19
	17	254	379	40	54	2.89	3.12
	18	93	242	40	60	2.79	3.03
150,000 Exsheathed L_3 GROUP D	19	297	481	29	39	2.85	3.52
	20	65	176	43	63	2.95	3.28
	21	326	449	29	39	2.78	3.09
	22	111	267	45	65	2.85	3.04
15,000 Adults GROUP E	23	146	258	44	66	2.81	3.07
	24	245	380	31	48	2.86	3.19
	25	240	364	31	50	2.73	3.04
	26	232	356	38	62	2.73	3.36
Control group Expt 3	27-32	222	467	38	66	2.81	3.27

Table 2.2 The mean serum pepsinogen, serum gastrin and abomasal pH change in response to feeding in parasite-naive sheep. The feeding response was determined by subtracting pre-feeding values from post-feeding values for control sheep. Pre-feeding samples were collected 30 minutes prior to a once daily feeding. Post-feeding samples were collected two (Experiment 2 and 3) or three (Experiment 1) hours after feeding.

Experiment	Feeding response		
	Serum pepsinogen (mU tyrosine/L)	Serum gastrin (pM)	Abomasal pH
1	17	-2	0.12
2	-37	18	-0.06
3	-9	6	-0.04

approximated a normal distribution (Fig. 2.1). The normal ranges for serum pepsinogen, serum gastrin and abomasal pH were calculated from values from uninfected sheep for each sheep and each group (Table 2.1) and for all animals. Overall, the μ and $\mu+2s$ were: abomasal pH, 2.80 and 3.26; serum pepsinogen, 223 and 454 mU/L; serum gastrin, 53 and 109 pM, but if Expt 2 were excluded 38 and 64 pM (serum gastrin levels were unusually high in the control sheep in Expt 2, See 2.3.2).

To determine whether abomasal pH were significantly elevated in individual sheep, the upper limit of the normal range for abomasal pH was taken as pH 3.26 for all animals as this was very constant among all control sheep. To determine whether serum pepsinogen concentration were elevated in individual sheep, their serum levels were compared against a single $\mu+2s$ value derived from the control group for that Expt. In some sheep, there was only a small increase in serum pepsinogen after infection. These sheep were classified as "low-responders" (Section 2.3.4.1) and were treated separately and their values not included in group means. To determine whether serum gastrin concentration were elevated, the $\mu+2s$ level derived from respective control groups was used for Expt 1 and Expt 3. In Expt 2, individual preinfection values for each sheep were used as their own control. These were still high relative to $\mu+2s$ for the control groups in the other Expts.

2.3.2 CONTROL GROUPS

No changes were evident in the serum pepsinogen, serum gastrin and abomasal pH measurements determined for the control groups in the three Expts with the exception of serum gastrin in Expt 2. The $\mu+2s$ for serum gastrin for this group was noticeably higher than the preinfection values for the larval-infected group from the same Expt and differed substantially from those determined (and used) to assess post-infection measurements in Expt 1 and Expt 3 (Table 2.1). This may have resulted from a conditioned response, particularly in some sheep: in one of the group the maximum gastrin concentration was 94 pM and μ was 56, while, in another sheep, eight measurements exceeded 100 pM and of these, seven were before rather than after feeding. The mean feeding response (Table 2.2) was principally an increase in serum gastrin of 18 pM and a decrease in serum pepsinogen after feeding in Expt 2. While other changes were relatively small, these were evident in individual sheep.

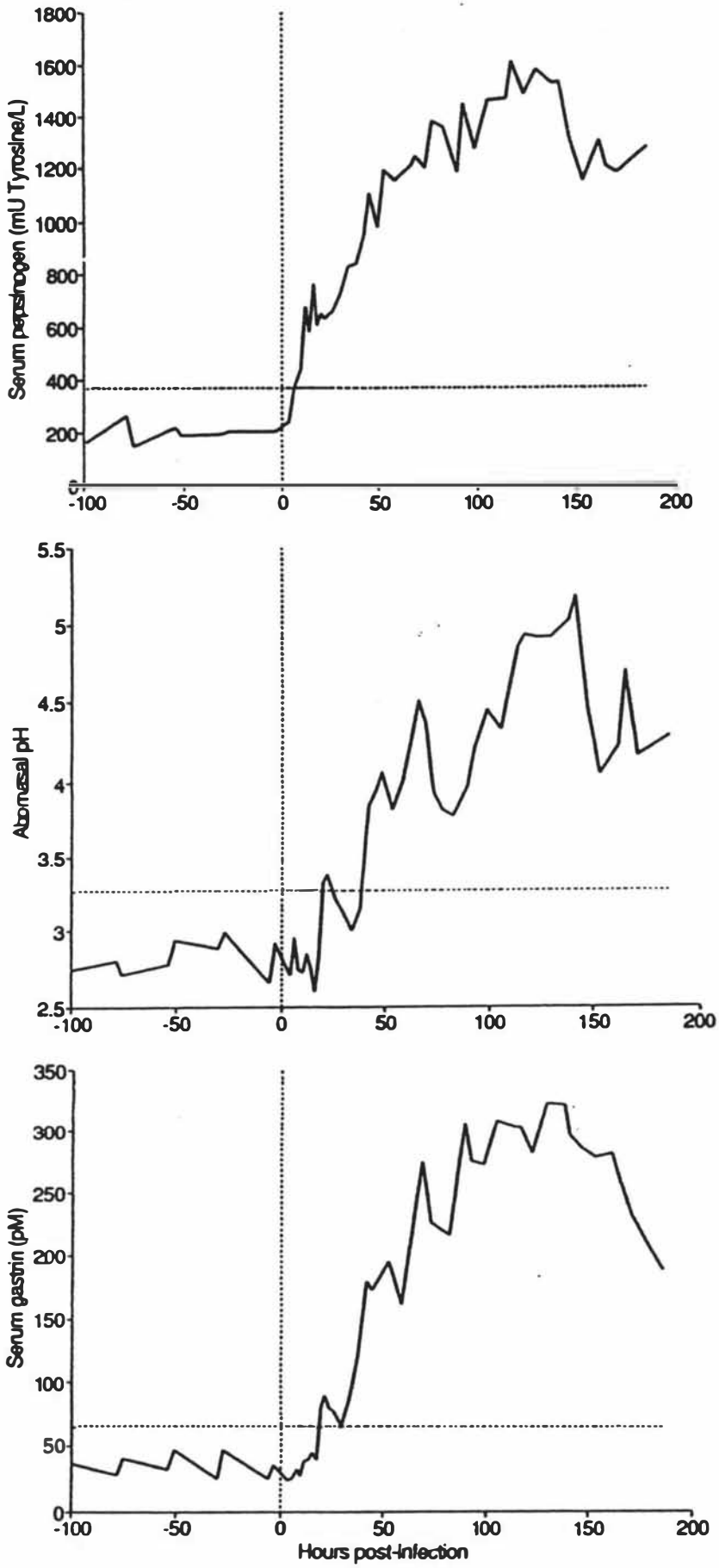

Figure 2.2 Mean serum pepsinogen, abomasal pH and serum gastrin values in sheep in Group E prior to and following direct transfer into the abomasum of 15 000 adult *O. circumcincta*. The horizontal dotted lines denote the upper limit of the normal range, defined as the mean plus two standard deviations ($\mu+2s$) of values determined in uninfected sheep.

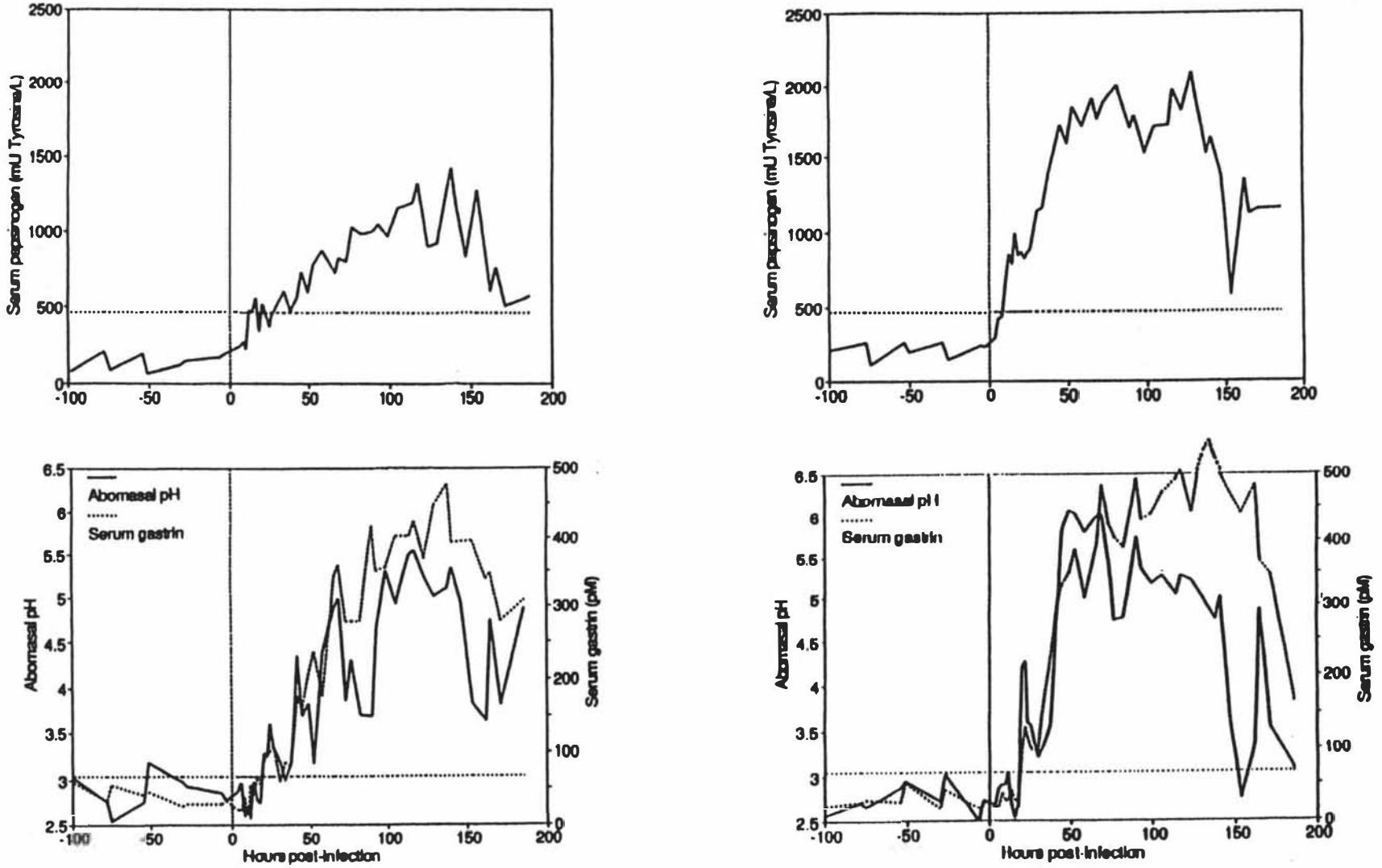

Figure 2.3 Serum pepsinogen, abomasal pH and serum gastrin in sheep 23 (left panel) and 25 (right panel) prior to and following direct transfer into the abomasum of 15 000 adult *O. circumcincta*. For pepsinogen and gastrin the horizontal dotted line denotes the upper limit of the normal range defined as the mean plus two standard deviations (μ+2s) of values determined in uninfected sheep. For pH the horizontal dotted line denotes μ+1s.

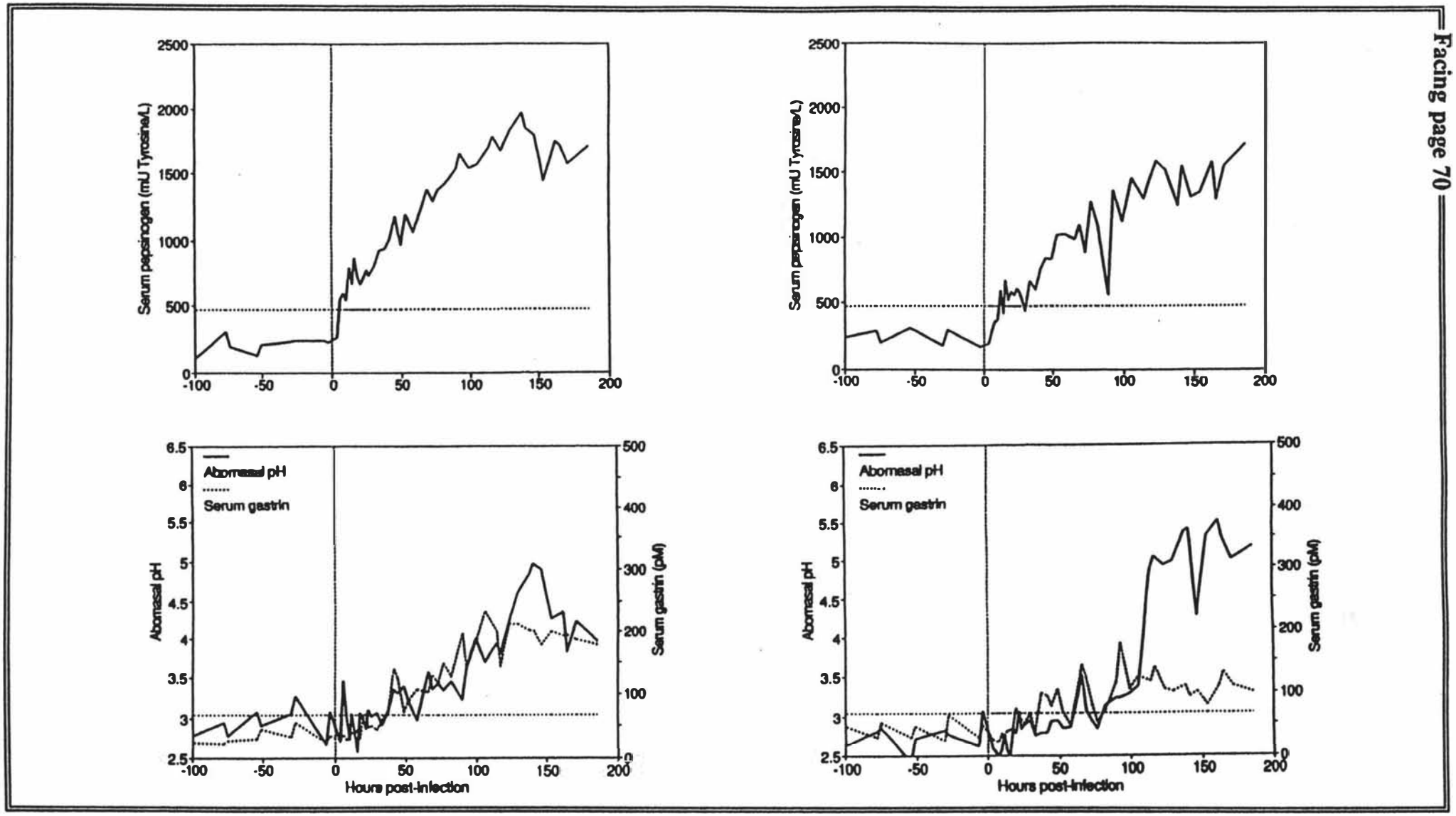

Figure 2.4 Serum pepsinogen, abomasal pH and serum gastrin in sheep 24 (left panel) and 26 (right panel) prior to and following direct transfer into the abomasum of 15 000 adult *O. circumcincta*. For pepsinogen and gastrin the horizontal dotted line denotes the upper limit of the normal range defined as the mean plus two standard deviations (μ+2s) of values determined in uninfected sheep. For pH the horizontal dotted line denotes μ+1s.

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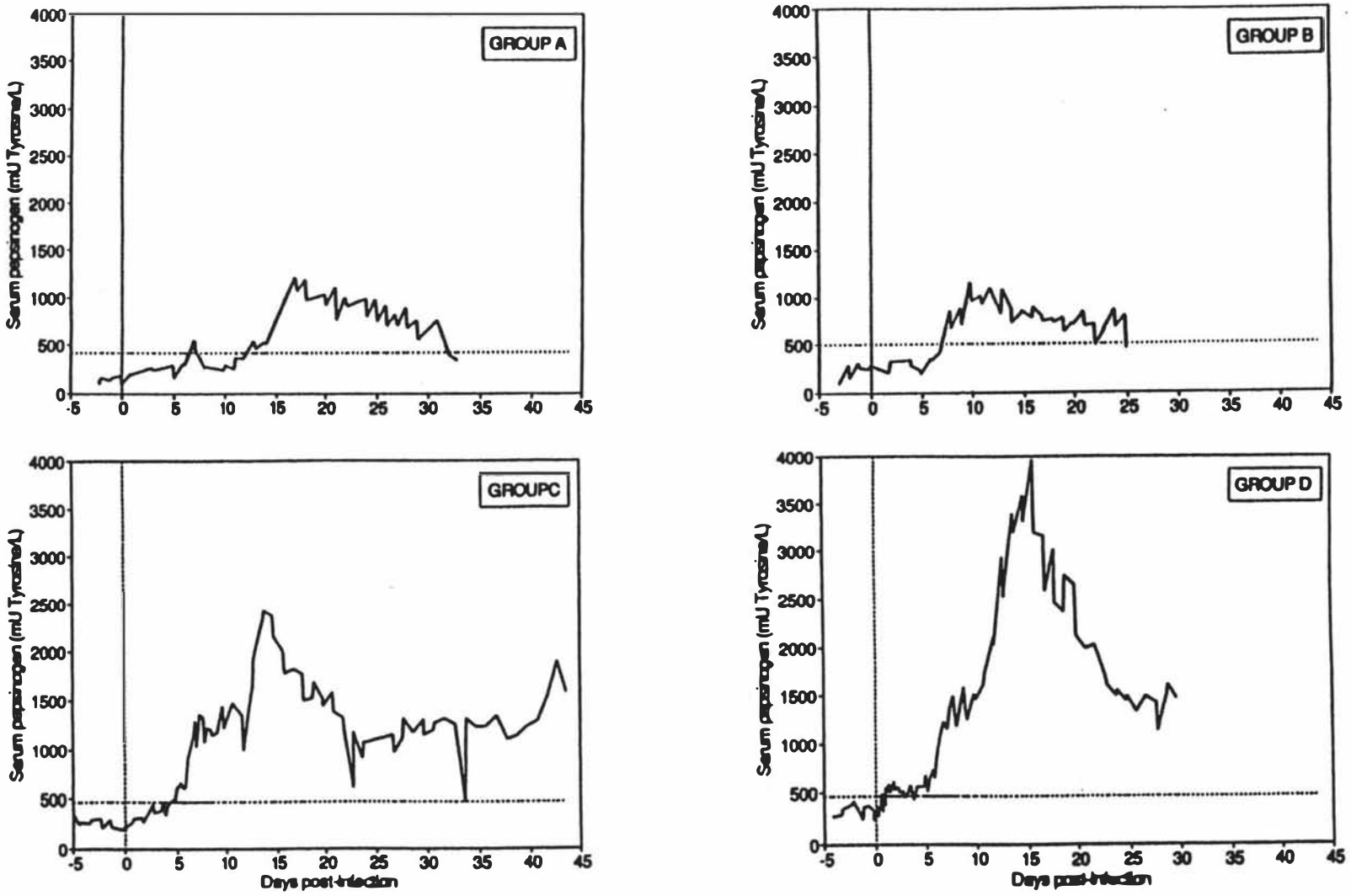

Figure 2.5 Mean serum pepsinogen concentrations of sheep in Group A (30 000 L_3, previously exposed), Group B (50 000 L_3), Group C (150 000 L_3) and Group D (150 000 exsheathed L_3) prior to and following infection with *O. circumcincta* larvae. The horizontal dotted line denotes the upper limit of the normal range, defined as the mean plus two standard deviations (μ+2s), determined for each group from control sheep.

2.3.3 INFECTION WITH ADULT *O. CIRCUMCINCTA*

The mean serum pepsinogen increased from the time of transfer of adult worms to exceed the normal range after 8 hours. It continued to rise until the maximum was reached after about 125 hours before declining slowly from about 140 hours and until the animals were killed on Day 8 (Fig. 2.2). In all individual sheep, the serum pepsinogen concentration exceeded the normal range before serum gastrin and abomasal pH (Figs 2.3 and 2.4) but began to decrease at the same times. The abomasal pH increased rapidly in all sheep: the group mean exceeded **μ+2s** after 19 hours and **μ+1s** shortly before. This result was biased toward two sheep in which the pH increased above 5.5 very rapidly (Fig. 2.3) whereas in the other two sheep, the pH rose gradually over the first four days before increasing to similar levels on Day 5 (Fig. 2.4). In one of these sheep, the pH was not raised until 90 hours, although the pH had been above **μ+1s** (3.04) for most of the preceding 30 hours. The pH slowly decreased from about 140 hours until euthanasia on Day 8. The increase in serum gastrin closely followed that in abomasal pH in individual sheep (Figs 2.3 and 2.4) as did the group mean values (Fig. 2.2).

2.3.4 INFECTION WITH LARVAL *O. CIRCUMCINCTA*

2.3.4.1 SERUM PEPSINOGEN CONCENTRATION

All infections elevated serum pepsinogen. Four sheep in which the increases were much smaller than in other group members (sheep #5, #18, #20, #22) were deemed 'low-responders' and the mean daily response of the Group was assessed without them. Serum pepsinogen levels did intermittently exceed their respective control **μ+2s** level in sheep #5 and #18 but not in sheep #20 or #22. In the latter two sheep, serum pepsinogen regularly exceeded the **μ+2s** derived from their own preinfection samples. The preinfection serum pepsinogen concentrations of the 'low-responders' were the lowest in their respective groups (Table 2.1). They were also all males. There were substantial serum gastrin and abomasal pH changes in all sheep that were deemed 'low-responders' on the basis of their serum pepsinogen levels. The mean serum pepsinogen concentrations for all infected groups after the exclusion of low-responders are shown in Fig. 2.5.

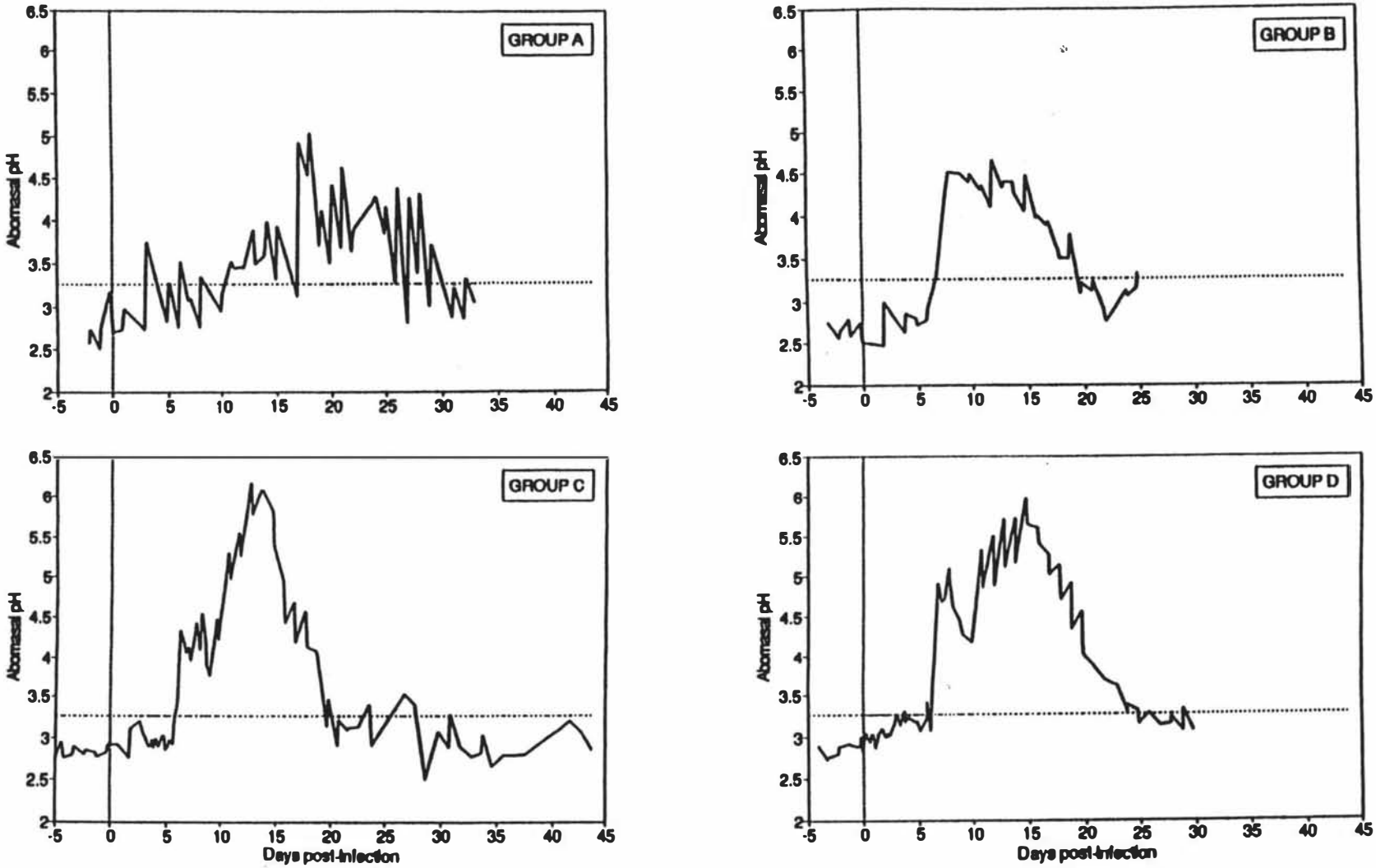

Figure 2.6 Mean abomasal pH of sheep in Group A (30 000 L_3, previously exposed), Group B (50 000 L_3), Group C (150 000 L_3) and Group D (150 000 exsheathed L_3) prior to and following infection with *O. circumcincta* larvae. The horizontal dotted line denotes the upper limit of the normal range, defined as the mean plus two standard deviations (u+2s) and determined from all control sheep.

Serum pepsinogen rose most quickly in those infected with exsheathed larvae via their cannula (22 hours), although this increase was relatively small until the rapid rise after about 140 hours. In those groups which received larvae intraruminally, the serum pepsinogen concentration was raised from: 102 hours Group C, 156 hours Group B and 290 hours (12 days) Group A. The small rise following infection with exsheathed larvae (Group D) was closely matched by a similar small disturbance in the group given 150,000 larvae intraruminally (Group C), although in the latter group this did not exceed $\mu+2s$ at this time (Day 2 to 5). The later rapid rise in pepsinogen concentration at about 140 hours after infection in Group D was matched exactly by the rapid rise which occurred in Group C. In Group B, serum pepsinogen also increased at about this time and there was a small transient disturbance in the previously-parasitised sheep (Group A). The greatest increase in serum pepsinogen concentration occurred in the two responder sheep receiving exsheathed larvae, in which a mean maximum of 3950 mU/L was recorded between Days 13 and 15. Of the groups infected intraruminally, pepsinogen levels were highest in those given 150,000 larvae (Group C).

After the mean maximum serum pepsinogen had been reached, there was a steady decline: the higher the concentration had become, the faster was the decline. In sheep which did not develop particularly high serum concentrations, the levels remained more constant, so that by about Day 25 greater parity was found between individual sheep. In Group A, the level decreased to below $\mu+2s$ at the end of the Expt (Day 33). In Group B (50,000 larvae), the concentration was at the $\mu+2s$ level on the last day (Day 25). Pepsinogen was still raised in Group D (150,000 exsheathed larvae) at 30 days. The subsequent trickle infection of Group C (150,000 larvae) from Day 21 onward was associated with a small gradual increase over the remainder of the Expt.

The increase in serum pepsinogen concentration preceded the rise in serum gastrin and abomasal pH in both groups given 150,000 larvae (Groups C and D) and occurred at about the same time (156 hours) as pH (157 hours) in those sheep given 50,000 larvae (Group B). In no individual sheep did there appear to be any particular association between the elevation in pepsinogen and abomasal pH, although this appears to be so for the mean group responses (Figs 2.5 and 2.6). The maximum levels for abomasal pH and serum pepsinogen occurred at approximately the same time, however, while pepsinogen remained elevated later in the infection, pH reverted towards normal. There was no discernable association between serum pepsinogen and gastrin in any sheep. In Group C, both gastrin and pepsinogen levels remained substantially elevated during the

Table 2.3 Time at which serum gastrin and abomasal pH were elevated after infection of parasite-naive sheep infected with *O. circumcincta* larvae. The upper limit of the normal range for serum gastrin was taken as the mean plus two standard deviations (**μ+2s**) and for abomasal pH, the time was assessed against both **μ+1s** and **μ+2s**.

Treatment and group	Sheep #	Time of elevation (hours)		
		Serum gastrin	Abomasal pH (**μ+1s**)	Abomasal pH (**μ+2s**)
GROUP B	5	80	136	140
50,000 larvae	6	81	77	186
	7	141	143	149
	8	166	159	186
GROUP C	15	150	125	144
150,000 larvae	16	166	88	141
	17	140	116	136
	18	125	45	170
GROUP D	19	135	25	131
150,000 exsheathed	20	76	38	61
larvae	21	312	128	185
	22	126	76	148

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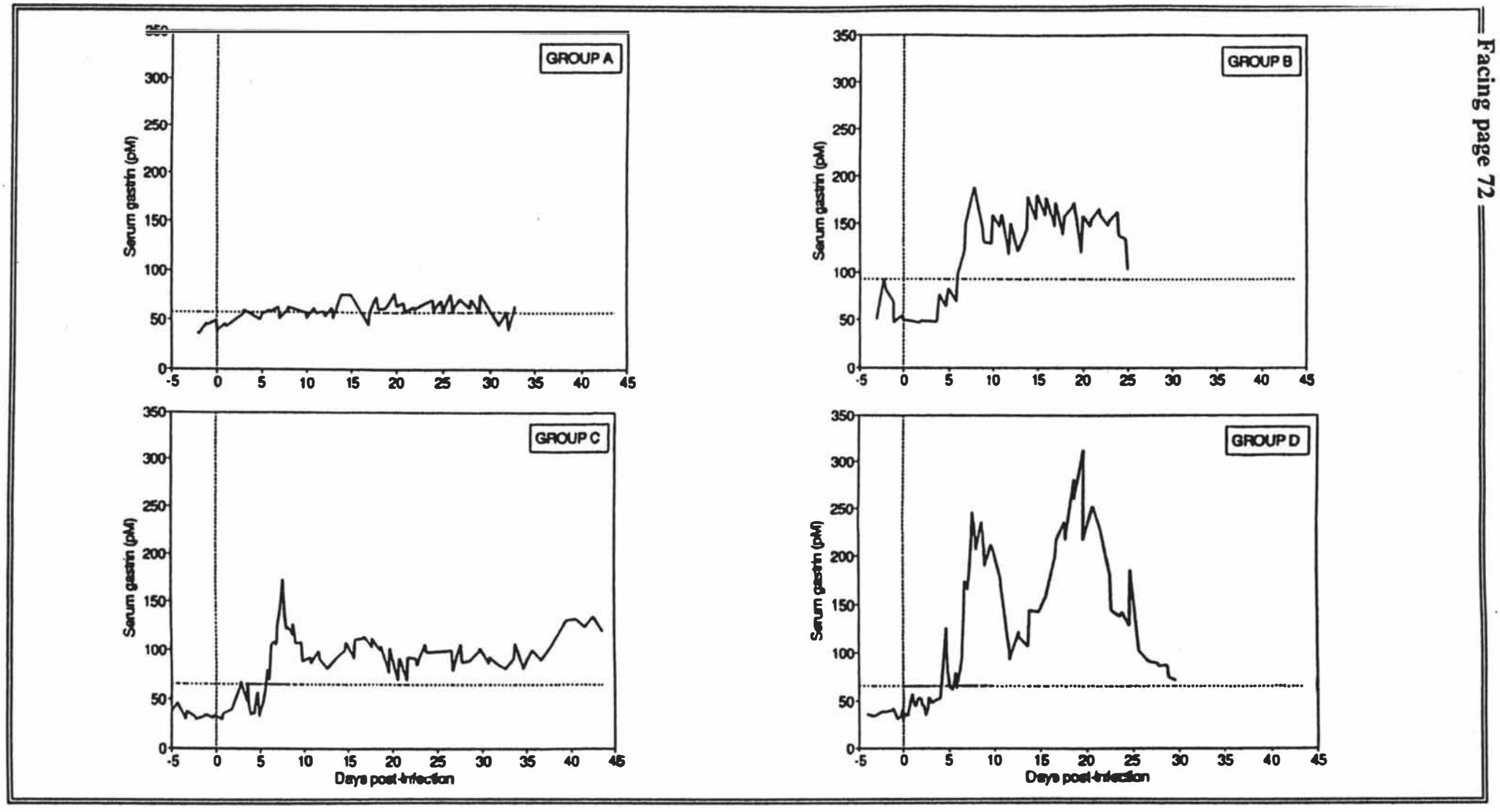

Figure 2.7 Mean serum gastrin concentration of sheep in Group A (30 000 L_3, previously exposed), Group B (50 000 L_3), Group C (150 000 L_3) and Group D (150 000 exsheathed L_3) prior to and following infection with *O. circumcincta* larvae. The horizontal dotted line denotes the upper limit of the normal range, defined as the mean plus two standard deviations (μ+2s), determined from control sheep, with the exception of Group B sheep, for which individual pre-infection values were used.

ensuing trickle infection.

2.3.4.2 ABOMASAL pH

Hypochlorhydria developed in all sheep (Fig. 2.6), the abomasal pH becoming highest in the two groups receiving 150,000 larvae (Groups C and D). In both, there was a small increase in pH between Days 2 and 4 which exceeded or remained at the **μ+1s** level, but the pH did not become significantly raised until the end of Day 5 (Group C at 138 hours and at 148 hours in Group D) when there was a dramatic rise in mean pH to about 6.0. The times at which pH increased to **μ+1s** and **μ+2s** in individual sheep are shown in Table 2.3. The abomasal pH remained very high for several days but returned towards normal from about Day 15 and was at the upper limit of the normal range from about Day 20 to 25 onward. During the ensuing trickle infection of Group C, the pH did not re-elevate, although the mean pH was higher (pH = 3.03) than the uninfected mean (pH = 2.81) from Day 21 onward (N.B. **μ+1s** = pH 3.04). In Group B (50,000 larvae), there was a distinct rise in pH between 142 (**μ+1s**) and 157 (**μ+2s**) hours, a time similar to the increase in the groups given 150,000 larvae. In Group B, the abomasal pH had returned to a relatively normal level by Day 19. In the previously-parasitised group that received 30,000 larvae (Group A), the abomasal pH rose least, first exceeding **μ+2s** 71 hours after infection and continuing to fluctuate at about this level until Day 10.

2.3.4.3 SERUM GASTRIN CONCENTRATION

Hypergastrinaemia occurred in all sheep infected with larvae (Fig. 2.7), to a lesser extent in Group A, but very markedly in the others, particularly in Group D (150,000 exsheathed larvae). Serum gastrin underwent a large increase late on Day 5 in Groups B, C and D and the route or number of larvae given had little apparent effect. In Group D, serum gastrin was raised from 102 hours post-infection and a rapid sustained increase occurred from 146 hours. The earlier rise at 102 hours derives from changes that occurred in only one of the four sheep (#20). In Group B (50,000 larvae), hypergastrinaemia developed suddenly from 135 hours and in Group C (150,000 larvae) the levels were raised from 136 hours. In Group A, the mean serum gastrin was first raised after 185 hours and sustained hypergastrinaemia occurred from Day 13 to 30. The times at which serum gastrin and abomasal pH were first considered raised in individual sheep are presented in Table 2.3.

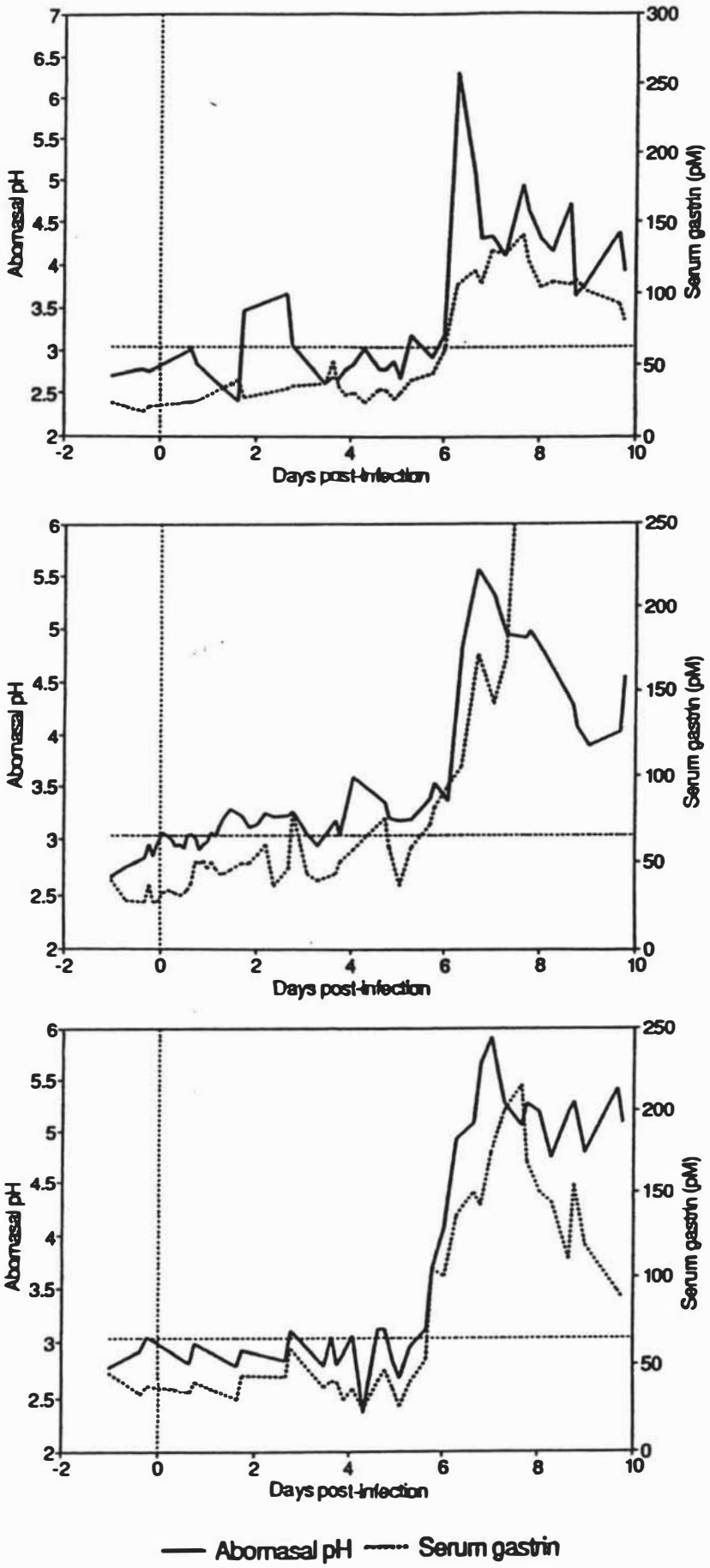

Figure 2.8 Abomasal pH and serum gastrin changes in sheep over the first 10 days after infection with *O. circumcincta* larvae. The horizontal dotted line indicates the upper limit of the normal range for gastrin (μ+2s), determined from values in control sheep. For pH the horizontal dotted line = μ+1s.

The period between infection and the steep increase in serum gastrin presents the most difficulty in interpreting whether or not the abomasal pH and serum gastrin were elevated or not. In all sheep receiving 150,000 larvae, there was a small increase in serum gastrin on about Days 2 and 3 which was more apparent in Group C, in which the group mean reached **μ+2s** on two occasions prior to becoming continuously raised (Fig. 2.7). This early disturbance was not seen in any of the sheep in Group B (50,000 larvae). In individual sheep, the times at which serum gastrin and abomasal pH increased (Table 2.3) were not always clear cut, as in some cases (e.g. sheep #16) gastrin levels were close to, or fluctuated about, **μ+2s** for some time before being clearly elevated. In addition, when the **μ+2s** value derived for each sheep from its preinfection samples was applied rather than the control group-derived value, the interpretation changed e.g. in sheep #21, serum gastrin was then raised from 144 instead of 312 hours, which conforms closely with the group mean. This problem was not relevant to Group B in which animal specific basal levels had been adopted for serum gastrin (Section 2.3.1).

Serum gastrin concentration and abomasal pH appeared to increase at about the same time in all sheep. This generalisation did not hold if the **μ+2s** values for both parameters were strictly adhered to (Table 2.3). When the changes in these two parameters were viewed more closely from individual graphs, it was apparent that significant data might be overlooked in the period leading up to the hypergastrinaemia and hypochlorhydria. Thus, in all sheep (except sheep #5 and #7 of Group B), the abomasal pH was between **μ+1s** and **μ+2s** at the time serum gastrin became raised, if it were not already raised. In sheep #7, serum gastrin rose only two hours before pH exceeded **μ+1s** and eight hours before it exceeded **μ+2s**, although samples were collected only twice daily in this Expt.

After the generally synchronous initial increase in serum gastrin and abomasal pH (as is exemplified in Fig. 2.8), the profile of each soon diverged in many sheep. In all groups, the elevation of serum gastrin persisted for the duration of the Expt. In those groups receiving only a single dose of larvae, the levels began to decline toward the end of the Expt from Day 25. In Group C, the subsequent trickle infection was associated with sustained elevation of serum gastrin at about double the control **μ+2s** value and there was an increase towards Day 40.

In all except two sheep in Groups B, C and D, after the abrupt initial increase in both

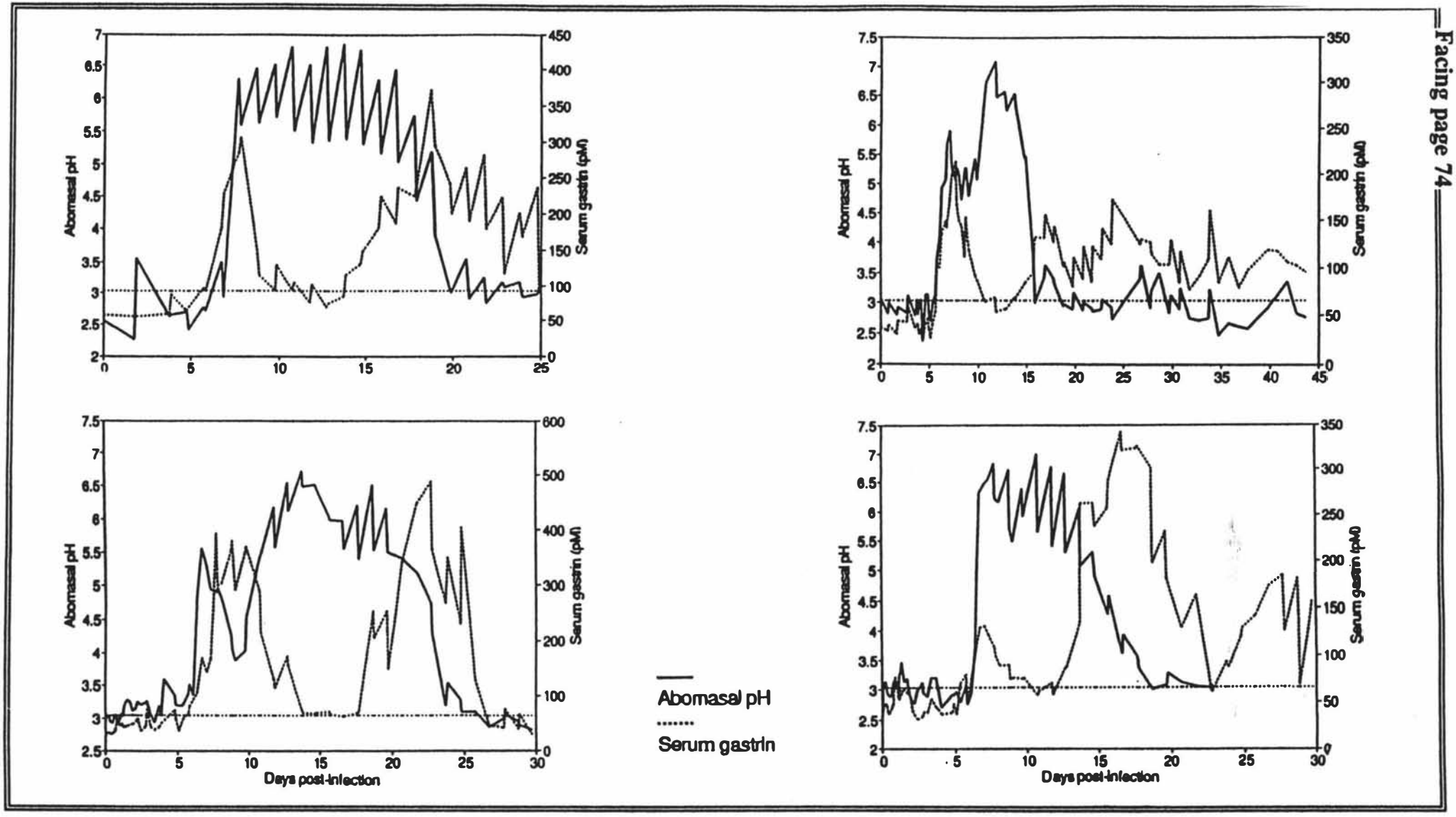

Figure 2.9 Abomasal pH and serum gastrin in sheep after infection with *O. circumcinta* larvae in which there was marked dissociation of the two parameters. The sheep shown in the top left is from Group B (50 000 L_3), top right from Group C (150 000 L_3 via tube) and lower left and right from Group D (150 000 exsheathed L_3). The horizontal dotted line denotes the upper limit of the normal range for gastrin (**μ+2s**) and **μ+1s** for pH.

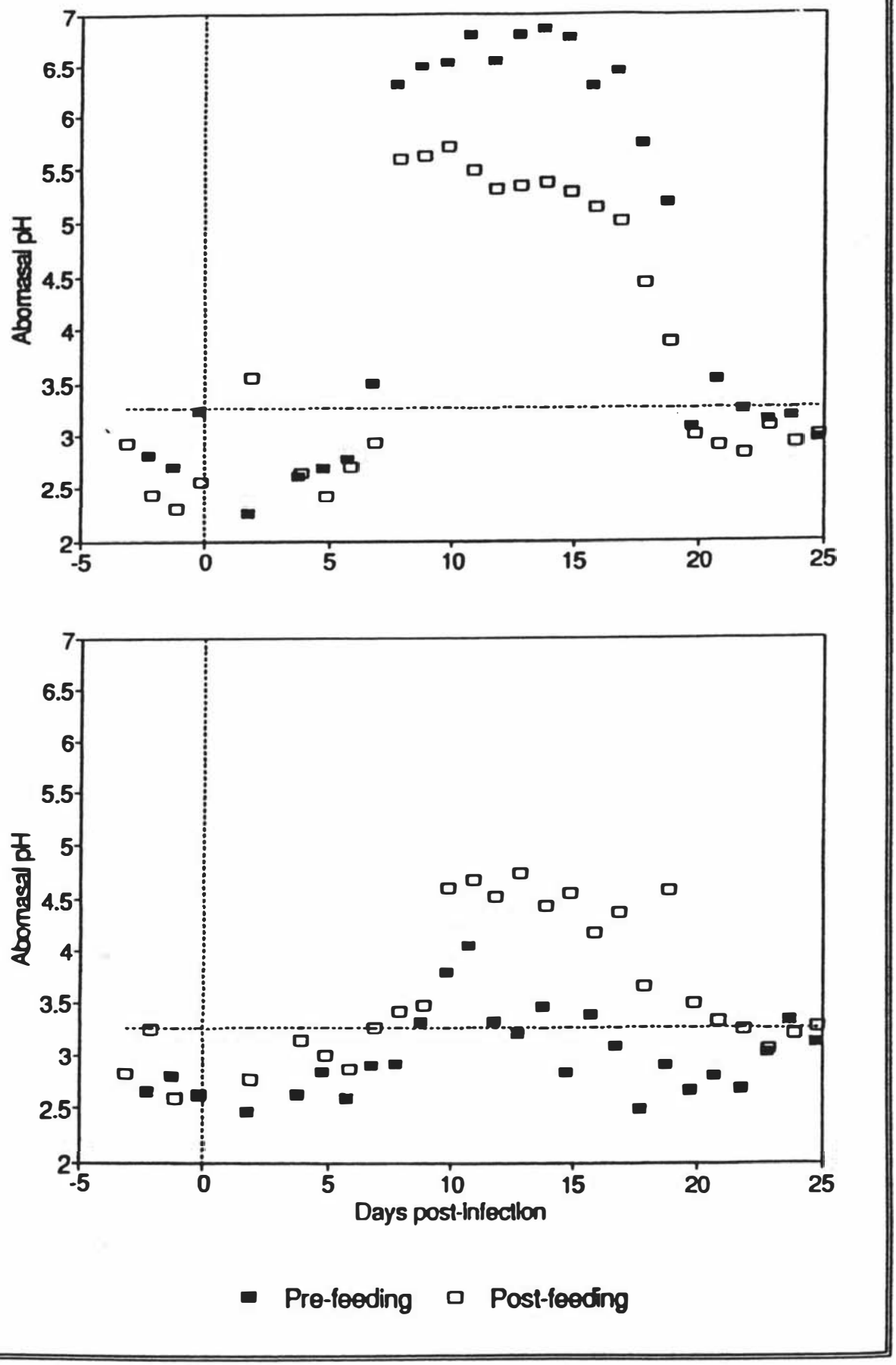

Figure 2.10 Effect of feeding on the abomasal pH in sheep infected with 50 000 *O. circumcinta* L_3: showing a post-feeding decrease (top) and a post-feeding increase (bottom). The horizontal dotted line denotes the upper limit of the normal range, defined as the mean plus two standard deviations ($\mu+2s$), derived from control sheep for pH and from individual preinfection samples for gastrin.

serum gastrin and abomasal pH around Day 5 there was clearly a dissociation between the two parameters for a variable period of time (Fig. 2.9) which occurred consistently under particular conditions. While abomasal pH was most raised, serum gastrin declined from a greatly elevated level, frequently to below **μ+2s**, and was followed by an equally dramatic rise again after about three days. In each case, the drop in serum gastrin was associated with severe hypochlorhydria of about pH 5.5 and above. Further, when the pH initially rose rapidly, the first major rise in gastrin essentially failed to occur. The association between high abomasal pH (>5.5) and a drop in serum gastrin was consistent in sheep infected with larvae. The abomasal pH exceeded 5.5 in only one sheep in Group E given 15,000 adult worms. In this sheep, the abomasal pH exceeded 5.5 for 22 hours but there was not a profound accompanying drop in serum gastrin.

2.3.4.4 FEEDING RESPONSE

In many infected sheep, abomasal pH was affected by feeding. In all sheep in which severe hypochlorhydria developed (pH>5.2), abomasal pH decreased after feeding (negative FR). As a group this was most evident in Group D from Day 10 to 15. In Group B, one sheep (#7) which developed marked hypochlorhydria, exhibited a FR of -1.18 during the 10 days of maximal disturbance (Fig. 2.10, top). In another sheep (#8, Group B) where hypochlorhydria was less severe (pH<5.2), the opposite occurred. In this animal there was a mean FR of +0.96 during the same 10 day period (Fig. 2.10, bottom). In a number of sheep, both phenomena were apparent: while pH was raised but below about 5.5, there was a positive FR but when the pH rose above about 5.5, this became a negative FR. In some sheep, this became a positive FR again when the pH declined below 5.5 later in the infection. A positive FR occurred also in one sheep in Group A which had a moderately raised abomasal pH.

Although serum gastrin was increased by feeding in the control animals only in Expt 2, a FR was evident in some infected animals (Fig. 2.7): those of Group B (also Expt 2) showed a group mean FR of +23pM two hours after feeding from Day 14 to 21; in Group D, a negative FR was predominant; and in Group A, the gastrin level initially fluctuated about **μ+2s** in response to feeding after infection, in one sheep the FR was positive while in the other it was negative.

Table 2.4 Relative wet weight of abomasum to body weight at post-mortem in parasite-naive sheep and sheep infected with *O. circumcincta* from Experiment 3.

Treatment	Time between post-mortem and infection	Sheep #	Abomasum weight (g)	Body weight (kg)	Relative abomasal weight
GROUP C		15	-	-	-
150,000 L_3, trickle	7 weeks	16	326	36.0	9.1
infection after 21		17	289	34.5	8.4
days		18	233	31.0	7.5
GROUP D		19	202	25.5	7.9
150,000 exsheathed	4 weeks	20	176	23.0	7.7
L_3 via cannula		21	221	27.0	8.2
		22	-	-	-
GROUP E		23	203	28.0	7.3
15,000 adult worms	8 days	24	183	23.0	8.0
via abomasal cannula		25	200	25.0	8.0
		26	167	23.0	7.3
CONTROL SHEEP		27	140	36.0	3.9
	Killed with	28	121	30.5	4.0
	Group C	29	173	36.0	4.8
		30	149	35.0	4.3
	Killed with	31	106	24.0	4.4
	Group E	32	99	21.0	4.7

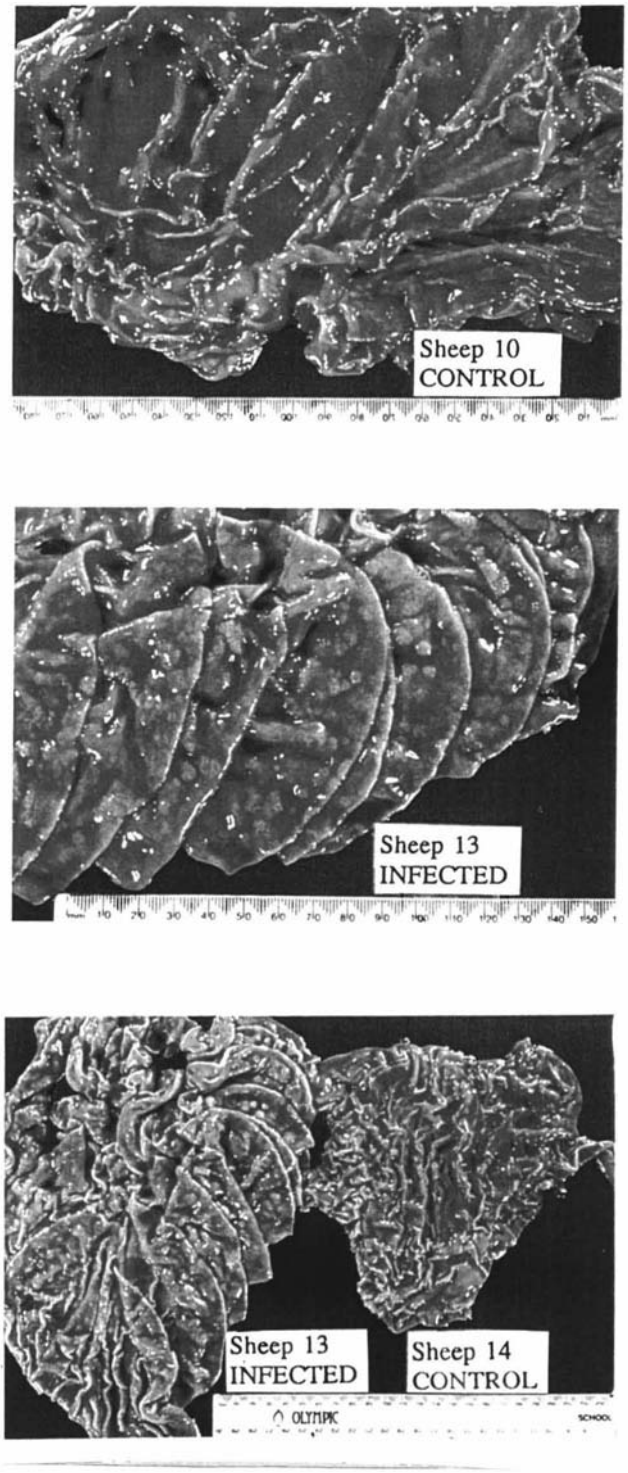

Figure 2.11 Photographs of the mucosal surface of the abomasum of a parasite-naive sheep (top), a sheep infected with 150 000 *O. circumcincta* larvae (centre) and of a parasite-naive sheep and infected animal (bottom). Nodular hyperplasia is evident in the infected abomasum (centre) and the increased size of infected abomasa when compared with those of a parasite-naive sheep (bottom). Sheep numbers in the photographs relate to numbers in the text as follows: Sheep 10 = 29, Sheep 13 = 17, Sheep 14 = 30.

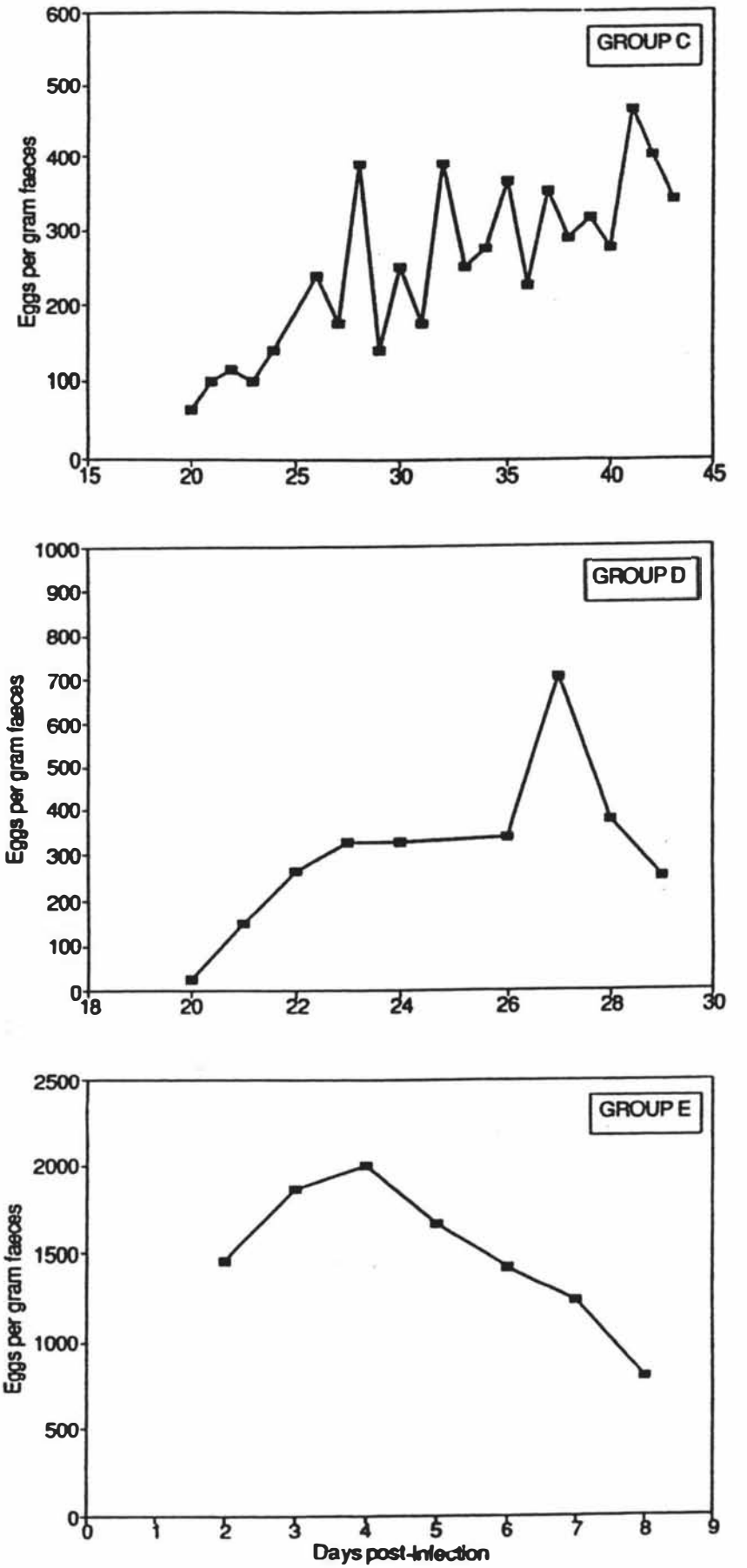

Figure 2.12 Mean faecal egg count of sheep in Experiment 3 after infection with either larval or adult *O. circumcincta*: Group C, 150 000 L_3 intraruminally; Group D, 150 000 exsheathed L_3 via cannula; Group E, 15 000 adult worms via cannula.

Table 2.5 Amount of gastrin extracted per gram of antral mucosa collected from parasite-naive sheep and sheep infected with *O. circumcincta*.

Group and treatment	Sheep #	Tissue gastrin content (pMoles/g)	Terminal plasma gastrin concentration (pM)	Relative abomasal weight (g/kg)
Group C 150 000 L_3, trickle infection after 21 days	16	200	73	9.1
	17	310	69	8.4
	18	740	79	7.5
Group D 150 000 exsheathed L_3 via cannula	19	280	30	7.9
	20	390	55	7.7
	21	340	44	8.2
Control sheep	27	2650	22	3.9
	28	2570	36	4.0
	29	2490	56	4.8
	30	2260	32	4.3
	31	2980	44	4.4
	32	1670	40	4.7

2.3.5 ABOMASAL WET WEIGHT

Where measured, infection increased the abomasal mass as a proportion of total body weight in all sheep (Table 2.4). For control groups, the mean abomasal weight was 4.35 g/kg body weight, compared with 7.65, 8.33 and 7.93 g/kg body weight for the groups infected with adults, larvae, and exsheathed larvae respectively. These were all significantly increased ($p<0.0001$).

The macroscopic appearance of the abomasal mucosa differed in parasite-naive and infected sheep (Fig. 2.11). In the former, it was pink coloured and was delicate and friable when dissected. In the sheep killed 45 days after infection, the abomasa were more brown in colour, larger, the walls and folds were thickened and the mucosa showed evidence of nodular hyperplasia.

2.3.6 TISSUE GASTRIN

The tissue gastrin content was significantly reduced ($p<0.0001$) in the infected sheep in Expt 3 compared with the control sheep (Table 2.5). For the control group, the mean (±SD) gastrin content per gram mucosa was 2440±440 pMoles/g compared with 420±290 and 340±60 in Group C and D respectively, a reduction to 17% and 15% of the control ratio.

2.3.7 PARASITOLOGY

2.3.7.1 PREVIOUS INFECTION (GROUP A)

The four sheep in Expt 1 were infected with parasites when acquired. Both *Monesia* segments and nematode eggs were seen. Following drenching, all returned negative faecal egg floats.

2.3.7.2 PARASITE-NAIVE SHEEP

Faecal egg floats revealed that some sheep used in Expt 3 were passing *Nematodirus* spp. eggs (Appendix 2.12), probably acquired from the aged hay. The mean number of eggs in each float from these sheep was 1.5 which indicates that only a very small

number of worms were present. Following drenching, all sheep returned negative floats.

2.3.7.3 FAECAL EGG FLOATS AND EGG COUNTS

During the experimental period, all control sheep returned negative floats. Sheep in the infected groups had negative floats until patency was reached. In Group B (50,000 larvae), eggs were first detected 18 days after infection and by the subsequent sample collected on Day 25, all four sheep were passing eggs. Sheep #5 to #8 were passing 150, 600, 2200 and 550 epg respectively on their final day (Day 25). The mean e.p.g obtained in Expt 3 are presented graphically in Fig. 2.12. Patency was first reached on Day 19 in Group C (150,000 larvae intraruminally) and on Day 20 in Group D (exsheathed larvae into abomasum). The mean egg production never exceeded 500 e.p.g and 700 e.p.g respectively. In Group E (adult transfer), sheep began passing eggs within 24 hours and egg production peaked on Day 4.

2.3.7.4 EXAMINATION OF ABOMASAL FLUID SAMPLES

The mean number of worms lost in the 1-2 ml abomasal sample taken for pH determination was 1.75 in Group E (adult transfer). No mature eggs were seen until Day 28 in any female worms found in samples taken from sheep intraruminally infected with 150,000 larvae. The maximum number of maturing eggs seen in any individual female worm was 11.

2.3.7.5 POST-MORTEM PARASITOLOGY

The worm population in the abomasal contents obtained at post-mortem was approximately 3000 per animal in Group B and 3300 in Group D. No other species of abomasal nematode was seen. A few *Cooperia* and *Nematodirus* were found in the caecum and small intestine of some sheep, but in very low numbers e.g. only two *Nematodirus* were found by examining the entire small intestinal contents from one sheep in Expt 3.

2.4 DISCUSSION

These experiments have reaffirmed that infection of sheep by the abomasal nematode *O. circumcincta* is associated with marked increases in serum pepsinogen, serum gastrin and abomasal pH. These occurred in all infected sheep, including two animals previously exposed to field parasitism and sheep given adult worms directly. The intensive sampling regime assisted in relating the aetiology of the pathophysiological changes to one another and to the life cycle of the parasite.

2.4.1 INTERPRETATION OF RESULTS

The control sheep which remained parasite-naive during the experimental period were used to define the normal ranges for serum pepsinogen, serum gastrin and abomasal pH against which values obtained after infection were compared. These parameters showed no major changes in uninfected animals, but fluctuated constantly within the normal range, with the exception of the unusually high serum gastrin values in Expt 2 controls, in turn, considered to be a conditioned response to the once daily feeding regime. Since the normal range (μ+2s) was derived using both pre- and post-feeding values, any normal feeding responses are included in s and effectively widen the normal range. The *normal* feeding response was never large enough to overshadow any effect of the parasitism.

Feeding responses occurred mainly in Expt 2, as well as in some individual sheep (Table 2.1). In Expt 2, serum pepsinogen and abomasal pH decreased and serum gastrin increased, which is consistent with previous observations in which there were increases in serum gastrin (Reynolds *et al.*, 1978) and the secretion of HCl and pepsin from fundic pouches (McLeay & Titchen 1970, 1977a,b) following feeding. Despite an increased acid output, however, the pH of the abomasal contents was found to rise by Reynolds *et al.* (1978). When the sensitivity of the assay procedures used is taken into account, only the effect on serum gastrin in Expt 2 (i.e. control group μ+2s = 139 pM) was of sufficient magnitude to necessitate consideration of individual levels rather than values derived from the control group. The high mean pre-feeding gastrin levels in these sheep also suggests a conditioned response. This may have been related to the more restricted diet given to these sheep (600 g of lucerne nuts compared with 800 g in Expt 3) which possibly heightened their anticipation of feeding; McLeay & Titchen (1970, 1975)

reported that teasing sheep with food produced a rapid increase in acid and pepsin secretion which they considered vagally-mediated and secondary to gastrin secretion.

The interpretation of the effects of *Ostertagia* on serum pepsinogen, serum gastrin and abomasal pH is heavily dependent on the criteria used to define elevated values: in this study those above two standard deviations from the mean. This criterion was also employed by Anderson *et al.* (1985). The data for all three parameters conformed to a normal distribution (Fig. 2.1), in spite of pH measurements being on a logarithmic scale, so that approximately 95% of sample measurements will fall within 2s of μ and only 2.5% of normal values would exceed the designated upper limit. Since two successive normal samples would exceed this level only 0.0625% of the time, the parameter was determined to be significantly raised from the earlier of these. Although values for μ+2s determined from the whole group may not best represent the 95% range for individual sheep, it was considered to be the best choice given the limited number of preinfection samples collected. Collection of a large number of samples from each sheep and examination of the data animal by animal may be the best procedure.

The values for the three parameters calculated from the overall data (μ and μ+2s) - serum pepsinogen, 223 and 454 mU tyrosine/L; serum gastrin, 38 and 64 pM (excluding Expt 2); abomasal pH, 2.80 and 3.26 - were similar to data reported by other authors. The serum gastrin levels were within a similar range to those recorded by others in parasite-naive sheep (e.g. Anderson *et al.*, 1976a, 1985). The mean serum pepsinogen concentration was similar to that reported by Holmes & MacLean (1971), Anderson (1972) and Coop *et al.* (1977). The assay used in the present study utilises serum protein as the substrate and any value below 500mU/L using this method should be considered normal. In assays in which additional substrate is added, substitution of haemoglobin for albumin approximately doubles the amount of hydrolysed product (Berghen, 1987), which may account for the wide range of pepsinogen concentrations used to indicate bovine ostertagiasis (Hilderson *et al.*, 1989). In the present study, all sera were assayed by the one method so that the absolute value is not critical, nevertheless, attempts that were made to quantify pepsinogen activity in sera using commercially-prepared porcine pepsinogen (Sigma Chemical Co., USA), but these were unsuccessful. Few publications that present pepsinogen data address the method of its determination in any detail and almost none determines the actual enzyme activity.
The mean abomasal pH values were within the range reported by Ash (1961a,b). The effect of diet (type and amount) observed by McLeay & Titchen (1974) was not

apparent here. The mean abomasal pH and the standard deviations in the control groups and in individual sheep were very similar in the three experiments despite differences in the diet and feeding logistics and the effect on serum gastrin in Expt 2. This similarity would suggest any minor differences in the position of the cannulae were unimportant, that the normal pH at the body/antral junction is very constant and that abomasal pH is closely regulated.

The criteria (μ+2s) used to define abnormal values are probably conservative, particularly for abomasal pH. Since antral pH regulates the secretion of gastrin, a small change in pH may have a significant effect on gastrin despite being well within the normal range. There are no data in sheep defining the actual relationship between gastric pH and gastrin secretion, although a reduction in acid secretion followed perfusion of antral pouches with solutions of pH 2.7 (McLeay & Titchen, 1977b). Therefore, it is likely that acid inhibition occurs well within 1s of the mean pH and, conversely, that gastrin secretion may increase with a comparably small increase in abomasal pH. The effect of abomasal pH changes within the μ+2s range on gastrin secretion is clearly of concern, since abomasal pH may well rise to a level *in vivo* that is associated with gastrin hypersecretion while not exceeding μ+2s, or even μ+1s. It was for this reason that the latter level was also considered when examining the data.

2.4.2 DIRECT TRANSFER OF ADULT WORMS

A dramatic rise in serum pepsinogen and gastrin and abomasal pH followed the direct transfer of 15,000 adult *O. circumcincta* into the abomasa of four sheep. This clearly demonstrated that the presence of larvae and the associated morphological disturbances are not essential to affect the secretory function of the abomasum. Serum pepsinogen was significantly elevated after 8 hours and appears to have began to increase almost immediately. A similar rapid rise was reported by Anderson *et al.* (1985) following the transfer of adult *O. circumcincta* into the abomasum of sheep and by McKellar *et al.* (1986, 1987) who transferred variable numbers of adult *O. ostertagi* into calves. The major discrepancy between the present study and the three previous ones concerns the timing of the changes in serum gastrin and abomasal pH and whether, in fact, the pH did increase in the calves. In this study, after the earlier increase in serum pepsinogen, all three parameters generally showed similar temporal changes, beginning to decrease again after Day 6. Since faecal egg counts decreased from Day 4, this may have been

related to the loss of worms from the abomasum.

In the present study, serum gastrin was raised 19 hours after infection and was accompanied by a simultaneous elevation of abomasal pH, in contrast to findings in the other studies. In none of the four sheep, irrespective of whether the rise were fast (Fig. 2.3) or slower (Fig. 2.4), could it be confidently concluded that either serum gastrin or abomasal pH increased before the other, particularly if pH values above **μ+1s** are considered physiologically elevated. Anderson *et al.* (1985), however, reported that abomasal pH increased only 5-7 days after the transfer of adult *O. circumcincta*, a time interval comparable to that following larval infection and a few days after they considered serum gastrin to be raised. Although some larvae were present in the mainly adult population, they estimated that less than 1% were early L_4. Also, the early rise in serum pepsinogen they reported indicates that there was significant adult worm activity as the present study has shown that such a rapid change is not produced by larvae alone. This applies too, to the work of McKellar *et al.* (1987), in which no subsequent rise in abomasal pH was considered to occur. Since serum gastrin was sustained above preinfection levels from 24 hours, 48 hours and four days in the three sheep given the 'mainly adult' population by Anderson *et al.* (1985), they concluded that serum gastrin increased before pH following adult transfer, just as they had done earlier (1981) following larval infection. This conclusion hinges on the definition of a raised abomasal pH which Anderson *et al.* (1985) determined from the preinfection samples since no control sheep were used. It was noted earlier (2.4.1) that they used a similar system to that used here (2 x the fiducial limits) although they did not present these values. However, from their data for one sheep receiving 'mainly adults' (a preinfection mean (μ) = 3.18, SEM = 0.235 and 8-10 preinfection samples), it can be estimated that s is between 0.66 and 0.74 and **μ+2s** is, therefore, between 4.51 and 4.66 for pH as defined in the present study. This is much higher than the **μ+2s** value of 3.27 determined for pH from over 1000 sample collections in the present experiments. It is not surprising, therefore, that in this animal they did not consider pH raised (by their definition) until Day 6. In addition, gross enlargement of their Figure 2 and subsequent scrutiny of this using values of **μ+2s** of 66pM (gastrin) and 3.27 (pH), as determined in Expt 3, indicates that in only one animal did serum gastrin rise before abomasal pH.

McKellar *et al.* (1987) supported the conclusion of Anderson *et al.* (1985) that hypergastrinaemia preceded hypochlorhydria when they found that the pH did not rise in any of six calves directly infected with adult *O. ostertagi*. Unfortunately, once again,

no reference was made to the definition of what constituted an abnormal value for any parameter or whether pre-infection samples were taken. The number of adult *O. ostertagi* given by McKellar was comparable to the dose of adult *O. circumcincta* given in the present study, which clearly caused pH to rise to between 5 and 6 in all four animals. The lack of response in the previously-infected 'Group 1' calves reported by McKellar *et al.* may be explained by the persistence of a significant worm population after fendendazole treatment. McKellar *et al.* (1987) ascribe a 95% efficacy to the fenbendazole treatment used to purge these sheep of worms, although this is calculated from an assumed 100% establishment of the 100,000 *O. ostertagi* L_3 given by trickle infection and no loss of worms over the six week period between commencing their infection and slaughter of the control animal. A more realistic level of establishment might be 30%. Indeed, it would be more appropriate to regard the anthelmintic treatment as ineffective rather than of high efficacy, and that a moderate worm burden was present in this group at re-infection (the control animal that was not re-infected after drenching had 3,700 worms, six weeks after initial infection). Results from the sheep in Group C in the present study have shown that when further larvae are given over and above a pre-existing infection there is not necessarily renewed hypochlorhydria. That no pH change occurred in the naive 'Group 2' calves is, however, of some interest, although there was also essentially no increase in serum gastrin in any of these animals. A marked gastrin response was seen in two calves but these were both from 'Group 1' which were probably already parasitised at transfer and cannot, therefore, be attributed to an effect of adult worms. The lack of both a gastrin and pH response in 'Group 2' may indicate that the activity level of the adult worms in their experiment or the response in cattle fundamentally differs from that in sheep.

The present study has clearly established that the transplantation of adult worms can mimic the secretory lesions seen in a naturally occurring or experimental infection by larval *O. circumcincta.* It has confirmed an almost immediate increase in serum pepsinogen concentration and that serum gastrin and abomasal pH increase almost together, but some 10 hours after serum pepsinogen. What was particularly astonishing in this study was that the 76% increase in relative wet weight which occurred after only eight days infection with adult worms was 84% of the total increase that followed seven weeks infection of larval origin. These observations raise questions concerning the role of larval invasion of the gastric glands in the aetiology of the disease.

2.4.3. PATHOPHYSIOLOGY AND PARASITE DEVELOPMENT

Administration of several doses of *O. circumcincta* larvae via the rumen and the direct transfer of exsheathed larvae into the abomasum has allowed correlation of the pathophysiological effects of the parasites with the different stages of the life cycle (described by Threlkeld (1934); Sommerville (1954); Armour *et al.* (1966); Denham (1969), Chapter 1, 1.5.1). These are supported by similar correlations between the life cycle and biochemical changes in cattle infected with *O. ostertagi* (Jennings *et al.*, 1966), although the timing differs because of the slower development of that parasite in cattle.

In all experimental groups infected with larvae, there was increased serum pepsinogen, serum gastrin and abomasal pH. As these changes also occurred in two animals previously exposed to field parasitism as well as in parasite-naive sheep, the effects of the parasites are not unique to primary infections. Expt 1 was primarily a pilot study and involved sheep with an unknown parasitological history. The magnitude and timing of the physiological disturbances differed from those in the naive sheep: there was a lesser, more erratic increase in abomasal pH; serum gastrin was barely raised; for all three parameters the disturbances took longer to develop. Since these sheep were given a lesser dose of larvae than the other groups, these differences cannot be attributed with certainty to the previous exposure to parasites. However, Anderson *et al.* (1976b, 1981) reinfected sheep which they had previously challenged and reported similar findings. Reinfection of sheep with larvae after anthelmintic treatment by Anderson *et al.* (1976b) resulted in a lesser and more transient pH disturbance. In 2 of 4 of these sheep, abomasal pH did not exceed 3.5, however, anthelmintic treatment of these 2 animals in particular was not completely effective. Further trickle infection of sheep in the present study did not lead to a further pH increase. The accompanying serum gastrin concentrations (subsequently published by Anderson *et al.* in 1981) were increased, but much less profoundly than following primary exposure, and plasma pepsinogen concentrations were re-elevated from four days after reinfection. In the present study, serum pepsinogen clearly did not increase until Day 12 although a small transient disturbance occurred at the time of the abrupt increase in the other groups. Interestingly, Anderson *et al.* (1976b) reported that this disturbance increased in severity 12 days after infection.

In the naive sheep infected with larvae, the disturbances to abomasal function involved some consistent pathophysiological features, regardless of the dose of larvae or the route of infection, which were more clearly seen in sheep receiving the highest number of larvae:

(1) a small increase in all parameters within the first 2-3 days that was not usually significant and was only clearly seen in Groups C and D;

(2) a sudden rapid increase in all three parameters on Day 5-6 regardless of dose or route of infection or whether already raised;

(3) between Days 10-12, a second phase of rapid increase in serum pepsinogen in Groups B, C and D and in abomasal pH in Groups C and D which coincided with the more delayed pepsinogen response in Group A and to a lesser extent with further gastrin and pH disturbances. The serum gastrin level was often markedly reduced in sheep with extremely high abomasal pH;

(4) from Days 12-18, there was sustained elevation of serum pepsinogen and abomasal pH, in many cases declining from about Day 15. Serum gastrin levels varied and, in part, this was dependent on the abomasal pH;

(5) By Days 19-21, abomasal pH was at or below the upper limit of normality whereas serum pepsinogen and gastrin remained elevated for the rest of the infection. The abomasal pH did not re-elevate, even with the ensuing trickle infection.

Previous studies, which involved the collection of relatively few samples over the initial post-infection period, are consistent with these observations. Anderson *et al.* (1976b) collected samples at 4 day intervals and found plasma pepsinogen was elevated on the fourth day post-infection, which is consistent with the minor increase in serum pepsinogen within the first 2-3 days. Anderson *et al.* (1976b, 1981) reported an increase in plasma gastrin which they considered preceded the rise in abomasal pH in sheep infected with 20,000 larvae thrice weekly and concluded that hypergastrinaemia arises from some form of parasite stimulation and not from a change in abomasal pH. They do not however clearly state what it is that they define as a normal or abnormal pH although they do refer to the abomasal pH in their sheep as being either above or below 4. Reference is also made to the work of Becker *et al.* (1973) in dogs in which plasma gastrin increased when the antrum was perfused with solutions at or above pH 4. In the absence of any other information, it appears that Anderson *et al.* (1976b, 1981), unlike Anderson *et al.* (1985), considered abomasal pH values below 4 to be normal. In the present study, values above 3.27 were considered abnormal and, as noted by Titchen & Reid (1988), the threshold pH for elevation of gastrin has not been defined in the sheep

(perhaps this is within one or two s of μ). Thus, in light of a more or less synchronous rise in abomasal pH and serum gastrin in the present study, the emphasis placed on the development of pre-hypochlorhydric hypergastrinaemia by Anderson *et al.* (1976b, 1981) may be unjustified. In addition, Anderson *et al.* (1981) took blood samples only at four day intervals while, in the present study, blood was collected every four or six hours over this stage of infection (4-10 days) in those groups given 150,000 larvae. Therefore, at least with respect to the initial development of these lesions, the present study rejects their conclusion and suggests rather that the development of hypochlorhydria and hypergastrinaemia are temporally associated, particularly if the μ+1s level for abomasal pH is considered (Fig. 2.8), although not necessarily interdependent.

The timing of the pathophysiological effects following larval infection can be related to the development of the parasite. Upon reaching the abomasum, the larvae invade the gastric glands and pits and four days after infection most larvae are within the glands and have completed the third ecdysis (Armour *et al.*, 1966; Denham, 1969). In this initial period, only small and generally insignificant disturbances occurred over the first three days and were apparent only in sheep given 150,000 larvae. Compared with the immediate increase in serum pepsinogen and the slightly later but still very rapid increase in serum gastrin and abomasal pH after the transfer of adult worms (Group E), larval invasion and growth to the L_4 stage has remarkably little effect on abomasal secretions. The direct introduction of exsheathed L_3 parasites into the abomasum (Group D) intensified only relatively slightly the effects on abomasal secretion during this period and that this was unrelated to the intensive sampling regime was not shown. It appears, therefore, that the failure to see profound effects on abomasal secretion over the first few days after infection with larvae is not due to their protracted arrival from the rumen so that the effect on the tissues is less than if there were a concentrated invasion of the glands. It seems that the L_3 stage has only mild affects on abomasal secretion in spite of the marked effects it can have on abomasal morphology. This may be because suspected ES products are not produced by this stage, the physical effect of larvae and adults on the tissues differs, the biomass or worms is too small or inflammatory processes are not so readily stimulated.

The apparently greater effect of exsheathed larvae on serum pepsinogen compared with larvae given intraruminally may be the result of either (1) the exclusion of two 'low responder' sheep from the group mean of those receiving exsheathed larvae, leaving only the data from two animals or, (2) a greater percentage establishment compared to

the group given 150,000 intraruminally. Unfortunately, the collection of parasites and tissues at post-mortem for other work did not afford retrospective analysis of the parasite burden. Dakkak *et al.* (1981) found that approximately 50% of *H. contortus* larvae placed in the rumen arrived in the abomasum without exsheathing, and as the exsheathing conditions are provided by the rumen (Rogers & Sommerville, 1960), it is possible that those sheep infected intraruminally are effectively given a smaller dose.

After ecdysis, larvae may either emerge from the glands as early fourth stage larvae (from Day 4 to 5), continue to grow in the mucosa to late fourth stage or immature adults before emerging, or enter a hypobiotic state (Sommerville, 1954, 1963). Although Armour *et al.* (1966) found that about 80% of all worms were still in the mucosa on Day 8, it has been personally observed that sheep infected with 100,000 *O. circumcincta* larvae and killed on Day 7 have a large number of worms in their abomasal contents and that these were all L_4. The substantial increase in all three parameters on Day 5-6 coincides with the start of L_4 emergence and may be attributed to disruption of the mucosa or alternatively to the presence of the parasite at a later stage of development at which it is capable of affecting abomasal function.

A second wave of emergence comprising mainly immature adults is completed by Day 12 (Armour *et al.*, 1966). In cattle, development of *O. ostertagi* to a similar stage usually takes 21 days (Armour & Ogbourne, 1982). The earliest that immature *O. circumcincta* adults occur in the "lumen" appears to be on Day 8 (Denham, 1969). Gravid females have been observed on Day 12 (Denham, 1969) and patency on Day 13 (McLeay *et al.*, 1973). This emergence probably accounts for the second disturbance to abomasal function with which the increase in serum pepsinogen in Group A sheep coincided. The maximal disturbances observed between Day 12 and 15 has been attributed by Anderson *et al.* (1981) to the presence of adults in the abomasum rather than with the emergence itself, as previous workers had proposed. This would seem likely given that the direct transfer of adult worms mimics this phase of the infection. However, it is curious that the disturbances following larval infection in this study were ameliorated and, at least abomasal pH, had returned to relatively normal levels while a substantial and nascent adult population was present and before patency was reached.

Other features of the secretory lesions following larval infection are of considerable interest. The effect of feeding on abomasal pH was dependent on the degree of hypochlorhydria and confirms the magnitude of the presumed acid inhibition in the

infected abomasum (it is possible that abomasal pH may increase due to increased HCO_3^- secretion and not by decreased H^+ secretion). In severely hypochlorhydric sheep (pH above 5.5), the inability to acidify the contents was nearly complete and the abomasal pH was lowered by the incoming digesta. In less severely hypochlorhydric sheep, there was an increase in abomasal pH immediately after feeding but the residual capacity for acidification allowed the pH to be lowered over the following 24 hours until the next feeding period. These observations also suggest that the pH of digesta entering the abomasum was between pH 5.0 and 5.5. In most infected sheep, serum gastrin increased after feeding indicating that despite high basal secretion levels the G cells were still able to respond to further stimulation and were not secreting maximally. The sheep infected with exsheathed larvae were an exception in showing a decrease in serum gastrin after feeding. However, due to spatial restrictions these sheep were housed separately from the others and as a group developed strong patterns of behaviour in anticipation of feeding and had high pre-feeding serum gastrin levels while infected which were unique to this group. In many sheep there was a decrease in serum pepsinogen after feeding. This is consistent with increased secretion at this time reducing the diffusion gradient into the circulation. There is no evidence to suggest that the secretory response to feeding of the body region (fundus) described by McLeay & Titchen (1970) is lessened during ostertagiasis, only *presumably* that its acid component declines.

A feature of the present experiments was the marked decrease in serum gastrin at the time of severe hypochlorhydria (Fig. 2.9). This may be the result of the release either from the parasites, the parasitised tissues or the abomasal microflora of a potent suppressor of gastrin release. The infection of sheep with *H. contortus* (Nicholls *et al.*, 1987) and cattle with *O. ostertagi* (Jennings *et al.*, 1966) caused microbial proliferation and a change the composition of the microbial species. Either the change in composition of the microflora or the generation of gastrin inhibitors at high abomasal pH but not at the normal pH may account for this drop in serum gastrin. The role of the parasites and of the microorganisms have been examined in *in vitro* studies (see Chapters 5 and 6) and strongly support the origin of this gastrin inhibition being the generation of chemicals by the abomasal microorganisms. The anomalous depression of gastrin-hypersecretion in almost all animals infected with larvae at some stage suggests the need for caution when considering the diagnostic value of serum gastrin.

2.4.4 AETIOLOGY OF THE SECRETORY LESIONS

Although the morphological and functional lesions caused by ostertagiasis are well known, there is still little knowledge of how these lesions are induced by the parasite. A number of fundamental questions remain unanswered. How closely related are the morphological and function lesions caused by the parasites? Is abomasal secretion disrupted by the physical effects of the parasites on the tissue or are parasite secretory products responsible? Do the parasites produce their effects directly on the parietal, chief and gastrin cells or are some or all of them induced indirectly by inflammatory or immune processes provoked in the host tissues? There is little direct evidence pertinent to these questions and much is circumstantial.

2.4.4.1 DISRUPTION OF THE INTRINSIC MUCOSAL BARRIER

Traditionally, increased serum pepsinogen has been attributed to a'leak lesion' (Jennings *et al.*, 1966) which allows the retrograde diffusion of accumulated non-activated pepsinogen into the blood (Murray *et al.*, 1970). Coincident with the increase in serum pepsinogen, there is an increase in luminal Na^+ (McLeay *et al.*, 1973) and loss of plasma into the gut (Holmes & MacLean, 1971). Direct observation of the lesion has been achieved histologically (Murray *et al.*, 1970). Murray (1969) found that 50 to 90% of the plasmalemmata forming the zonular occludens were either completely or partially separated in cattle with ostertagiasis. The failure by Stringfellow & Madden (1979) to detect an increase in mucosal permeability using horseradish peroxidase as a tracer in calves after primary infection with *O. ostertagi* led McKellar *et al.* (1987) to suggest that chief cells may be stimulated to secrete pepsinogen directly into the blood by the parasites or their products. Subsequently, McKellar *et al.* (1990a) demonstrated that presumed ES products prepared from *O. ostertagi* were slightly stimulatory to pepsinogen secretion from isolated bovine gastric glands. Despite less than 9% stimulation they concluded that these products were 'extremely potent'.

The increased permeability may result from physical disruption of the intrinsic mucosal barrier while larvae grow within the gastric glands as proposed by Jennings *et al.*, (1966) or, alternatively, as McKellar *et al.* (1987) have suggested, increased permeability may be associated with products of the parasite. If so, this might account for the rapid increase in serum pepsinogen observed after the transfer of adult worms. Certainly, the

speed of the response tends to suggest that it is chemically and not physically mediated. This does not, however, in anyway preclude a physical effect associated with larval development, nor does it indicate that if a chemical effect is associated with the response to adult worms, that this is restricted to this mature stage. Although 15,000 adult worms were transferred to the sheep in Group E, the biomass of these adults is many times greater than 150,000 larvae.

Another suggestion proffered by McKellar *et al.* is that adult parasites may migrate into and out of the mucosal layer. This seems unlikely as only very rarely are adults found within the mucosa, although they commonly are closely associated with its surface (W.A.G. Charleston, personal communication). However, the sensitivity of the adult stages to acid was demonstrated by Eiler *et al.* (1981), suggesting that the adult worms, in order to survive after transfer in this and other studies, may require rapid establishment of the worms within a niche in which the pH is more moderate, at least until hypochlorhydria develops. Such an environment would be provided by the mucus and unstirred layer. The mucus in highly effective in maintaining a measurable pH gradient between the lumen and the surface of the mucosal cells, at least while it is intact: there is a gradient across the mucus layer from pH 2 in the lumen to neutrality at the cell surface in the pig (Williams & Turnberg, 1980). By establishing themselves within this niche, the adult worms may disrupt the layer and thus expose the surface epithelial cells to the harsh luminal environment. Whether this initiates an inflammatory reaction in the sheep or some other host response is purely speculative, although it is most probable as noxious stimuli in general provoke inflammation. It is interesting that Durham & Elliot (1975), who consider the morphological changes inflammatory, describe the mucosal response to *O. circumcincta* infection as more like that caused by an irritant toxin than by parasitism. Substantial inflammation may have caused the increase in mucosal wet weight that occurred in all infected sheep and within eight days of adult transfer. Sodium cromoglycate (a mast cell stabilizer) did not reduce the pepsinogen response in sheep infected with *O. circumcincta* (McKellar & Bogan, 1987) which suggests that, if such a reaction occurs, mast cell-derived histamine is unlikely to be a major factor. This assumes that sodium cromoglycate is effective in the sheep. Localization of the adult worms in the mucus layer would also enhance the effectiveness of their ES products as these would be concentrated in the vicinity of their putative action.

The minimal pepsinogen response in some sheep, which were designated 'low

responders', was of interest. These sheep had the lowest preinfection serum levels of their respective groups and raise the question whether the serum concentration is an indicator of the magnitude of pepsinogen release by the abomasum or whether it is an index of mucosal permeability. If it is the latter, these sheep may prove a useful tool in studying the leak lesion associated with parasitism. These sheep also serve as a reminder that serum pepsinogen (on its own) is not a reliable indicator of ostertagiasis in the individual animal.

2.4.4.2 PARIETAL CELL INHIBITION

Inhibition of acid production could be induced by the parasite either directly by the loss of mucosal integrity or by production of secretory products or indirectly through inflammatory processes. As yet it is not known which of these inhibits parietal cell activity or effectively inactivates them, although McKellar *et al.* (1990b) were unable to demonstrate any effect of presumed *O. ostertagi* secretions on acid production by isolated bovine parietal cells *in vitro*. It is known that the disturbance is effected locally and requires the presence of the parasite. Acid secretion by separated pouches prepared from the body of the abomasum actually increased when the main portion of the abomasum that was infected with *O. circumcincta* became hypochlorhydric (McLeay *et al.*, 1973). Inhibition of acid secretion has been attributed to a loss of function of the parietal cells. In the present experiments, the effect of feeding demonstrated the almost complete inability to reduce the pH of the digesta in some animals (Fig. 2.10).

The parietal cells in parasitised tissues have an altered appearance typical of non-secreting cells. Ritchie *et al.* (1966) and Murray *et al.* (1970) describe how larval growth in the glands and their emergence from these is associated with replacement by hyperplastic and undifferentiated cells and Jennings *et al.* (1966) state that it is this that results in failure to secrete acid in primary bovine ostertagiasis. However, it is highly unlikely that these cells are actually replaced, as parietal cell renewal takes several months (Lipkin, 1973; Willems, 1991) despite rapid renewal of other epithelial components (Attaix *et al.*, 1984). The entire surface epithelium is renewed within three to six days in most adult mammals (Willems, 1991). Perhaps, the apparent *undifferentiated* appearance of the parietal cells reflects their inactivity or inhibition. McLeay *et al.* (1973) observed that the parietal cells of infected sheep lacked canaliculi and displayed other features they considered suggestive of inactivity or inhibition. This

interpretation is perhaps the most reasonable explanation as it has been found that the ability of these cells to secrete acid returns within 2-3 days after anthelmintic treatment (Anderson *et al.*, 1976b). The recovery of function of the parietal cells from about Day 15 in the present experiments, especially the return of abomasal pH to near normal values before patency, was surprising particularly when the transfer of adults produced such potent effects. Why did the recently transferred adult worms cause the abomasal pH to rise while abomasal pH declined at what was likely the peak of adult worm numbers in larvae infected sheep? Similarly, the ineffectiveness of the trickle infection to re-elevate the abomasal pH contrasts with the reinfection of previously-parasitised animals (Group A) and conflicts with other reports. Fourteen weeks after infection with *O. circumcincta*, five of eight sheep that were trickle infected still had abomasal pH values above 4.7 (Sykes & Coop, 1977).

Functional secretion of acid by the parietal cell is dependent on its polar orientation (Forte & Soll, 1989). Loss of mucosal integrity, from any of the causes discussed above, may thus compromise the ability of these cells to function effectively. Christie (1970) suggested that the loss of integrity may result in increased pH but through consequential equilibrium with tissue fluids. The cause of increased abomasal pH was not determined in the present study. In the present study, abomasal pH returned to relatively normal levels before serum pepsinogen, which suggests that a permeability lesion still persisted. However, assuming that damage to the intrinsic barrier affects the secretory function of parietal cells, there need only be a partial loss of the intrinsic barrier for pepsinogen to enter the blood while an almost complete disruption of the barrier would be necessary to prevent the acidification of the contents. While the performance of some cells would be compromised, others would probably secrete acid effectively. The work of Holmes & MacLean (1971) is interesting in this regard. They considered that their work confirmed the 'leak lesion' proposed by Jennings *et al.* (1966) in which pepsinogen enters the blood and plasma proteins leak into the gut during the early stages of *O. circumcincta* infection. However, there was a more prolonged elevation of plasma pepsinogen compared with the loss of plasma. They, therefore, concluded that pepsinogen does not reflect accurately the permeability of the mucosa. Their profile of plasma loss was similar to that of abomasal pH in the present study.

Compromise of the intrinsic mucosal barrier may affect the parietal cells in other ways. Release of inflammatory mediators, perhaps responsible for the lesion, or processes initiated to counteract its effects may directly affect acid secretion. For example,

prostaglandins are credited with a cytoprotective function in the alimentary tract (Robert *et al.*, 1979). As well as promoting epithelial renewal, these substances inhibit the secretion of acid by parietal cells (Hirst, 1989). After primary infection of the rat with *N. brasiliensis*, prostaglandin-E increased 10-fold in intestinal tissues (Dineen & Kelly, 1976). Shaw *et al.* (1987) suggest that epidermal growth factor may be released from activated platelets during acute injury to the gastric mucosa which may, through its inhibitory effect on acid secretion, prevent the accumulation of acid under the protective cap and mucus and fibrin which forms over the damaged area. IL-1 (α and ß) have both been shown to inhibit acid secretion (Wallace *et al.*, 1991). If IL-1 is the most potent inhibitor of gastric acid secretion known (Chapter 1, 1.3.3.4.5) (Robert *et al.*, 1991) and its effect is mediated through prostaglandin-dependent pathways (Saperas *et al.*, 1990) or by inhibition of gastrin-stimulated histamine release (Wallace *et al.*, 1991), then hypochlorhydria in the face of hypergastrinaemia may be explained. The role of these and other host derived putative substances in the aetiology of hypochlorhydria during ovine ostertagiasis is purely speculative and untested.

2.4.4.3 GASTRIN HYPERSECRETION

Elevation of serum gastrin could be entirely secondary to the elevation of abomasal pH or caused by any of the mechanisms suggested for serum pepsinogen or abomasal pH. Certainly, the ovine G cell will hypersecrete when abomasal pH is raised by infusion of sodium bicarbonate or by rumen contents (Reynolds *et al.*, 1991). Controversy remains over whether the hypergastrinaemia of ostertagiasis is secondary to acid inhibition or whether it is partially or completely independent, depending on whether the two appeared to increase simultaneously or not in each particular study. The difficulty, as previously discussed (2.4.1) is in the definition of an abnormal value, particularly for abomasal pH. In the present study, the initial increase in the two parameters could not be considered independent both after infection with adult and larval *O. circumcincta*, however, later in the infection there was an apparent independence. First, while pH levels were at their most raised, serum gastrin was lowered and, secondly, after the abomasal pH had returned to relatively normal levels later in the infection, serum gastrin remained elevated, most noticeably during the trickle infection that began on Day 21 in Group C. This dissociation indicates that other factors must be involved in the hypersecretion of gastrin. Anthelmintic treatment of lambs experimentally-infected with *O. circumcincta* resulted in a gradual decrease in serum gastrin over 10 days (Anderson

et al., 1981). This is a considerably longer recovery time than they reported for abomasal pH. Perhaps following the severe disturbances associated with primary infection the sensitivity of parietal cells to gastrin is reduced and a new basal serum gastrin level is established that is slow to revert.

The possible effectors of the serum pepsinogen and abomasal pH rises, such as inflammatory mediators or parasite ES products, may equally well be applied to the hypersecretion of gastrin but have not been subject to experimental examination. Prostaglandins either stimulate or inhibit gastrin secretion in the dog depending on the type of prostanoid and the experimental model (Schepp *et al.*, 1994).

The relative abomasal weight markedly increased as has also been observed by McLeay *et al.* (1973), Anderson *et al.* (1988) and Fox *et al.* (1993). The relative wet abomasal weight of control calves reported by Fox *et al.* was greater than in this study which may be because the calves were younger than the lambs used here or differences in the relative rate of gut development in the two species. Nevertheless, the ratio had increased in infected calves killed only 10 days after infection with larvae, although not as dramatically as in the sheep of Group E infected with adult *O. circumcincta*. Anderson *et al.* (1988) found that the relative abomasal weight increased more in sheep subjected to a longer and heavier infection regime which is consistent with the present study. A 76% increase in relative wet weight occurred after only eight days infection with adult worms, which was 84% of the total increase that followed seven weeks infection with larvae. This may be due in part to the trophic action of gastrin on the mucosa of the body and fundic regions (Johnson, 1980, 1987): cell proliferation begins within 24 hours of the exogenous administration of gastrin (Willems *et al.*, 1972) and the abomasum responds quickly to infection. The fundic mucosal weight of calves increased within 10 days of infection and before blood gastrin (Fox *et al.*, 1993), suggesting the likelihood of other factors or processes. To what extent oedema, the infiltration of leucocytes and other processes, versus hyperplasia or hypertrophy of the normal abomasal cell constituents accounts for this change is not known, although certainly increased mucosal thickness was only a small component in the present study.

Concomitant with the increased serum gastrin concentration, the gastrin content of the antral mucosa decreased to about one sixth of that in control sheep in infected animals. This decrease is about double that reported by Fox *et al.* (1993) 28 days after infection

of calves with 60,000 *O. ostertagi*. In part, this may be due to the greater increase in wet abomasal weight in the present study.

2.4.6 PARASITE EXCRETORY/SECRETORY PRODUCTS

The mechanism by which the parasite communicates its presence to the host tissues remains the subject of speculation. The involvement of ES mediators of *Ostertagia* has often been proposed in the physiological disturbances associated with the parasitism of sheep and cattle (McLeay *et al.*, 1973; Titchen, 1982; McKellar *et al.*, 1987). These authors have variously suggested that worm products may stimulate pepsinogen and/or gastrin secretion or inhibit parietal cell activity. A large number of substances have been identified as parasite ES products (Chapter 1, 1.5.4). Ogilvie *et al.* (1973) have demonstrated the presence of acetylcholinesterase in *O. circumcincta* and although there was no evidence that it is secreted while in the host, moderate levels were found in the maintenance medium *in vitro*. A comparison of third stage, fourth stage and adult worm extracts of *D. viviparus* showed that acetylcholinesterases were only produced by later developmental stages of this parasite (McKeand *et al.*, 1994b). There may be a parallel in the apparently greater potency of later stages of Ostertagia on abomasal secretory activity suggested in this study.

Components isolated from the ES mix (Chapter 1, 1.5.4) clearly are involved in the inflammatory response of the host tissues and the suggestion has also been made by Murray *et al.* (1970) that adult *O. ostertagi* may secrete enzyme inhibitors. An eosinophil chemotaxin has been found in soluble extracts of *O. ostertagi* larvae (Klesius *et al.*, 1985) which has subsequently been identified as a lectin. Similar substances secreted by the parasite are believed to interfere with T cell communication, thus delaying the onset of an effective immunological response (Klesius, 1993). It is possible that parasite ES products modify the secretory function of the abomasum in an indirect manner via the inflammatory and immune processes rather than by acting directly on parietal, chief or G cells. Alternatively, they may have direct effects on these cells. Few studies have examined the physiological action of ES products and there has been little positive result. McKellar *et al.* (1990a) demonstrated that presumed ES products prepared from *O. ostertagi* caused a 9% stimulation of pepsinogen secretion from isolated bovine gastric glands but were unable to demonstrate an effect of *O. ostertagi* secretions on the production of acid by isolated bovine parietal cells *in vitro* (McKellar

et al., 1990b). Since acutely prepared parietal cells are well known to be difficult to study and are usually used after short-term culture (Chew, 1994), this does not rule out such an action. There appear to be no reports of *in vitro* studies on the effect of secretions prepared from *Ostertagia* on G cell secretory behaviour.

The role of *Ostertagia* ES products, either direct or indirect, on abomasal secretion needs thorough examination. First, appropriate *in vitro* preparations derived from ovine tissue need to be developed and are described in Chapter 3. Since little is known about the control of abomasal secretion in ruminants (Chapter 1, 1.3), the responsiveness of these preparations to pharmacological agents needs to be verified (Chapter 4).

Chapter 3

IN VITRO TECHNIQUES

3.1 INTRODUCTION

Regulation of gastric exocrine and endocrine secretion involves multiple hormonal, neuronal and paracrine factors (Chapter 1, 1.3). While *in vivo* investigations, such as those described in Chapter 2, may best reflect the response to a treatment (e.g. hypergastrinaemia induced by infection with *O. circumcincta*), the processes involved in effecting the lesion are often best revealed by *in vitro* techniques where the number of variables is more limited. Many different *in vitro* methods have been developed or adapted for the study of gastric function and hormone release, each with its own applicability, advantages and disadvantages.

Whole organ perfusion (e.g. vascular perfusion of the mouse and rat stomach by Schubert and co-workers Chapter 1, 1.3.3, 1.3.4) employs a relatively intact system that preserves the intrinsic nerve supply, mucosal integrity and paracrine relationships. Such a preparation is relatively intact and retains much of the complexity of *in vivo* systems. One difficulty with such preparations is determining the origin in the stomach of substances, such as somatostatin, which are recovered in the venous effluent. It was for this reason that Schubert *et al.* (1988b, 1991b) performed later studies in which segments of rat antral and fundic mucosa were examined separately using a perifusion technique.

Perifusion of tissue segments has been used successfully for many years to study the secretion of gastrointestinal and pancreatic peptides and is a technique noteworthy for its simplicity. As early as 1975, Hayes & Williams studied the effect of various agents on gastrin secretion by perifused rat antral tissue segments. These segments, which retain paracrine connections and much of the local neuronal connections, behave in a very similar way to more intact preparations (Schubert *et al.*, 1988b). A variation of the

perifusion system is that described as antral tissue or organ culture in which the tissue is incubated statically in a volume of medium (Harty & McGuigan, 1980; Wolfe *et al.*, 1984; Harty & Franklin, 1986; Wolfe & Reel, 1986). Whatever the system, preparations which use tissue pieces may be affected by inadequate diffusion of oxygen and nutrients into the tissue and the number of times each segment can be used to test secretagogues is limited.

Tissue complexity may be further reduced by dispersion into individual glands. These no longer are influenced by neuronal elements but retain intact paracrine connections. Richelsen *et al.* (1983) prepared human or rat antropyloric glands nonenzymatically and embodied these in a BioGel column and perifused these with control or test media. McKellar *et al.* (1990a), on the other hand, prepared isolated bovine or ovine glands with collagenase. They used this system to investigate the secretion of pepsinogen in response to products prepared from *O. ostertagi* and *O. circumcincta*. Whereas a perifusion system is limited by the number of observations that can be made, dispersed glands or cells can be used for multiple studies, as aliquots of the gland/cell suspension *should* provide identical replicates which allow direct comparison of the response to a variety of treatments.

The ultimate focus is provided by the individual isolated cell. For many gastroenterologists, the preparation and short-term cell culture of isolated cells is the *in vitro* method of choice for release and receptor studies. Not only are separated cells independent of the blood supply, neural elements and hormonal and direct paracrine influences, but cell enrichment partly or almost completely, depending on the cell type, removes the influence of other cell populations and their products. The method used by Soll (1978) to isolate parietal cells forms the basis of most current methods for the preparation of isolated gastric cells. His method involved the complete digestion of the mucosa with crude collagenase and an interruptive calcium chelation step. The parietal cell concentration within the resulting suspension was then increased by counterflow centrifugation before studies on oxygen consumption were performed later on the same day. Subsequent methods that have been described for the preparation of isolated suspensions of gastric mucosal cells have few fundamental differences from that reported by Soll (1978). Many workers use the cells immediately after isolation, although the isolation procedures are recognised as being stressful and compromise cell responsiveness to agonists or antagonists. In part, this may be due to hyper-stimulation by the dispersal processes. To overcome these limitations associated with acutely

isolated cells, many workers now maintain their cell preparations in short-term culture before performing release studies (reviewed by Chew, 1994). Enriched cell populations and their short-term culture, while providing study material in its purest and least complicated form, requires the most sophisticated and elaborate equipment of the methods described.

Three *in vitro* techniques were tested for the purpose of establishing a method for studying the pharmacology of the ovine G cell and the effects of products derived from *O. circumcincta* on gastrin secretion:

1. the perifusion system described by Richelsen *et al* (1983);

2. a novel static incubation system with some similarity to that of Wolfe *et al.* (1984) (reported in abstract form by Lawton & Simpson (1993));

3. incubation of freshly dispersed cell suspensions similar to the dispersed gland preparation of McKellar *et al.* (1990a).

In this Chapter, preliminary studies to assess the applicability and limitations of each of these methods are presented, while the results of pharmacological studies (Chapter 4), experiments with parasite secretates (Chapter 5) and microbial products (Chapter 6) are reported in detail separately.

3.2 GENERAL PROCEDURES

3.2.1 BASAL MEDIUM (BM)

The basal medium consisted of Hank's Balanced Salt Solution (HBSS, with added $NaHCO_3^-$) (GIBCO BRL, Life Technologies Inc., USA) containing 0.25% bovine serum albumin (Fraktion V, Boehringer Mannheim GmbH, Deutschland), 0.1% D-glucose (Sigma Chemical Co., USA) and 10 mM HEPES (GIBCO BRL, Life Technologies Inc., USA). The pH was adjusted and maintained at pH 7.38 ± 0.02 by the addition of 1.0 M HCl or NaOH solution. The BM was gassed with either 100% oxygen or carbogen ($95\%O_2$:$5\%CO_2$) depending on the *in vitro* method to be used.

3.2.2 ABOMASAL TISSUE

Sheep were killed either with Pentobarb 500 (Chemstock Animal Health) or stunned by captive bolt and then exsanguinated. The abomasum was immediately removed and immersed in BM. The abomasum was opened along its greater curvature and the mucosa cleaned by gentle wiping with cotton wool swabs soaked in BM. Antral mucosa was separated in sections from underlying tissue by blunt dissection and the sections submerged in BM until used in one of the three methods. Antral tissue was obtained from the distal antrum, particularly around, although excluding, the torus. Fundic tissue was obtained from the mid-section of abomasal folds, about half way along their length and was similarly submerged in BM until further dissection.

3.2.3 ASSAYS

The pepsinogen concentration was estimated as previously described (2.2.3.3. and Appendix 1.1). Forty replicate samples were assayed and the mean coefficient of variation (CV) was 17%.

The gastrin concentration was determined by RIA as previously described (2.2.3.4 and Appendix 1.2). The mean sensitivity of the assay was 3.8 ± 0.4 pM. Within assay variation was 17% ± 11 (n = 77). Between assay variation was not calculated as all samples from each experiment were assayed within the one assay and results from each experiment were processed internally.

The somatostatin concentration was estimated by RIA as described in Appendix 1.3. The mean sensitivity of the assay was 98 ± 44 pg/ml. Within assay variation was 18.5% ± 8 (n = 28). Between assay variation was not calculated as all samples from a given experiment were assayed within the one assay and results from each experiment were processed internally.

3.3 METHOD 1:

PERIFUSION OF TISSUE SEGMENTS

This method was an adaptation of that of Richelsen *et al.* (1983) which, in turn, was based on that reported earlier by Lowry (1974) in which corticotrophin releasing factor was secreted by a perifused pituitary cell column. The system permits the study of kinetics and dose-response characteristics using the glands or tissue as its own control.

3.3.1 PRINCIPLE OF METHOD

Pieces of chopped mucosal tissue or nonenzymatically-isolated antropyloric glands were suspended in a column of BioGel which was perifused by a continuous stream of medium and the effluent perifusate collected at 2 minute intervals. Up to five columns were perifused in parallel. In general, 20 minute periods of perifusion with BM alternated with 10 minute periods of perifusion with BM containing the test substance.

3.3.2 PREPARATION OF DISPERSED GLANDS OR TISSUE PIECES

Tissue pieces were prepared by cross-cutting the mucosa with razor blades. Dispersed glands were prepared by placing tissue pieces in BM and agitating them by hand for two minutes at room temperature. After rinsing, the tissue was transferred to cation-free Hank's balanced salt solution (GIBCO BRL, Life Technologies Inc., USA) and agitated at 4°C for a further 15 minutes. The tissue remnant was then removed and the suspension centrifuged lightly (1000 rpm) to sediment the dispersed glands. The glandular pellet was resuspended in BM.

3.3.3 EXPERIMENTAL PROCEDURE

The perifusion medium consisted of BM held at 37°C in a water bath and continuously bubbled with carbogen (95%O_2:5%CO_2). The pH was continuously monitored with a stomach pH electrode (Radiometer, Copenhagen, Denmark) and the pH was adjusted as necessary.

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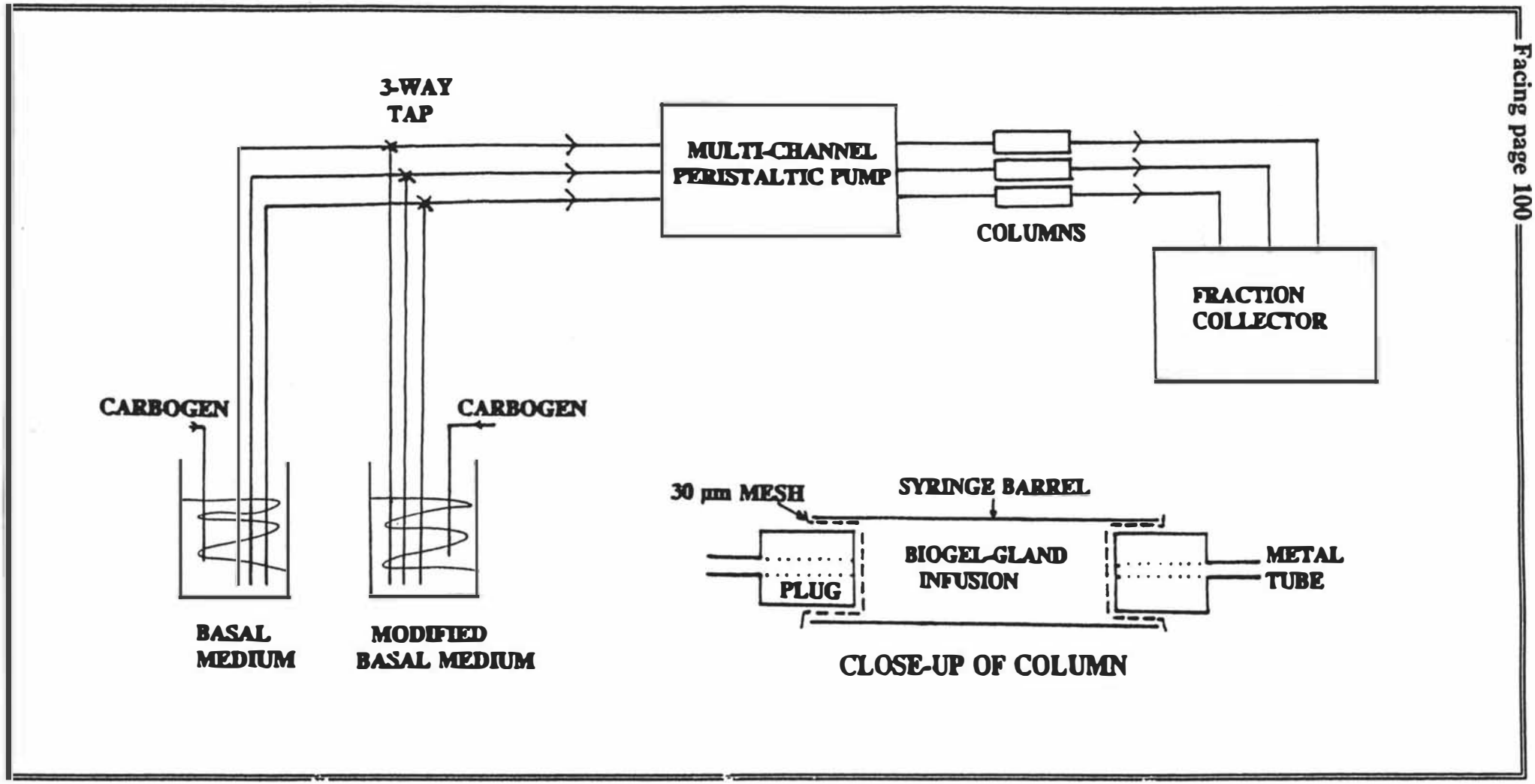

Figure 3.1 Schematic drawing of column perifusion apparatus. Column was constructed from a 3 ml disposable syringe, containing BioGel P2 beads and antropyloric glands or tissue pieces (see text for details).

Each column was made from the barrel of a three ml syringe with the nozzle removed to leave only a tube. The base was sealed with a rubber bung which was covered with 30 µm nylon mesh and through which a metal tube had been inserted. The bung provided a tight seal to the column, and the perifusion medium was pumped into the column through the metal tube. The mesh retained the BioGel within the column (Fig. 3.1). Resuspended glands or chopped tissue were mixed with BioGel P-2 beads (BioRad Laboratories, Richmond, U.S.A.) that had been preswollen overnight in BM at 4^0C and the mixture was poured into the column. About five mm of the mixture was removed from the top of the column in order to allow a second rubber bung similarly fitted with mesh and a metal tube to complete the column. BM was pumped into the bottom of the column from a reservoir through tubing passing through a multi-channel peristaltic pump (Minipuls 2, Gilson, France). A three-way tap between the reservoir and the pump allowed switching to another reservoir containing modified BM (Fig. 3.1). Tubing connected to the top of the column allowed the collection of the column effluent into tubes in a fraction collector. Up to five columns were clamped in parallel in a perspex frame and the reservoirs, tubing and columns placed in a water bath at 37^0C.

After the columns had been prepared, the perifusion medium was pumped at a flow rate of 0.5 ml/min. After a 30 minute equilibration period of perifusion with BM, the columns were perifused alternately with BM for 20 minutes and a modified medium for 10 minutes. Effluent was collected at two minute intervals. The dead space in the tubing caused a time delay, as determined by a dye marker, of four minutes between switching of the tap and effluent collection.

Test substances added to the BM were carbachol (C-4382, Sigma Chemical Co., USA) in concentrations from 10^{-8} to 10^{-4} M and bombesin acetate salt (B-4272, Sigma Chemical Co., USA) in concentrations from 10^{-10} to 10^{-4} M.

3.3.4 ASSESSMENT OF RESPONSE

The gastrin concentration of successive perifusate collections was plotted against time using the software package Cricket Graph (Computer Associates International Inc., U.S.A.). The data points corresponding to effluent collected during the perifusion with modified medium and the first three subsequent samples were deleted and a curve fitted to the remaining values (normally a three factor polynomial). This curve was taken to

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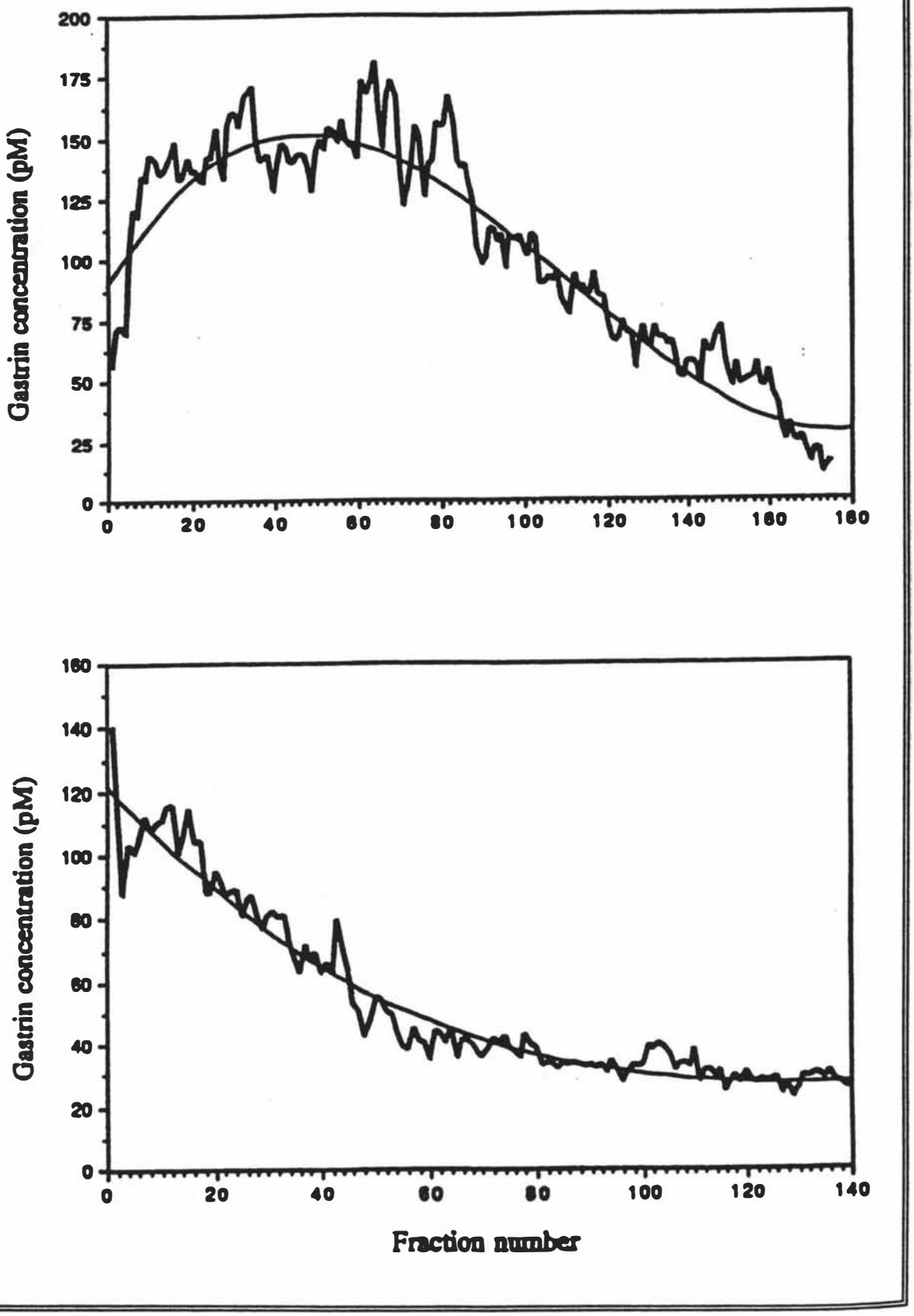

Figure 3.2 Gastrin concentration in the effluent from two BioGel/antral chopped tissue columns perifused with BM at a flow rate of 0.5 ml per minute. Fractions were collected at two minute intervals. The curve fitted to each graph represents the basal secretion by the tissue.

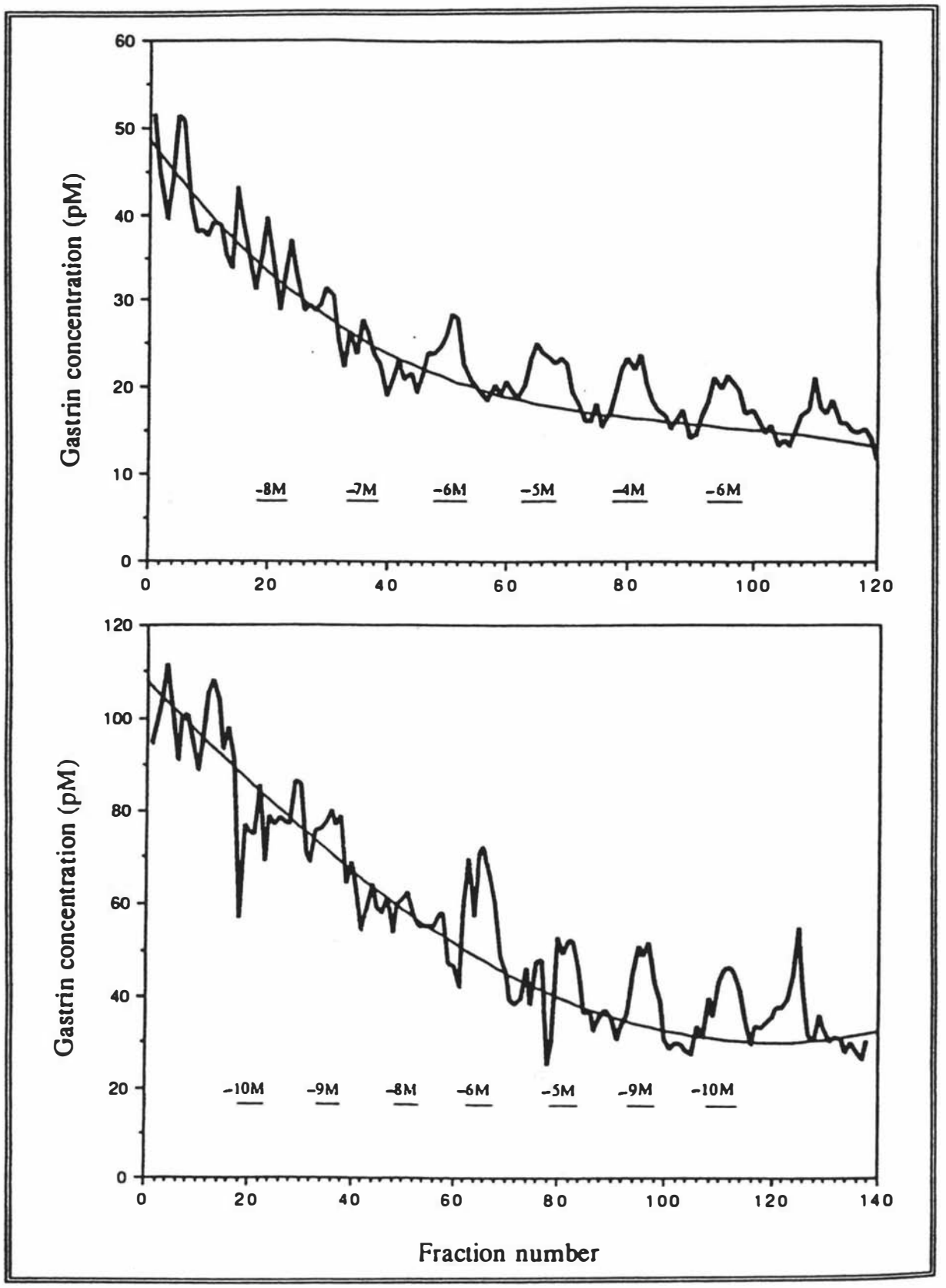

Figure 3.3 Gastrin concentration in the effluent from two BioGel/antropyloric gland columns perifused with BM containing a range of bombesin concentrations. Medium was perifused through the column at 0.5 ml per minute and fractions were collected at two minute intervals. Horizontal bars indicate periods of exposure of the column to the concentration of bombesin shown (xM = 10^{x}M).

Table 3.1 Effect of bombesin on gastrin secretion by perifused ovine antropyloric glands. The gastrin response is expressed as a percentage of the predicted basal secretion during the test period. In three columns, 10^{-10} and 10^{-9} M bombesin were re-perifused: (♥) represents those results relating to their initial perifusion, while (●) relate to their subsequent perifusion.

Bombesin concentration	% gastrin response (mean ± SD)	Number of columns
10^{-10} M♥	-14 ± 6	3
10^{-9} M♥	12 ± 4	3
10^{-8} M	16 ± 15	8
10^{-7} M	7 ± 9	5
10^{-6} M	29 ± 14	13
10^{-5} M	27 ± 16	8
10^{-4} M	31 ± 9	5
10^{-9} M●	48 ± 28	3
10^{-10} M●	53 ± 31	3

represent the basal gastrin secretory activity of the glands or tissue. Deleted data points were returned and a line drawn to connect all successive data points. From enlarged printed copies of the graphs, the area between the plot and the basal secretion curve during collections periods relating to perifusion with modified medium was measured using a digitiser and the software package Sigma Scan (Jandel Scientific, U.S.A.). This was divided by the area below the basal secretion curve during the corresponding period to give the % response relative to basal secretion.

3.3.5 RESULTS

In every case, basal gastrin secretion decreased over the duration of the experiment (Fig. 3.2), with the rate of decline progressively lessening as the tissue approached a steady state. In some columns, the decline was preceded by an initial period of increasing basal secretion.

The responses to carbachol and bombesin were examined in tissue derived from a number of different sheep and after plotting the results, only some resulted in meaningful response curves which could be analysed. All were from columns containing dispersed glands and none contained chopped tissue. It was also subjectively observed that columns with moderate basal gastrin secretion rates (i.e. gastrin concentration in the perifusate of between 20 and 80 pM), gave the most clearly identifiable responses. Central to the identification of a response was the fitting of the basal secretion curve. For many columns, the secretion of gastrin during basal perifusion periods was so erratic that no basal secretion curve could realistically be fitted to the data. Consequently, from the many columns examined, a limited number were selected and the response to carbachol and bombesin in these determined. These columns were **not**, however, selected on the basis of the magnitude of their response to specific test substances.

The responses to the perifusion of bombesin in concentrations of 10^{-10} to 10^{-4} M of two columns prepared from tissue from different animals are shown in Fig. 3.3 and the overall response to bombesin is presented in Table 3.1. The sequence in which the doses were tested was not randomised, although in all cases some concentrations were re-tested later in the experiment. (Note that the results of the re-perifusion of 10^{-10} and 10^{-9} M bombesin are given at the bottom of Table 3.1 while the response to the initial

Table 3.2 Effect of carbachol on gastrin secretion by perifused ovine antropyloric glands. The gastrin response is expressed as a percentage of the predicted basal secretion during the test period.

Carbachol concentration	% gastrin response (mean ± SD)	Number of columns
10^{-8} M	16 ± 11	2
10^{-7} M	31 ± 20	6
10^{-6} M	31 ± 14	8
10^{-5} M	31 ± 16	11
10^{-4} M	41 ± 26	6

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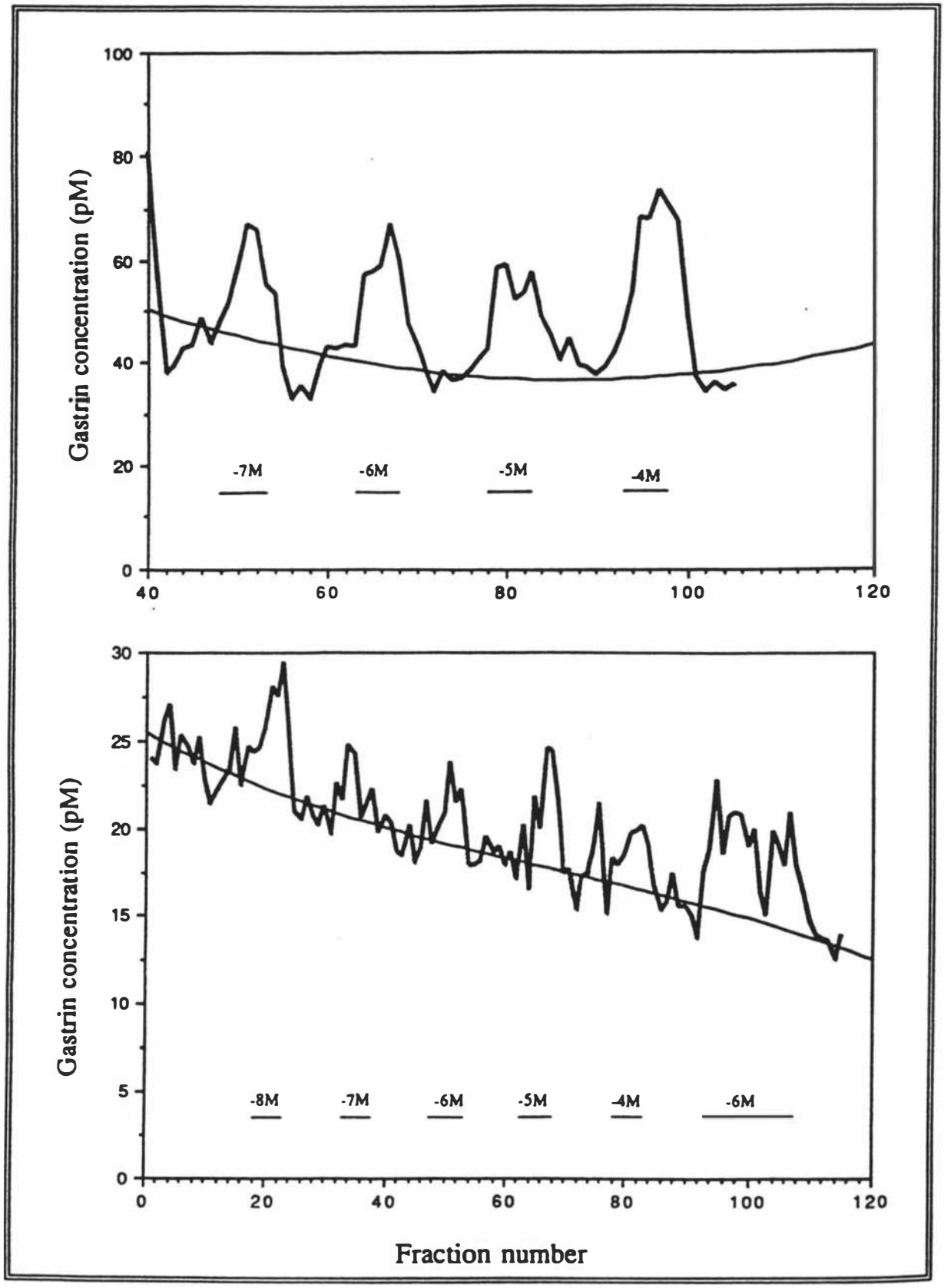

Figure 3.4 Gastrin concentration in the effluent from two BioGel/antropyloric gland columns perifused with BM containing a range of carbachol concentrations. Medium was perifused through the column at 0.5 ml per minute and fractions were collected at two minute intervals. Horizontal bars indicate periods of exposure of the column to the concentration of carbachol shown (xM = 10^{x}M).

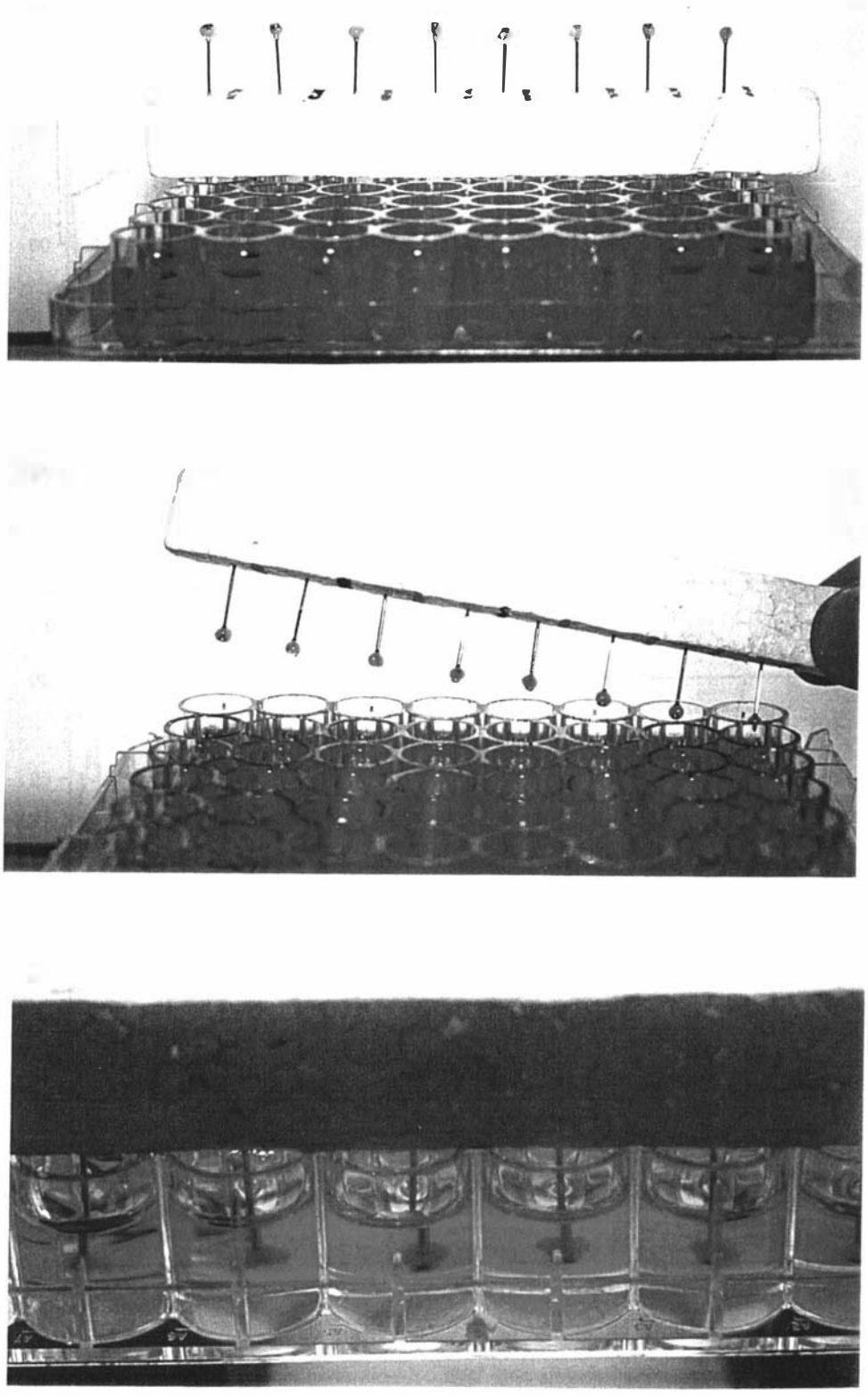

Figure 3.5 Arrangement of ovine abomasal tissue segments for use in the static incubation method: (top) showing segments mounted in rack of polystyrene; (bottom) showing pins conforming with the wells of a Costar 48 well cell culture plate; (bottom) showing tissue suspended in 1 ml of BM.

perifusion is given at the top.) In every individual case, the response to the subsequent re-treatment was greater than to the initial perifusion, as is evident in both columns illustrated in Fig. 3.3. In the five columns in which the sequence depicted in the upper graph was followed, there was an increase in the percentage response to 10^{-6} M bombesin during the latter perifusion period compared with the earlier one of 83 ± 56% (mean ± SD). Similarly, in the three columns in which the sequence depicted in the lower graph was followed, the response to the subsequent dose of 10^{-9} M bombesin was 233 ± 128% greater than to the earlier exposure. These increases were evident when the absolute responses were considered, although the percentage difference was less.

The responses of two columns prepared from different animals to the perifusion of carbachol in concentrations between 10^{-8} and 10^{-4} M are shown in Fig. 3.4 and the overall response to carbachol is presented in Table 3.2. As is evident in the lower graph, subsequent repeated perifusion with 10^{-6} M carbachol was associated with a greater response.

3.4 METHOD 2:

STATIC INCUBATION OF TISSUE SEGMENTS

Perifusion systems have several limitations, notably the variable basal secretion leading to difficulty in quantifying the response. A static system using small volumes of BM was developed, principally to overcome the limitations associated with the perifusion system and because of the small amounts of parasite secretions available.

3.4.1 PRINCIPLE OF METHOD

Tissue segments were transfixed on pins and mounted on a polystyrene strip in a row of eight, spaced so that each piece of tissue was suspended in the centre of 1 ml of BM in a well along one side of a 48 well cell culture cluster (Fig. 3.5). After each successive incubation period at 37^{0}C, either for 10 or 20 minutes, the polystyrene rack was moved to the next row until 6 incubation periods were completed (Rows A-F). Test substances were added to the BM in the wells in rows D, E, and F and the response compared with those in control plates containing only BM in all 6 rows.

3.4.2 PREPARATION OF TISSUE SEGMENTS

Separated mucosa (which included variable amounts of submucosa) was cut into segments approximately 4 mm^2 (antrum) or 25 mm^2 (fundus) with a scalpel on a cork board. Tissue was maintained in a viable condition by intermittent irrigation with BM. The tissue segments were transfixed on pins and submerged in BM until all had been prepared. The pins were randomly selected and mounted in a row of eight on strips of polystyrene approximately 10 cm x 1 cm x 1 cm in size. These were then placed on top of 48 well cell culture cluster plates (Costar Corporation, USA; see Fig. 3.5) in which each well contained 1 ml of BM. When all rows had been assembled, the plates were taken to a room at a temperature of 37°C and each rack was transferred to fresh BM and left undisturbed for a 30 minute pre-experimental equilibration period.

3.4.3 EXPERIMENTAL PROCEDURE

The BM and BM containing test substances were prepared within 24 hours of each experiment. The pH was adjusted to 7.38 ± 0.02 by the addition of NaOH or HCl and the osmolarity was measured using an osmometer (Digimatic Osmometer, Advanced Instruments (Inc.), U.S.A.). Media were gassed with oxygen and maintained at 4°C under a 100% oxygen atmosphere until immediately prior to the experiment when the media were preheated to 37°C and 1 ml volumes dispensed into the prewarmed plates in the temperature controlled environment. Unless tissue were being incubated, the lids were kept on all plates to minimise evaporation and gaseous exchange.

After the equilibration period, each piece of tissue underwent six sequential incubations (A to F): 10 minutes for antral tissue or 20 minutes for fundic tissue. The racks were randomly selected for transfer to the different plates. The time delay in moving racks during the sequential incubations was compensated for by always moving them in the same order and at the same rate. After the incubations were completed, the plates were transferred to 4°C. The 1 ml incubates were aspirated from the wells into plastic tubes, subsampled, sealed and stored at -20°C for subsequent analysis of pepsinogen, gastrin or somatostatin concentration. Incubates A to F were either stored and assayed individually or pooled (A to C) and (D to F) before storage. A 10 µl aliquot of the proteinase inhibitor Trasylol (Bayer, Deutschland) was added to all samples collected for somatostatin analysis prior to storage.

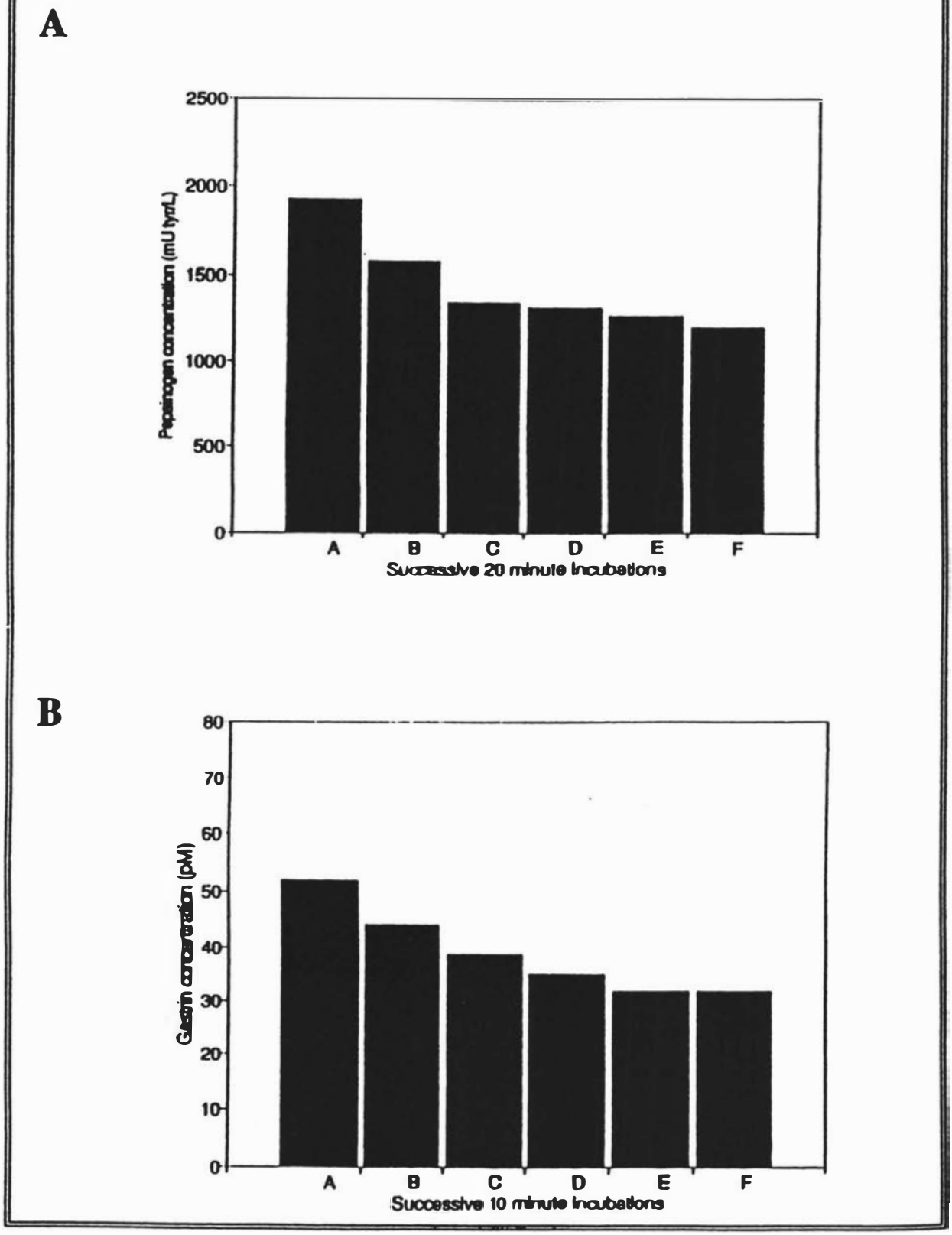

Figure 3.6 Concentration of pepsinogen (**A**) and gastrin (**B**) in the medium used to incubate control tissue for six successive periods using the static incubation method. Control ratio (CR) equals the mean secretion during incubations D-F divided by the mean secretion during incubations A-C. Pepsinogen was released from fundic mucosal tissue during six consecutive 20 minute incubations (A-F). The number of tissue pieces was 16 and the CR = 0.7613. Gastrin was released from antral mucosal tissue during six consecutive 10 minute incubations (A-F). The number of tissue pieces was 16 and CR = 0.7391.

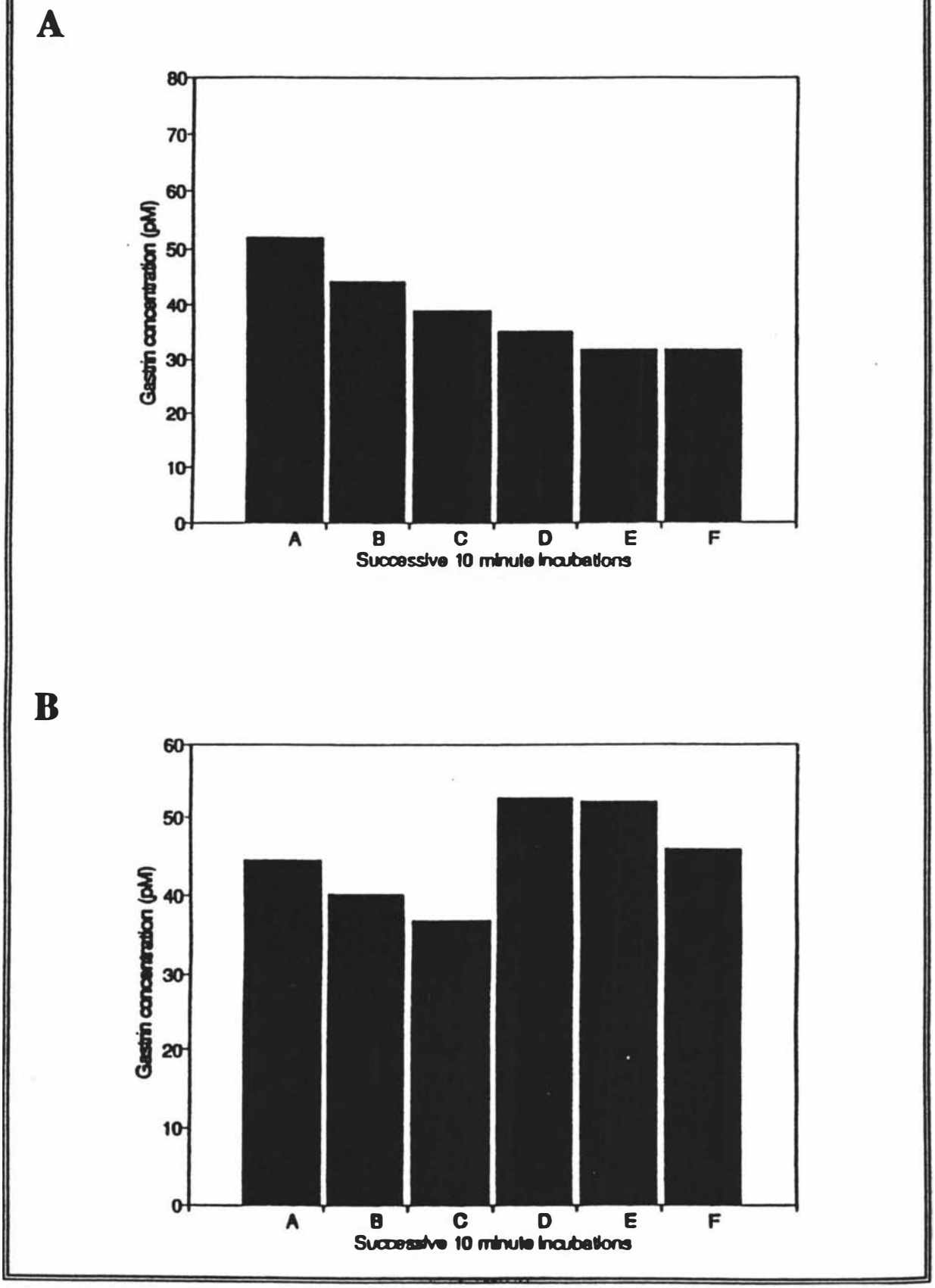

Figure 3.7 Effect of 10^{-4} M carbachol on gastrin secretion by ovine antral tissue segments using the static incubation method. (A) gastrin secretion by control tissue over six consecutive 10 minute incubations. The number of tissue pieces was 16 and control ratio = 0.7391. (B) gastrin secretion by test tissue over the same period. Carbachol was included in the medium during incubations D, E and F. The number of tissue pieces was 8 and test ratio = 1.2452.

3.4.3.1 EFFECT OF A SECRETAGOGUE: CARBACHOL

Carbachol (Sigma Chemical Co., USA) was added to BM to provide solutions with final concentrations of 10^{-4} to 10^{-9} M for testing on both antral and fundic tissue.

3.4.3.2 EFFECT OF EXPERIMENTAL CONDITIONS: OSMOLARITY AND pH

The osmolarity or pH of the BM was varied as a treatment and tested on antral tissue. Solutions of 125%, 87.5% and 80% of the osmolarity of BM were prepared by making the solution up to a lesser volume or by the addition of excess water. The osmolarity (mOsm/L) of the solutions was checked. Solutions of pH 7.18, 7.28, 7.48 and 7.58 were prepared by the addition of NaOH or HCl as appropriate.

3.4.4 ASSESSMENT OF RESPONSE

Tissue incubated in BM for six successive periods showed a decreasing basal rate of gastrin, somatostatin or pepsinogen secretion (Figs 3.6 and 3.7A). This basal secretion served as the experimental control against which the secretory behaviour of tissue pieces exposed to a test substance during incubations D-F (Fig. 3.7B) could be compared. The secretory behaviour of test tissue pieces was defined by the three internal control incubation periods (A-C) which preceded exposure to the test substance in incubations D-F. Similarly, the A-C incubation periods allowed the secretory behaviour of the control tissue to be determined.

(1) Calculation of the response to a treatment (Rt)

The response to a treatment (Rt) was calculated as:

$$Rt\ (\%) = (\text{test tissue secretory behaviour} \div \text{control tissue secretory behaviour} \times 100) - 100$$

where, the secretory behaviour of control tissue = CR (control ratio) = Y/X,

and Y = mean secretion by control tissue in wells D-F
X = mean secretion by control tissue in wells A-C

Similarly, secretory behaviour by test tissue = TR (test ratio) = Y/X,

and Y = mean secretion by test tissue in wells A-C

X = mean secretion by test tissue in wells D-F

The secretory behaviour of any individual (i) piece of tissue is derived by

Y_i/X_i (for either a control (CR_i) or test (TR_i) tissue segment)

Thus, $Rt_i\ (\%) = (TR_i \div CR \times 100) - 100$

The CR used to determine either the response by an individual tissue segment (Rt_i) or the response of a set of tissue segments (Rt) to a test substance within an experiment was the mean value of the CR_i set.

This value was calculated by dividing the average Y value by the average X value,

$$(\Sigma Y_i/n) \div (\Sigma X_i/n)$$

However, as the size and secretory capacity of tissue segments was not uniform. To prevent bias towards the effects occurring in those pieces which released high concentrations of the substance (gastrin, pepsinogen), equal weighting must be given to each piece when calculating CR (or TR). The weighted values of X and Y (X_i and Y_i) were calculated such that $X_i + Y_i = 1$

$$X_i = X_i(1/(X_i+Y_i)) \quad \text{and} \quad Y_i = Y_i(1/(X_i+Y_i))$$

This method of calculating the mean CR (or TR) value is more resilient to the effects of potentially deviant outlying values than is the averaging of CR_i (or TR_i) sets, as no tissue segment can contribute more than 1/n to CR. It was always used to calculate CR and TR for the determination of Rt:

$$Rt\ (\%) = (TR \div CR \times 100) - 100$$

(2) Multiple determination of Rt

The Rt values derived from individual experiments for a given treatment were then

combined for the assessment of the overall response. The size of the data set equaled the number of experiments in which a particular agent was tested and the standard deviation (SD) reflects the variation of the Rt among experiments. As the response to a treatment (Rt) was rationalised internally against control tissue, it could be combined with responses to similar treatments in other experiments, or compared with different treatments without further reference to the experimental CR.

(3) Rt for individual experiments

Since the method used to calculate Rt provides only a single percentage response for each treatment within each experiment, where only a single experiment was performed, the Rt for individual pieces of tissue was also calculated to provide a data set for statistical analysis. The mean of this data set differed from the previously calculated Rt only when there were large variations among values for individual tissue pieces (i.e. outlying values).

For the analysis of the response to a treatment within an individual experiment, however, an Rt_i set was calculated using individual TR values and the mean CR value,

$$Rt_i test\ (\%) = (TR_i \div CR \times 100) - 100$$

A corresponding control data set was determined,

$$Rt_i control\ (\%) = (CR_i \div CR \times 100) - 100$$

(4) Calculation of CV

The Rt_itest and the Rt_icontrol sets were determined to generate the %CV for that experiment. This value reflects the variation in the response by individual tissue segments within an experiment to a particular treatment (control or test).

3.4.5 STATISTICS

All statistics were performed using the software package MINITAB (Minitab Inc., USA). The data set included Rt values, each derived from separate experiments, if an agent or

Table 3.3 Effect of carbachol on pepsinogen secretion by segments of ovine fundic mucosa. The response to treatment (Rt) was derived from four experiments (n = 4); for each n there were 24 control and 8 test tissue pieces; CV is the coefficient of variation calculated from raw data. The mean CV for control tissue was 13%.

Carbachol concentration	% pepsinogen response (mean ± SD)	Mean %CV
10^{-9} M	-1 ± 2	15
10^{-8} M	-3 ± 3	9
10^{-7} M	2 ± 6	9
10^{-6} M	4 ± 5	10
10^{-5} M	11 ± 6	10
10^{-4} M	8 ± 12	12

solution was tested in more than one experiment. For intraexperimental comparisons, and where an agent or solution was only tested in one experiment, the data set included Rt_i values. Where related treatments were tested (e.g. a range of BM concentrations) the data sets were compared using the Tukey (equal sample sizes) or Dunnett (unequal sample sizes) multiple comparison method which follow a preliminary one-way analysis of variance. Where the effect of a treatment (Rt) within an experiment was tested against the null hypothesis (H_0) of Rt = 0, and only two variables were involved (e.g. Rt_itest and Rt_icontrol sets), two sample t-tests were used. Differences were considered significant at the 5% level.

3.4.6 RESULTS

3.4.6.1 CONTROL TISSUE

The secretion of pepsinogen and gastrin by control tissue is illustrated in Fig. 3.6. The respective CR values of 0.7613 and 0.7391 are typical. The CR (mean ± SD) for pepsinogen, gastrin and somatostatin were 0.7604 ± 0.0329, 0.7137 ± 0.0821 and 0.7756 ± 0.0831 respectively. In every case CR was less than 1.0 since the secretion during periods D, E and F was less than that during A, B and C and was indicative of decreasing basal secretion, consistent with that observed using the perifusion technique.

3.4.6.2 EFFECT OF CARBACHOL ON GASTRIN AND PEPSINOGEN SECRETION

Gastrin secretion was stimulated by 10^{-4} M carbachol by 68%. Gastrin secretion during each of the six incubation periods in a control plate and in the presence of 10^{-4} M carbachol are shown in Fig. 3.7.

Carbachol did not significantly stimulate pepsinogen secretion overall (Table 3.3). There was no response in three experiments but in the fourth (Expt 3), significant increases of about 19% and 26% were associated with 10^{-5} and 10^{-4} M carbachol respectively.

3.4.6.3 EFFECT OF OSMOLARITY AND pH ON GASTRIN SECRETION

The effects of altering the osmolarity and pH of the medium on gastrin secretion are

Table 3.4 Effect of the osmolarity of the incubation medium on the secretion of gastrin by segments of ovine antral mucosa. The gastrin response (Rt) was derived from n experiments; %CV is the coefficient of variation calculated for raw data; (*) indicates a significant difference from control at 5% level (Dunnett method).

Osmolarity (mOsm/L)	% gastrin response (mean ± SD)	Mean %CV	n	Tissue pieces per n
400	-42 ± 8*	19	6	8
320	0	14		
280	71 ± 16*	13	4	8
256	155 ± 46*	17	6	16

Table 3.5 Effect of the pH of the medium on gastrin secretion by segments of ovine antral mucosa. Each gastrin response (Rt) was derived from 3 experiments (n); for each n the number of tissue pieces = 24; ; %CV is the coefficient of variation calculated for raw data; (*) indicates a significant difference from control at 5% level (Tukey method).

Medium pH	% gastrin response (mean ± SD)	Mean %CV
7.18	-27 ± 8*	14
7.28	-18 ± 16	12
7.38 (basal medium)	0	10
7.48	1 ± 1	11
7.58	4 ± 7	9

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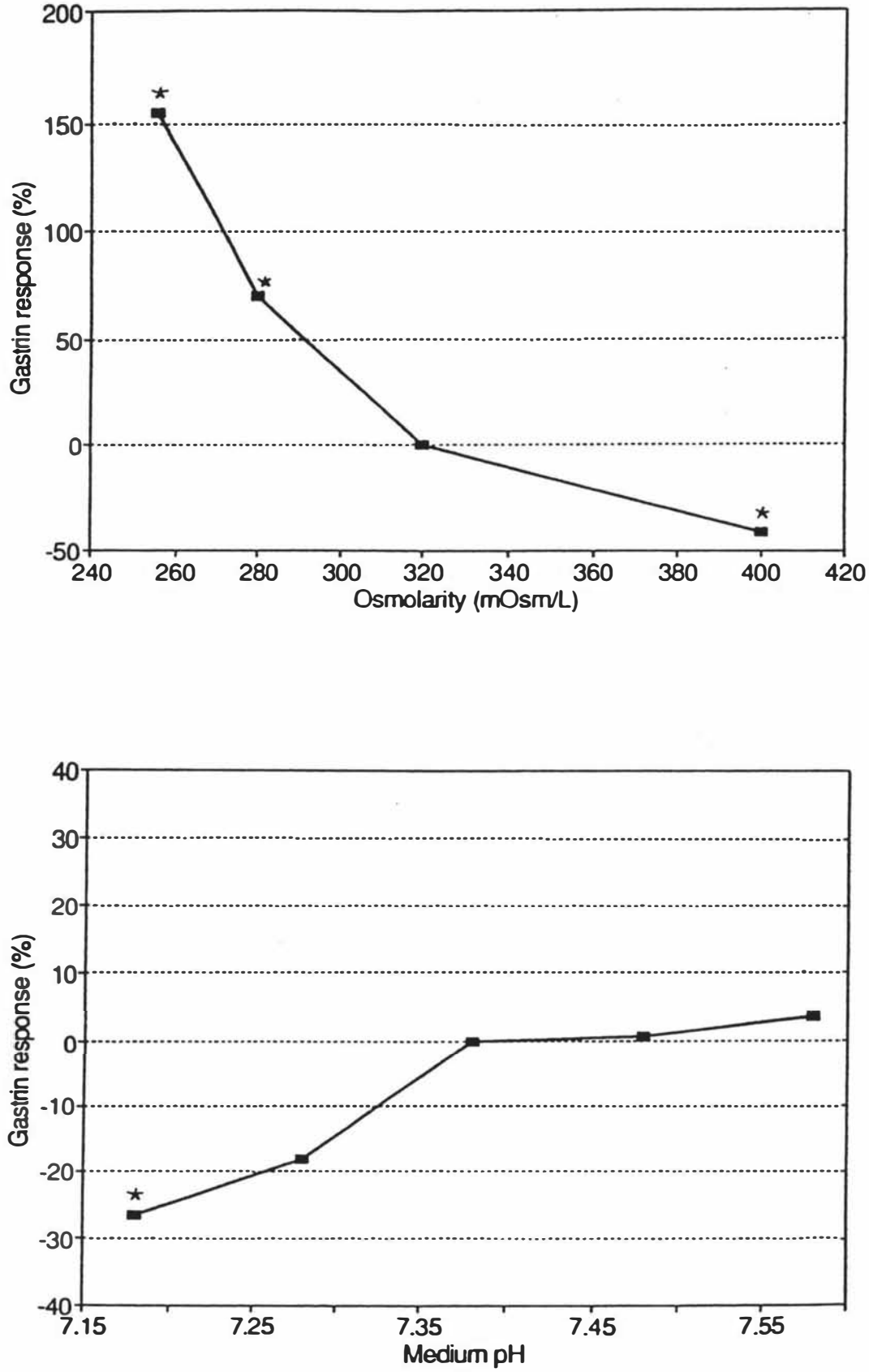

Figure 3.8 Effect of osmolarity and pH changes on gastrin secretion by segments o ovine antral mucosa. (*) indicates a significant difference from control (Dunne method).

presented in Tables 3.4 and 3.5 and are shown in Fig. 3.8. In both cases, there were significant effects. Dilute solutions increased the release of gastrin while the hypertonic solution reduced the secretion of gastrin. Lowering the pH of the BM by 0.1 and 0.2 units decreased gastrin secretion. The inhibition at pH 7.18 BM was not significantly greater than the inhibition at pH 7.28. Alkalinization (+ 0.2 pH units) did not significantly affect gastrin secretion.

3.5 METHOD 3:

DISPERSED CELL PREPARATIONS

The usefulness of crudely dispersed cell suspensions of the ovine antral or fundic mucosa to examine abomasal secretion was assessed. The mucosa was digested with collagenase into a suspension of dispersed cells and the release of pepsinogen or gastrin by these cells in response to secretagogues and inhibitors during a 30 minute incubation time studied. No attempt was made to enrich the population of any particular cell type within the suspension nor was any attempt made to short-term culture the cells prior to the release studies.

3.5.1 PRINCIPLE OF METHOD

Fresh abomasal mucosa was digested with collagenase into a suspension of dispersed cells. Aliquots of this suspension were then added to all tubes which contained BM with or without the addition of a treatment (e.g. carbachol). The resulting mixtures were incubated at 37^{0}C for 30 minutes. After incubation, the tubes were centrifuged to sediment the cellular component and a sample of the supernatant was collected for pepsinogen or gastrin estimation.

3.5.2 PREPARATION OF DISPERSED CELL SUSPENSION

The mucosa was cleaned by gentle wiping with cotton wool swabs soaked in BM. Mucosa was scraped free from the underlying tissue with a glass slide and placed in a petri dish with a small volume of cation-free HBSS containing 0.03% EDTA (di-sodium ethylenediaminetetraacetate, May & Baker Ltd., England) where it was minced using

two scalpel blades. The finely chopped mucosa was suspended in 100 mls of cation-free HBSS containing 0.03% EDTA, agitated for 15 minutes at 37°C in a shaking water bath then centrifuged at 1000 rpm for 5 minutes. The supernatant was discarded and the tissue pellet was resuspended in 100 mls of normal BM containing 480 U/ml collagenase (C-0130, Sigma Chemical Co., USA).

The tissue was digested by the collagenase for one hour in a shaking water bath at 37°C while being further stirred and gassed by bubbling oxygen through it. After one hour, the mixture was poured through a tea strainer and any tissue pieces retained were discarded. The filtrate was centrifuged at room temperature for 5 minutes at 1000 rpm and the supernatant discarded.

The tissue-cell pellet was resuspended in cation-free BM and filtered through 200 µm nylon mesh. The filtrate was then centrifuged at 1000 rpm for 5 minutes and the supernatant discarded. This process was repeated before the pellet was resuspended in normal BM and washed a further two times (centrifugation and resuspension). After the total of four washings, the resuspended mixture was filtered through 20 µm nylon mesh and resuspended in 35 mls BM (antral cells) or 100 mls BM (fundic cells) to give the dispersed cell suspension to be used in the experiment.

3.5.3 ASSESSMENT OF CELL VIABILITY

Two aliquots of the dispersed cell suspension were added to a volume of BM equal to that in which aliquots of dispersed cells were incubated in experiments. These duplicate mixtures were used to count the cell density with a haemocytometer, observe the degree of separation and to estimate the viability of the cells by trypan blue exclusion. One drop of 5% trypan blue was added to the cell-BM mixture. After two minutes, the proportion of cells that had failed to exclude the dye were considered to be nonviable.

3.5.4 EXPERIMENTAL PROCEDURE

From a dispersed cell suspension that was being mixed, aliquots were taken and added to replicate tubes containing pre-warmed medium. The tubes were incubated at 37°C for 30 minutes and then centrifuged at 1750 rpm and 1.0 ml of the supernatant retained at -20°C for the subsequent determination of gastrin or pepsinogen concentration. The

omogeneity of a cell suspension was assessed by adding 0.5 ml aliquots of cell uspension to eighteen tubes containing 3.0 ml BM. The responsiveness of dispersed ell preparations was tested by the addition of either GRP, carbachol, bethanachol or *O. ircumcincta* secretate to the BM.

ntral cell suspensions were tested with 10^{-8} to 10^{-4} M GRP (synthetic porcine GRP-28; -1649, Sigma Chemical Co., USA), 10^{-8} to 10^{-4} M carbachol (Sigma Chemical Co., SA) or 10^{-8} to 10^{-4} M bethanechol (Carbamyl-ß-methycholine chloride, C-5259, Sigma hemical Co., USA). 0.25 ml aliquots of cell suspension were added to tubes ontaining 2.0 mls of pre-warmed solution: 15 control tubes contained normal BM and here were 10 replicates of each test solution. Carbachol was tested on cell suspensions rom five sheep, GRP on two and bethanechol on one.

arbachol was tested on one fundic cell suspension. One ml aliquots of the cell uspension were added to each of 15 tubes containing 1.0 ml of pre-warmed BM or 9 ubes of each concentration of carbachol in BM (10^{-8} to 10^{-4} M).

our worm-derived solutions that consistently inhibited gastrin secretion using the static ncubation method (Chapter 5, 5.3.2) were tested on an antral cell suspension. Each olution, itself based on BM, was mixed with BM in the ratio 1:4. Aliquots of 0.25 ml of the cell suspension were added to 15 tubes containing 2.0 ml of pre-warmed BM and 10 tubes containing each of the inhibitory solutions. Solutions of 10^{-8} to 10^{-4} M arbachol was also tested on this same cell suspension.

The response to the treatment was calculated:

$$Rt = ((T \div C) \times 100) - 100$$

where C = mean concentration in control tubes and T = mean concentration in treatment tubes.

The Rt sets for each dose were then compared with the control set (zeros) to determine if there were any response to the treatment (number of Rt values = n) using the Tukey multiple comparison method, or by comparison of Rt_i sets with C_i sets where n = 1.

Table 3.6 Effect of carbachol, bethanechol and GRP on gastrin secretion by dispersed ovine antral mucosal cells. The gastrin response (Rt) was derived from 15 control and 10 replicates for each concentration. The mean Rt to 10^{-6} M carbachol does not include the response by suspension 3. CV is the coefficient of variation calculated from raw data.

Cell suspension	% gastrin response to carbachol (mean ± SD)						%CV control	Cell density
	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M		
1	11 ± 11	14 ± 15	7 ± 10	5 ± 7	4 ± 6	3 ± 6	5	5×10^6
2	-4 ± 11	-8 ± 5	-1 ± 4	-11 ± 4	-2 ± 2	-4 ± 11	8	3×10^6
3	-2 ± 5	-0 ± 7	4 ± 6	-94 ± 2	3 ± 5	3 ± 6	5	14×10^6
4	8 ± 7	0 ± 8	-7 ± 13	3 ± 11	0 ± 7	-9 ± 8	10	4×10^6
5	10 ± 6	6 ± 10	-4 ± 6	0 ± 11	-2 ± 8	-4 ± 6	13	4×10^6
Mean ± SD	4 ± 7	2 ± 8	0 ± 6	-1 ± 7	0 ± 3	-2 ± 5	8	6×10^6
Cell suspension	% gastrin response to bethanechol (mean ± SD)						%CV Control	Cell density
	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M		
1	-6 ± 6	-2 ± 5	-2 ± 4	-2 ± 9	-1 ± 7	-2 ± 8	4	1×10^6
Cell suspension	% gastrin response to GRP (mean ± SD)						%CV Control	Cell density
	10^{-10} M	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	-		
1	17 ± 15	-7 ± 14	-6 ± 13	-16 ± 10	11 ± 17	-	14	5×10^6
2	-11 ± 4	-16 ± 4	-19 ± 9	-14 ± 10	6 ± 18	-	16	5×10^6
Mean ± SD	3 ± 20	-11 ± 7	-13 ± 9	-15 ± 1	8 ± 4	-	15	5×10^6

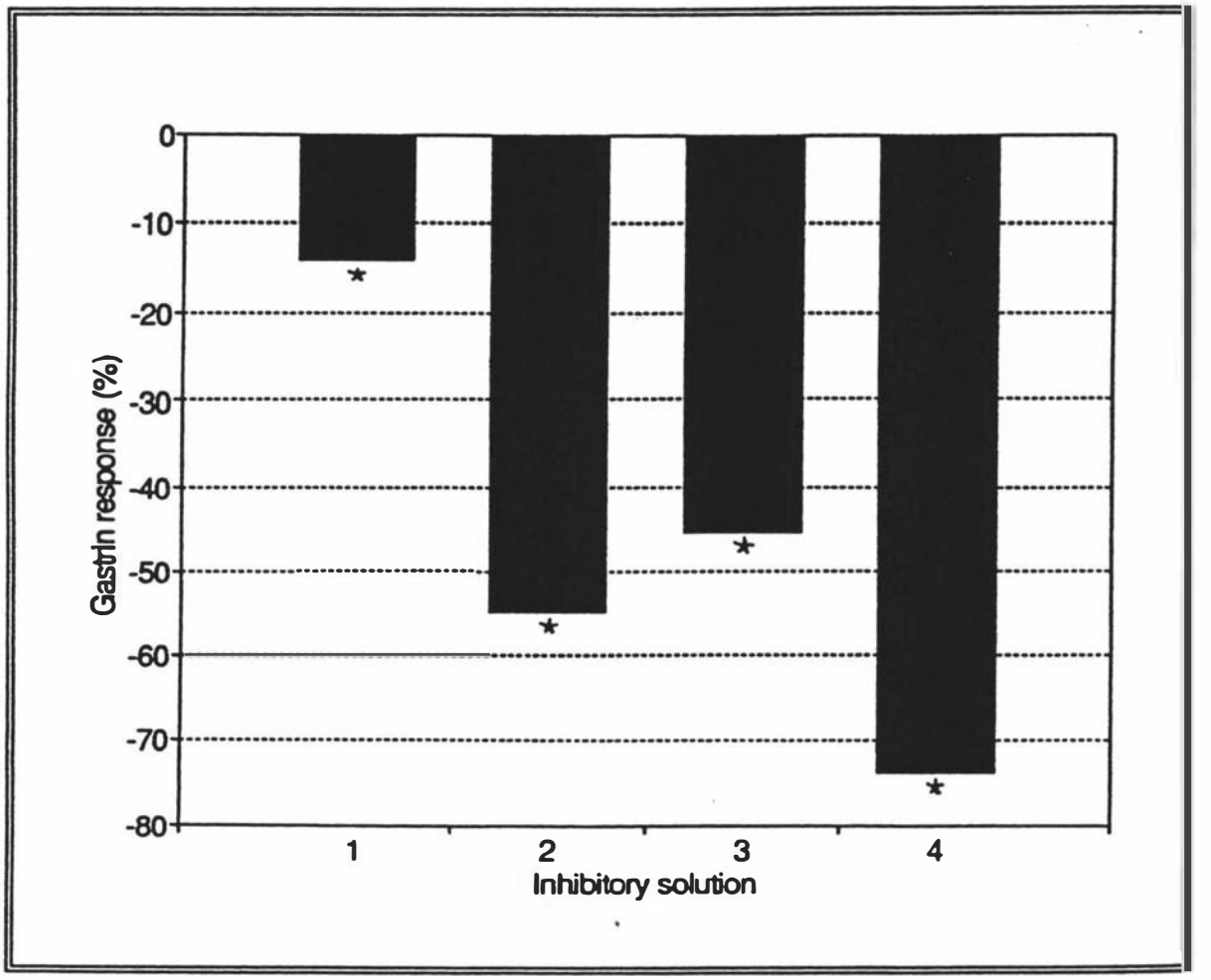

Figure 3.9 Effect of inhibitory solutions on the secretion of gastrin by dispersed ovine antral mucosal cells. (*) indicates a significant difference from control secretion (Dunnett method). The inhibitory solutions were derived from *O. circumcincta* larvae and adult worms and are described in detail in Chapter 5, 5.2.5.5, they were: (1) larval secretions (a combination of first and second pools); (2) larval extract; (3) excretory/secretory adult worm solution (Batch 1); (4) adult worm extract (Batch 1).

3.5.5 RESULTS

3.5.5.1 HOMOGENEITY OF ANTRAL CELL SUSPENSIONS

The gastrin release from 18 tubes was used to assess the homogeneity of the suspensions and two tubes were used to estimate cell density. The cell density was approximately 10^7 cells per ml and the cell viability was at least 95%. The gastrin concentration was 30.9 ± 4.0 pM (mean ± SD) with a CV = 13%. Microscopic examination of the cell suspension revealed that the cells were mostly single although clusters of two or three were commonly seen.

3.5.5.2 EFFECT OF SECRETAGOGUES ON GASTRIN SECRETION: CARBACHOL, BETHANECHOL, GRP

The results are presented in Table 3.6. None of the drugs at the doses tested significantly increased gastrin secretion (Dunnett method, two sample t-test), however, two of the 30 (6 doses in 5 experiments) carbachol responses were significant when assessed individually. One of these, the response to 10^{-6} M carbachol (cell suspension 3) was omitted from the mean as it was 13 standard deviations from the mean of the other four. The other result falls within the expected 5% error. For GRP, while no individual response differed from its control group, some responses did differ from each other, although not when the two experiments were considered together.

3.5.5.3 EFFECT OF INHIBITORY SUBSTANCES ON GASTRIN SECRETION: PARASITE INCUBATES

The cell density of the incubated mixtures of antral cells was estimated to be about 14 x 10^6 per ml, of which 90% were considered viable. Gastrin secretion by these cells was inhibited by all four solutions (Fig. 3.9). Note, all concentrations of carbachol tested on the same cell suspension had no effect.

3.5.5.4 EFFECT OF A SECRETAGOGUE ON PEPSINOGEN SECRETION: CARBACHOL

The one cell suspension tested had a cell density of approximately 8 x 10^6 cells per ml

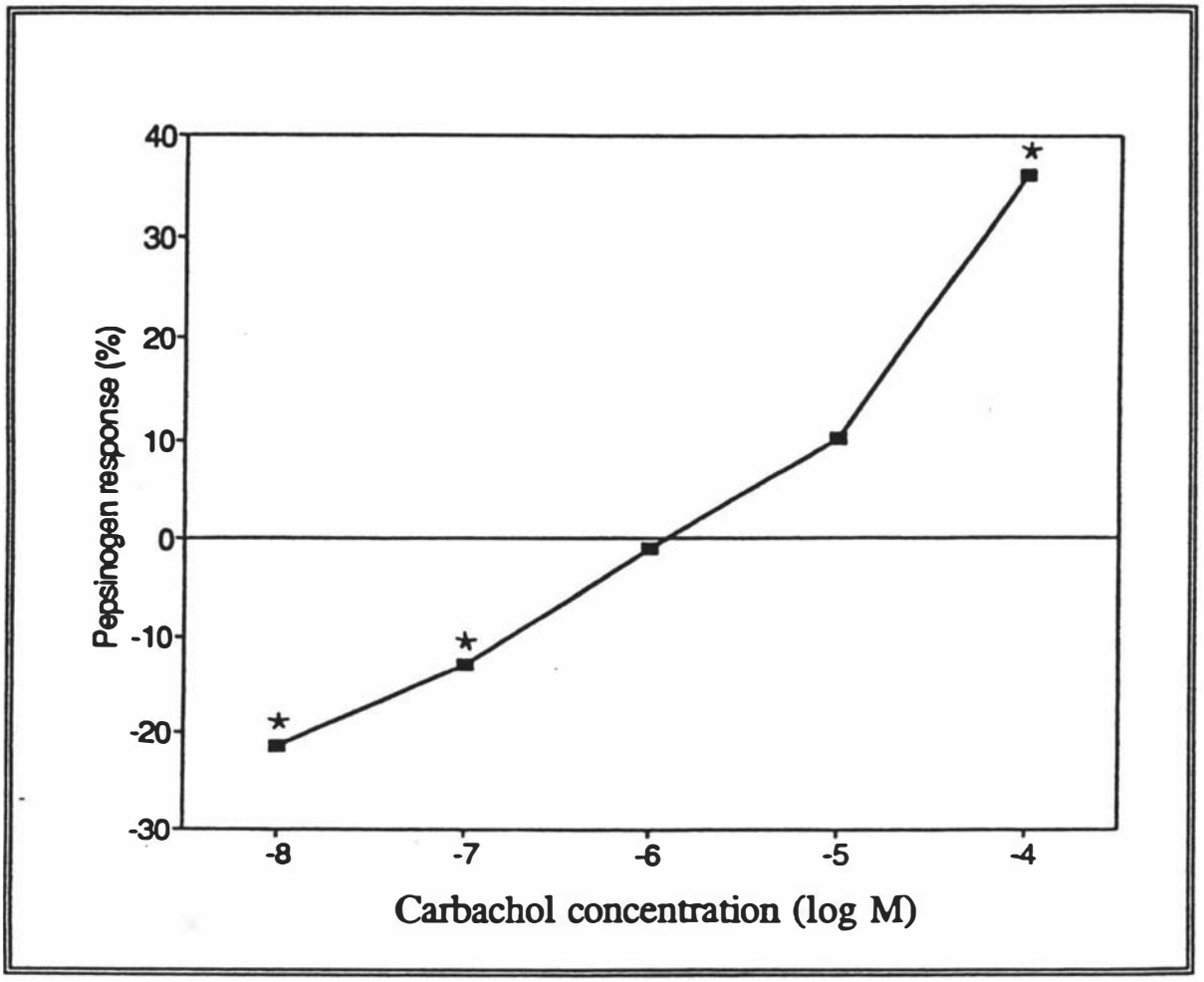

Figure 3.10 Effect of increasing carbachol concentration on pepsinogen secretion (Rt) by a dispersed ovine fundic mucosal cell preparation. (*) indicates a significant difference from secretion by controls (Dunnett method).

and about 90% were viable. Pepsinogen secretion was stimulated by 10^{-4} M carbachol while 10^{-7} and 10^{-8} M carbachol inhibited its release (Fig. 3.10). Tukey analyses revealed that the response to 10^{-8} M differed from that to 10^{-6}, 10^{-5} and 10^{-4} M; the response to 10^{-7} differed from that to 10^{-5} and 10^{-4} M; the response to 10^{-5} M differed from that to all but 10^{-6} M.

3.6 DISCUSSION

3.6.1 PERIFUSION METHOD

The perifusion method has shown that both bombesin and carbachol may stimulate the secretion of gastrin from perifused ovine antropyloric glands (Figs 3.3 and 3.4). Columns containing chopped mucosa failed to give any meaningful results. Whereas the responses that were identified were qualitative, the behaviour of individual columns was erratic resulting in responses which were not reproducible and especially difficult to quantitate. Difficulties experienced with this preparation were of two types: problems of carrying out the perifusion (column failure) and difficulties in assessing the response.

The principal cause of failure of the columns was the blocking of the mesh followed by leakage through the bung or tubing. Successive modification of the apparatus largely overcame the problem of blockages, but the difficulties with assessment of the response indicated a more fundamental problem with the method. The pH was continuously monitored and showed a tendency to decrease slowly, possibly because the gas used was carbogen, as was used originally by Richelsen *et al.* (1983). Oxygen may not have caused this problem. The pH was adjusted with NaOH, which in itself would increase the osmotic pressure slowly. Changes in pH or osmolarity of media may have influenced the response to subsequent re-perifusion of test media since changes in either of these conditions were seen to alter gastrin release in the static incubation method (3.4.5.3).

Quantitation of the responses is dependent on the basal/tonic secretory behaviour of the tissue. The basal secretion continued to decline over the course of the experiment, thus requiring the fitting of a baseline to the data. To minimise subjective influences, a computer programme was used to do this. The baseline was calculated from the control periods after the exclusion of the first three fractions collected subsequent to a period

of perifusion with test medium, in order to eliminate the influence of any delayed or persistent stimulation by the treatment. This approach seemed to work adequately and in most cases resulted in baselines which appeared subjectively to fit the data points. The basal secretory behaviour was unique to each column and could only be assessed retrospectively. If the tissue had been left until the basal secretion was changing more slowly, some of the difficulties associated with the baseline may have been reduced and the tissue may also have been more responsive to test substances. This was confirmed by the apparently greater absolute secretory response where basal secretion was lower, suggesting that the responsiveness of the tissue may be inversely related to the rate of basal secretion. In addition, for the same absolute response, the percentage increase will be less when the basal rate is high. Both of these factors probably account for the increased response to subsequent repeat perifusions of the same dose of secretagogue in every case.

The response to the drug was calculated from the area under the curve above (or below) the baseline for the five fractions associated with the treatment so that only the magnitude and not the duration of a response was considered. This approach was applied consistently but may not be ideal. In some cases, the gastrin secretion did not return to the calculated baseline before the subsequent test period began, however, due to the variable nature of the basal secretion this need not be due to the previous treatment. Nevertheless, in some instances the response did appear to continue beyond the period of treatment and in these cases the response may have been underestimated.

The responses were expressed as a percentage of the basal secretion rate. Concentration-response curves were no more reproducible when absolute responses were considered. Because of the falling baseline, it is only possible to quantify the response of a column at a particular moment in time and absolute responses of equal magnitude will give different results. It is interesting that others such as Schubert *et al.* (1991b), who also calculate the secretory response with respect to basal secretion, do not report any variability in this factor, yet it was the source of most of the problems associated with the method in the present study. Since the response of individual columns, as presented in Tables 3.1 and 3.2, is clearly not quantitatively reliable, statistical evaluation of the data was not carried out.

This method appeared to provide useful qualitative observations, but clearly was not appropriate for extensive routine pharmacological and other *in vitro* studies, at least

without extensive modification.

3.6.2 STATIC INCUBATION METHOD

This method was simple, a large number of replicates was possible for each treatment and the method gave reproducible results. The antral tissue was responsive to stimulation by carbachol and the behaviour of the control tissue was consistent between experiments. The technique appeared to provide a suitable method for further detailed studies of abomasal secretion. A disadvantage of a static system compared with a flow-through method is that substances released by the tissue may accumulate in abnormally high concentrations, but restriction of the incubation period to 10 or 20 minutes per well would likely keep this to a minimum. The responses are easily and justifiably quantified since the calculation requires no subjective interpretation and in this way differs fundamentally from the perifusion system.

The basal secretion of pepsinogen, gastrin and somatostatin decreased over time, all three having similar control ratios, suggesting that for all of these basal secretion decreased at about the same rate. The static incubation method is performed over an hour period that fits at some instant along the basal secretion curve seen in the perifusion method. As the basal secretion curve declines most rapidly initially, before flattening off as it becomes more constant, it is likely that experiments with lower CR values fall more to the left of the basal secretion curve, while experiments in which CR approaches one indicate that the experimental period fits to the right. The declining basal secretion is effectively not involved in the calculation as it is assumed that the basal secretion of the control and treated tissue is comparable and that the release study is done at the same instant on the basal secretion curve. The control basal secretion is used only to compare the responses within experiments. Since the individual pieces of tissue are derived from the same organ, are randomly assigned to treatments and are tested at the same time, it would seem to be valid to assume that the control and test groups to be in a similar state of dis-equilibrium. At least for the result presented in Figs 3.6 and 3.7, this appears to be valid. The method also assumes that the control group is sufficiently large as to be representative. The 68% increase in gastrin secretion stimulated by carbachol (10^{-4} M) accurately reflects how the tissue responded in absolute terms (Fig. 3.7).

As was suggested for the perifusion method, the responsiveness of the tissue may be influenced by the level of basal secretion, which will vary between experiments. In retrospect, it may have been wise to perform all experiments at the same time after the death of the sheep rather than as rapidly as possible and with only one 30 minute equilibration period. Had there been two 30 minute equilibration periods instead of one, the experiment would be pushed to the right of the basal secretion curve (increased CR) and there may have been less variation between experiments. However, there is no evidence that the time delay after death is the main variable influencing basal secretion.

All tissue would be vulnerable to secretory influences associated with accidental variations in the methodology (e.g. composition of the BM, physical stress to the tissue in moving the plates, variation in the temperature or evaporative loss in different parts of the warm room). The effects of changes in osmolarity and pH were consistent and in some cases substantial (Tables 3.4 and 3.5) and attention to both of these conditions is important. Hypotonic solutions caused much greater effects than did hypertonic BM: a reduction in osmolarity of 12.5% increased gastrin secretion by 50 to 90% but an increase in osmolarity of 25% reduced gastrin secretion by 30 to 50%. Decreases in pH below 7.4 cause greater changes in gastrin release than did comparable increases in pH (Fig. 3.8). As the phenol red in HBSS undergoes clearly visible colour changes within the pH range of 7.4 ± 0.1, undetected pH variations are unlikely to occur.

Whereas the method was clearly useful for studying gastrin secretion, this was not so certain for pepsinogen secretion. Carbachol stimulated pepsinogen secretion in only one of four experiments although cholinergic stimulation is a relatively consistent feature of pepsinogen secretion in other species both *in vivo* and *in vitro* (Chapter 1, 1.3.2). This result may accurately reflect the pharmacology of the chief cell in the sheep or, alternatively, the concomitant stimulation of somatostatin release may inhibit any direct stimulation of pepsinogen secretion. Such an effect may be enhanced by the static nature of this method in which the concentration of an inhibitor may accumulate during each incubation period to a level that completely blocks any net stimulatory effect. The perifusion system would prevent this type of effect as secreted substances would be removed in the effluent. This effect may be more apparent during the 20 minute incubation period and with the larger tissue size that was found to be necessary for fundic tissue in order for the pepsinogen concentration to rise to levels suitable for assay purposes. Further development of the method is required but it potentially is as applicable to the study of chief cell pharmacology as it is to study gastrin secretion.

3.6.3 DISPERSED CELLS

The presence of carbachol, bethanechol or GRP did not increase gastrin release by dispersed antral mucosal cell suspensions. This may result from either a general lack of cell responsiveness frequently seen after the tissue digestion and dispersal process (discussed by Chew *et al.* (1989)) or because the ovine G cell is not stimulated directly by cholinergic agonists or GRP. As reviewed in Chapter 1 (1.3.4), work with other species indicates that the gastrin-stimulating effect of cholinergic agonists, and to a lesser extent GRP, may be associated with inhibition of somatostatin release. However, GRP and bombesin have been shown to stimulate gastrin release from enriched or cultured human and canine G cells (Sugano *et al.*, 1987; Giraud *et al.*, 1987; Campos *et al.*, 1990). Similarly, carbachol stimulates gastrin secretion from canine antral mucosal cells in primary culture (Schepp *et al.*, 1994) and from dispersed antral gland preparations from the rabbit and human (Richelsen *et al.*, 1983). Nevertheless, none of the evidence presented by these authors precludes an action mediated via the D cell as in all cases somatostatin cells were present in small numbers, and, until highly enriched cultures can be prepared, doubt for a direct action of either stimulant, and particularly by cholinergic mechanisms, will remain.

Inhibition of gastrin secretion by all of the inhibitory (worm-derived) solutions tested in 3.5.5.3 indicates that the ovine G cell can be inhibited by the unknown active substances in these incubates. This responsiveness may support the contention that neither GRP or cholinergic agonists act directly on the G cell. Alternatively, it may further reflect the relatively disturbed state of the cell population, which, if hyper-secreting as a result of the dispersal process, while not having the potential to further respond to stimulatory factors, may have retained, if not increased, their potential for a reduced secretory rate. Unfortunately, no estimate of the total gastrin concentration of each aliquot was made. Such information may have given some indication of the relative rate of secretion with respect to the total cell content. To overcome some of the compromised responsiveness of acutely isolated cells, many workers now maintain their cell preparations in short-term culture before performing release studies. A detailed account of one method of primary culture of mucosal cells is provided by Chew *et al.* (1989). Short-term culture almost without exception is associated with enriched cell fractions prepared through elutriation (fibroblasts would overrun any other cell types if not excluded) and necessitates elimination of microorganisms (largely achieved through

elutriation) and maintenance of aseptic conditions. Equipment and conditions necessary for the culture of cells were not available to the present studies.

The dispersal method itself was effective and the cell viability over 90%. In all the final cell suspensions, cell dispersal was relatively complete. When examined under the microscope for cell counting or for assessing viability, most cells were present as single cells, fewer were in pairs and fewer still in clusters of three or more. Either the digestion process was efficient or larger cell groupings were excluded in the various filtration steps. Direct comparison with the yield obtained by Soll (1978) (7×10^7 per g of mucosa digested) is not possible as the amount of mucosa digested was not estimated and the resuspension volume differed. However, it is likely that the smaller gauge mesh (20 µm) used in these experiments to filter the suspension may have reduced the final cellular concentration.

The concentration of cells in the cell suspensions and the aliquot volume taken were appropriate to release either gastrin and pepsinogen in concentrations which could be assayed. The cell suspension was relatively homogeneous (3.5.5.1) indicating that the gentle mixing of the suspension during subsampling was adequate. The CV of 13% was not considered likely to mask any significant responses to test substances. This CV was higher than in most of the other experiments in which the mean CV was 9%. The variation in CV suggests that either the disturbance to the cells or the homogeneity/mixing of the suspensions prepared varied from day to day. This is perhaps to be expected as the mucosal tissue from different animals showed gross variation in its thickness, fat content and colour.

The freshly dispersed chief cells, unlike the G cells, were responsive to carbachol and thus may be more resilient to the dispersal process. The evidence supporting direct cholinergic stimulation of pepsinogen secretion via M_3 receptors was presented in Chapter 1, 1.3.2.1. The pepsinogen response to 10^{-4} M carbachol of about 40% is less than the 100% increase by rabbit cells (Koelz *et al.*, 1982). McKellar *et al.* (1990a) tested 3×10^{-6} M carbachol on bovine and ovine abomasal glands that had been dispersed by a procedure very similar to that used in this work. They reported a significant increase in pepsinogen release of 6.5% and 7.1% for bovine and ovine glands respectively. These increases fall between the secretion level found in this study for 10^{-6} and 10^{-5} M.

The procedure used to prepare the cell suspensions reported here appears to provide a cell population that is well separated and viable. The chief cell population appears to be responsive to stimulation while it was not possible to conclude from this work whether the lack of a gastrin response to agents that are known to stimulate gastrin secretion in other systems was due to their disturbance and consequent unresponsiveness or was a true reflection of their physiology.

3.6.4 CONCLUSIONS

The methods reported in this Chapter allow the study of the secretory activity of tissue pieces, dispersed glands and dispersed cells, each retaining a different complexity of control mechanisms. Whereas the static methods may allow unusually high levels of secretates to accumulate (e.g. somatostatin), the perifusion technique, being a flow-through system, may be particularly useful as a qualitative method to lessen the effects of any somatostatin accumulation. As a general method, its use is limited by the need for subjective interpretation and the difficulty in quantitating the response due to the declining and unique level of basal secretion particular to each column. Although the perifusion technique provided useful qualitative observations, it clearly was not appropriate for extensive routine pharmacological and other *in vitro* studies.

Freshly prepared dispersed cells are well known to be unreliable and thus short-term culture techniques are now preferred by many workers. The chief cells appeared to be reasonably responsive to stimulation and could be a useful preparation. The failure of the G cells to respond to stimulatory agents may not reflect their condition but rather their pharmacology. In contrast, the G cells retained their potential to be inhibited.

The static incubation method appeared to be the most suitable for extensive investigation of a wide range of pharmacological agents and also for screening worm preparations for active substances. For the study of gastrin secretion, the static incubation method was considered most appropriate, principally as the results could be easily and precisely quantified without subjective interpretation. Further modification of the method could reduce the quantity of medium required, which is an important consideration where expensive commercially-prepared peptides or small amounts of worm-derived solutions are to be tested. Results of studies using the *in vitro* methods described in this Chapter, principally the static incubation method, are described in detail in Chapters 4, 5 and 6.

Chapter 4

PHARMACOLOGICAL STUDIES OF THE OVINE G AND D CELL

4.1 INTRODUCTION

In the sheep, as in other species, the major source of gastrin is the mucosa of the pyloric antrum (Reynolds *et al.*, 1991). Without evidence to the contrary, it is reasonable to assume that gastrin release into the circulation is regulated by the same mechanisms as in other mammals: principally cholinergic, VIP and GRP-containing neurons and the paracrine mediator somatostatin (Schubert, 1993, 1994; Chapter 1, 1.3.4). *In vivo*, these regulators of the G cell are sensitive to input from the central nervous system (cephalic phase), the degree of gastric distension and the chemical composition of the luminal contents, in response to which they mediate changes in gastrin secretion. Interference with these regulators by alkalinization of the stomach contents, its perfusion with tetrodotoxin, atropine, GRP and VIP antagonists, infusion of somatostatin or its analogue, or vagal stimulation has measurable effects on gastrin release and affirms their mediatory function (Seal *et al.*, 1982; Wolfe *et al.*, 1983; Saffouri *et al.*, 1984a; Schubert *et al.*, 1988a; Ryberg *et al.*, 1990; Greenberg *et al.*, 1992; Meijer *et al.*, 1993).

Somatostatin is believed to exert continuous, direct, tonic inhibition on the G cell (Chapter 1, 1.3.4.2.5). This has been demonstrated by the use of somatostatin antiserum, which generally leads to an increase in gastrin secretion (Saffouri *et al.*, 1979; Holst *et al.*, 1992a; Martin *et al.*, 1994) and by the presence of somatostatin receptors on the G cell (Gable *et al.*, 1989). The tonic inhibition, or restraint that somatostatin exerts, is considered to be optimal as Hayes *et al.* (1975) failed to inhibit basal gastrin release from segments of rat antral mucosa perifused with 2×10^{-7} M somatostatin and Saffouri *et al.* (1984a) did not depress gastrin secretion with VIP, although somatostatin secretion was increased. Consequently, in order to obtain a maximal gastrin response to stimulants such as GRP, it appears to be necessary to inhibit or negate somatostatin secretion (thus removing tonic inhibition of the G cell) with cholinergic agonists or somatostatin antibody (DuVal *et al.*, 1981; Sugano *et al.*, 1987; Campos *et al.*, 1990).

Atropine converts a cholinergically-induced decrease in somatostatin secretion to an increase above basal levels (Makhlouf *et al.*, 1989).

Cholinergic agonists, such as methacholine and carbachol, dose-dependently inhibit somatostatin and stimulate gastrin secretion in a range of species (Saffouri *et al.*, 1980; DuVal *et al.*, 1981; Richelsen *et al.*, 1983). Studies that have used somatostatin antibodies indicate that, for the most part, the gastrin response to parasympathomimetics is mediated through reduced somatostatin secretion (Chapter 1, 1.3.4.2.1).

GRP (or bombesin) infusion into dogs, rats, humans and pigs induces significant increases in plasma gastrin (Bertaccini *et al.*, 1974; DuVal *et al.*, 1981; de Jong *et al.*, 1987; Holst *et al.*, 1987a). GRP is believed to act directly on the G cell as it stimulates gastrin release from enriched G cell cultures (Campos *et al.*, 1990) by a calcium-dependent mechanism (Sugano *et al.*, 1987; Giraud *et al.*, 1987) and is, therefore, independent of somatostatin. GRP mediates a large portion of the gastrin response to electrical field or vagal stimulation. This action is resistant to atropine and sensitive to hexamethonium (Chapter 1, 1.3.4.2.3). The remainder of the gastrin response to electrical or vagal stimulation is largely removed by atropine. The combination of GRP antiserum and atropine, therefore, effectively abolishes the response (Schubert *et al.*, 1985), implicating muscarinic cholinergic mechanisms.

VIP, catecholamines and various other substances such as prostaglandin-E_2, adenosine, GIP, GABA, PHI and amines may affect gastrin secretion either through interaction with enteric neurons, or through affects on somatostatin release (Chapter 1, 1.3.4). VIP is released on vagal stimulation in calves, cats, pigs and lambs (Bloom *et al.*, 1978; Fahrenkrug *et al.*, 1978; Holst *et al.*, 1983; Reid *et al.*, 1988). Vagal-stimulated VIP release, like that of GRP, appears to be atropine-resistant and hexamethonium-sensitive (Fahrenkrug, 1989), and results in stimulation of somatostatin secretion (Saffouri *et al.*, 1984a; Schubert & Hightower, 1989b). The action of catecholamines appears to be largely indirect and to be mediated by their effects on enteric neurons, from which catecholamines are believed to influence the release of substances such as GRP (Bloom & Edwards, 1982; Short *et al.*, 1985b). It is possible that B_2-receptors are present on the G cell and that their activation stimulates gastrin secretion directly (Buchan, 1991).

In the sheep, regulatory mechanisms similar to those outlined above appear to operate.

In vivo, sheep may be induced to release gastrin in response to cephalic stimuli (Reynolds *et al.*, 1978) and to chemical conditions within the abomasum (van Bruchem & van 'T Klooster, 1980; Reynolds *et al.*, 1989, 1991). Somatostatin, VIP and GRP have all been identified in the abomasum (Section 1.2.2) and their physiological roles in the abomasum have been investigated in a number of *in vivo* studies (Bloom *et al.*, 1978; Shulkes & Hardy, 1980, 1982; Bloom *et al.*, 1983; Bladin *et al.*, 1983; Barry *et al.*, 1985; Reid *et al.*, 1988; Shulkes *et al.*, 1994). The release of gastrin and somatostatin has been initiated by vagal (Bloom *et al.*, 1978; Reid *et al.*, 1988) or splanchnic (Bloom & Edwards, 1982) nerve stimulation in ruminants. Atropine administration to sheep either inhibited or stimulated post-prandial gastrin release depending on the dose (Reynolds *et al.*, 1991). *In vivo* studies such as these reveal little about the mechanisms involved in the control of gastric secretion. Indeed, a number of studies have reported responses that seem to be at variance with those in other species, e.g. GRP infusion had no effect on gastrin release in conscious calves (Bloom *et al.*, 1983) or sheep (Shulkes & Hardy, 1980; McDonald *et al.*, 1988), omeprazole-induced hypergastrinaemia (three times basal levels) developed in sheep without a change in plasma somatostatin (Read *et al.*, 1992) and infusion of somatostatin into sheep increased basal plasma gastrin (Barry *et al.*, 1985; Reynolds *et al.*, 1991). It is possible that, rather than physiological differences between ruminants and monogastrics, apparent discrepancies reflect the complexity of the *in vivo* preparation or the barely detectable changes on which some of these interpretations were based. Thus, although GRP infusion did not stimulate gastrin secretion in normal sheep (Shulkes & Hardy, 1980; McDonald *et al.*, 1988), a response occurred in animals that had been immunized against somatostatin (Shulkes *et al.*, 1994). While this subsequent study by Shulkes *et al.* does suggest a role for GRP in the sheep, it reinforces the suggestion made previously by McDonald *et al.* (1988), that ruminants may differ from other mammals in their sensitivity to GRP, and in particular its effect on somatostatin secretion. Interpretation of the gastrin and somatostatin response, particularly *in vivo*, may be confounded by the reciprocal effect that they are believed to have on their respective cell types and by possible autocrine regulatory mechanisms (see Chapter 1, 1.3.4.2.5).

In this Chapter, *in vitro* experiments on ovine tissues which examine the mechanisms regulating gastrin secretion and the interaction with somatostatin are reported. Most experiments used the static incubation method (Chapter 3, 3.4), although reference is made to some results from dispersed cell preparations and perifused antropyloric glands

which were presented in Chapter 3.

4.2 MATERIALS AND METHODS

4.2.1 EXPERIMENTAL DESIGN

The effects of pharmacological agents on gastrin release by ovine G cells, and in some experiments the simultaneous release of somatostatin by antral D cells, has been studied using the *in vitro* techniques described in Chapter 3:

(1) bombesin and carbachol were tested using the perifusion technique;

(2) bombesin, GRP (with and without somatostatin antiserum), VIP, GIP, adrenaline, carbachol and bethanechol (in combination with atropine and hexamethonium), eserine, nicotine and prostaglandin-F2α were tested by the static incubation method;

(3) GRP, carbachol and bethanechol were tested on dispersed antral mucosal cells.

4.2.2 PHARMACOLOGICAL AGENTS

The following agents were used in the concentrations listed:

(1) bombesin acetate salt (B-4272, Sigma Chemical Co., USA) in concentrations of 10^{-10} to 10^{-4} M for perifusion experiments and from 10^{-12} to 10^{-5} M for static incubations;

(2) GRP (Synthetic porcine GRP-28, G-1649, Sigma Chemical Co., USA) in concentrations of 10^{-12} to 10^{-6} M for static incubations and from 10^{-10} to 10^{-6} M for dispersed cells;

(3) carbachol (Carbamylcholine chloride, C-4382, Sigma Chemical Co., USA) in concentrations of 10^{-8} to 10^{-4} M for the perifusion method, from 10^{-9} to 10^{-4} M for static incubations and from 10^{-9} to 10^{-4} M for dispersed cells;

(4) atropine (A-0257, Sigma Chemical Co., USA) in a concentration of 10^{-5} M;

(5) hexamethonium (hexamethonium chloride, H-2138, Sigma Chemical Co., USA)

in a concentration of 10^{-5} M;

(6) bethanechol (Carbamyl-ß-methycholine chloride, C-5259, Sigma Chemical Co., USA) in concentrations from 10^{-9} to 10^{-4} M;

(7) nicotine ([-]-1-Methyl-2-[3-pyridyl]-pyrrolidine, N-3876, Sigma Chemical Co., USA) in concentrations of 10^{-6}, 10^{-5}, 10^{-4}, 5×10^{-4} and 5×10^{-3} M;

(8) eserine (Physostigmine salicylate salt, E-8500, Sigma Chemical Co., USA) in concentrations of 10^{-6} and 10^{-5} M;

(9) VIP (synthetic porcine VIP (V-3628, Sigma Chemical Co., USA) in concentrations from 4×10^{-10} to 4×10^{-6} M;

(10) GIP (synthetic porcine GIP, G-5512, Sigma Chemical Co., USA) in concentrations from 6×10^{-10} to 6×10^{-7} M;

(11) adrenaline (David Bull Laboratories, Australia) in concentrations of 10^{-8} to 10^{-4} M;

(12) prostaglandin-F2α (Upjohn Co., USA) in concentrations from 10^{-10} to 10^{-6} M;

(13) somatostatin antiserum (monoclonal antibody from mouse ascites; control - keyhole limpet haemocyanin monoclonal antibody, Dr J.H. Walsh, UCLA) in final dilutions of 1:500, 1:1000 and 1:2500.

4.2.3 GASTRIN AND SOMATOSTATIN ASSAYS

These were estimated as described in Chapter 3, 3.2.3 and the methods are described in detail in Appendices 1.2 and 1.3. Each experiment was assayed within a single assay to eliminate interassay variation.

4.2.4 STATISTICS

All statistics were performed using the software package MINITAB (Minitab Inc., USA).

Table 4.1 Effect of bombesin on gastrin and somatostatin secretion by ovine antral mucosa. CV = the coefficient of variation calculated from raw data (mean control CV = 13% (gastrin), 19% (somatostatin)); Rt values were derived from 4 experiments; in each experiment there were 32 control and 8 test tissue pieces.

Bombesin concentration	% Gastrin response		% Somatostatin response	
	Rt (mean ± SD)	Mean %CV	Rt (mean ±SD)	Mean %CV
10^{-12} M	4 ± 4	12	4 ± 13	23
10^{-11} M	5 ± 6	13	1 ± 20	24
10^{-10} M	1 ± 7	13	-7 ± 11	24
10^{-9} M	0 ± 2	11	-10 ± 14	20
10^{-8} M	-2 ± 21	13	-4 ± 26	25
10^{-7} M	8 ± 8	12	-7 ± 18	20
10^{-6} M	7 ± 10	13	4 ± 9	22
10^{-5} M	7 ± 7	13	-6 ± 16	21

Table 4.2 Effect of GRP on the gastrin and somatostatin secretion by ovine antral mucosa. CV = the coefficient of variation calculated from raw data (mean control CV = 13% (gastrin), 22% (somatostatin)); Rt values were derived from 9 of the 10 experiments performed for 10^{-12} to 10^{-7} M; in each experiment, there were 24 or 32 control and 16 test tissue pieces; for 10^{-6} M GRP, Rt was derived from 6 experiments, in each of which there were 8 tissue pieces; (*) = a significant difference from control at the 5% level (Dunnett method).

GRP concentration	% Gastrin response		% Somatostatin response	
	Rt (mean ±SD)	Mean %CV	Rt (mean ± SD)	Mean %CV
10^{-12} M	5 ± 6	13	-13 ± 14	24
10^{-11} M	-3 ± 13	10	-8 ± 9	20
10^{-10} M	4 ± 9	14	5 ± 11	23
10^{-9} M	0 ± 11	12	4 ± 9	22
10^{-8} M	1 ± 12	13	14 ± 23	24
10^{-7} M	22 ± 11*	14	5 ± 29	24
10^{-6} M	110 ± 43*	24	3 ± 12	33

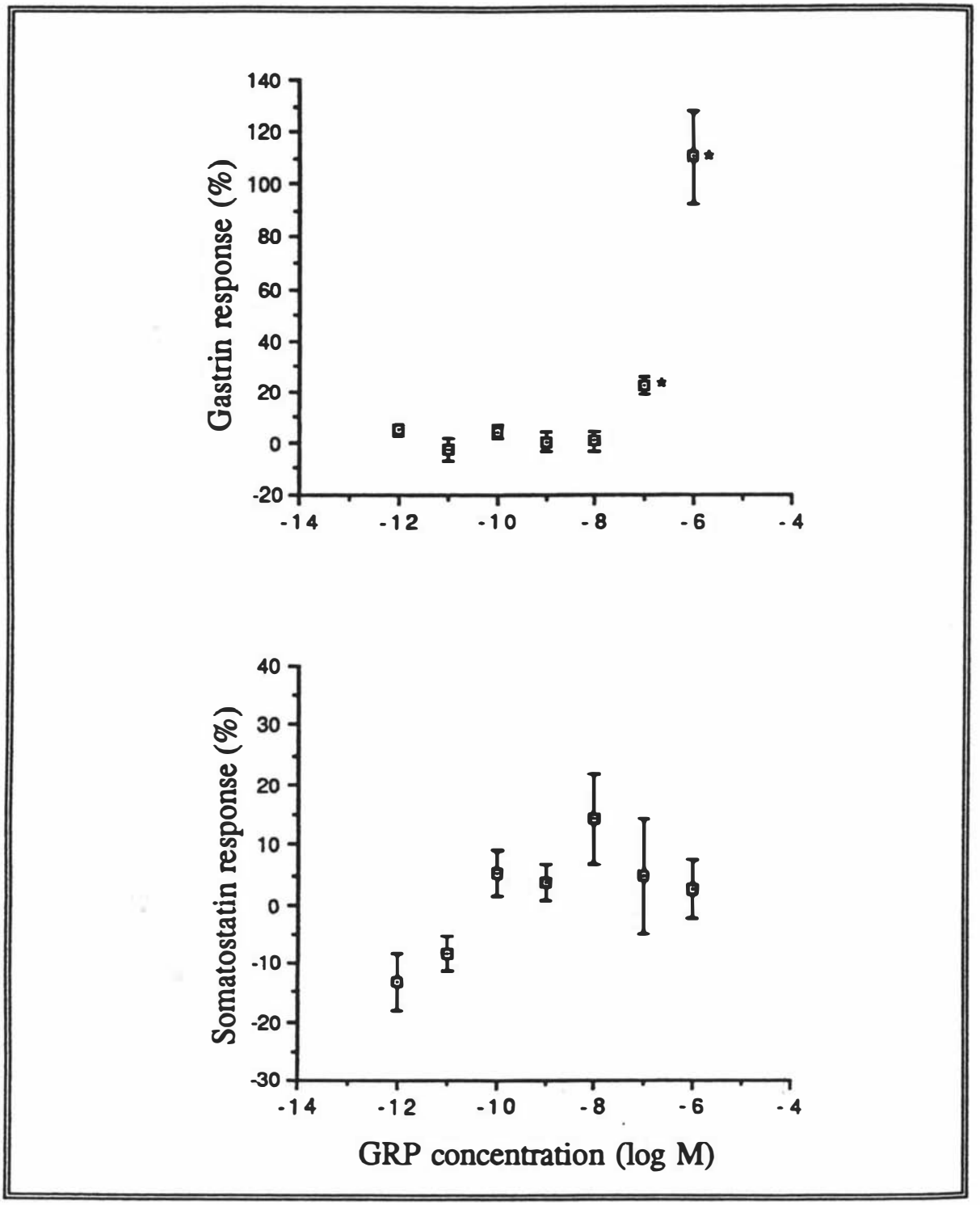

Figure 4.1 Effect of GRP on gastrin and somatostatin secretion by segments of antral mucosa (mean ± SEM). The gastrin and somatostatin response to treatment with GRP over the concentration range 10^{-12} to 10^{-6} M was determined using the static incubation method. (*) indicates a significant difference from control (Dunnett method).

The data set included Rt values derived from separate experiments. Where related treatments were tested (e.g. a range of concentrations of the same agent) the data sets were compared using either the Tukey (equal sample sizes) or Dunnett (unequal sample sizes) multiple comparison method. Where the effect of a treatment (Rt) within an experiment was tested against H_0 and only two variables were involved (e.g. Rt_itest and Rt_icontrol sets), two sample t-tests were used. The single sample t-test was used to test single data sets (e.g. difference between response to carbachol with and without atropine) against the null hypothesis. Where multiple treatments were involved in one experiment the Tukey or Dunnett multiple comparison methods were used. Differences were considered significant at the 5% level.

4.3 RESULTS

4.3.1 BOMBESIN-LIKE PEPTIDES

4.3.1.1 BOMBESIN

In four experiments using the static incubation method, bombesin in concentrations between 10^{-12} and 10^{-5} M had no significant effect on either gastrin or somatostatin release (Table 4.1). In contrast, perifusion of antropyloric glands by bombesin in concentrations from 10^{-10} to 10^{-4} M appeared to increase gastrin release above basal levels (Fig. 3.3 and Table 3.1), where gastrin release was stimulated by an estimated 30% for all three bombesin concentrations from 10^{-6} to 10^{-4} M.

4.3.1.2 GRP

GRP in concentrations from 10^{-10} to 10^{-6} M did not significantly alter the gastrin release from two dispersed antral cell suspensions (Table 3.6).

In the nine of the 10 experiments performed using the static incubation method that are presented in Table 4.2, GRP stimulated gastrin secretion by 22% at 10^{-7} M and by 110% at 10^{-6} M. The gastrin response to 10^{-6} M was significantly greater than that to 10^{-7} M. The effects of increasing GRP concentration on gastrin and somatostatin secretion are shown in Fig. 4.1. The 14% somatostatin response to 10^{-8} M GRP was significantly greater than the response to 10^{-12} M, but was not significantly different from control.

Table 4.3 Effect of somatostatin antiserum on the gastrin response (Rt) to 10^{-7} M GRP. The effect is expressed as response to GRP (10^{-7} M) minus the response to GRP (10^{-7} M) plus somatostatin (SMS) antiserum (1:500 dilution). Experiment 5 is appended as both its gastrin and somatostatin response to GRP alone deviated significantly from the mean. CV = the coefficient of variation calculated from the raw data (mean control CV = 12% (gastrin), 19% (somatostatin); in each experiment there were 32 control tissue pieces, 24 for GRP plus antiserum and 16 for GRP; (*) indicates a significant difference between the response to GRP and to GRP plus somatostatin antiserum at the 5% level (two sample t-test).

Experiment	GRP 10^{-7} M				GRP and antiserum		Rt (GRP) - Rt (GRP + SMS antiserum)
	% Gastrin response		% Somatostatin response		% Gastrin response		
	Rt	%CV	Rt	%CV	Rt	%CV	
1	17	11	-37	32	19	13	-2
2	32	12	-30	24	27	13	5
3	23	21	8	22	23	14	0
4	22	12	4	18	20	16	2
5	-12	10	330	43	15	9	27*

The results from the tenth experiment (Expt 5, Table 4.3) were excluded from the overall mean values in Table 4.2 because the gastrin and somatostatin responses to 10^{-7} M (the maximum concentration tested in that particular experiment) were outside 3 SD of the mean of the other nine experiments.

Somatostatin antiserum, in final dilutions up to 1:500, did not affect the gastrin response to 10^{-7} M GRP in four of the five experiments in which it was tested, either as a group or individually (Table 4.3). The control antiserum (1:500) also had no significant effect on the gastrin ($p = 0.11$) or somatostatin ($p = 0.4$) response to GRP in the experiment within which it was tested. The tissue in one experiment (Expt 5, Table 4.3, which is the experiment excluded from Table 4.2), behaved differently in that there was a significant increase of 27% in the gastrin response to 10^{-7} M GRP when 1:500, but not 1:1000 or 1:2500 dilutions, somatostatin antiserum was included in the BM. In that experiment, 10^{-7} M GRP inhibited gastrin release by 12% and increased somatostatin release by 330%; the inclusion of somatostatin antiserum converted the gastrin response to an increase which was not significantly different from the response to the same concentration of GRP in the other experiments, with or without somatostatin antiserum.

In the one experiment in which carbachol (10^{-4} M) and atropine (10^{-5} M) were combined with GRP (10^{-7} M), neither treatment produced a gastrin or somatostatin response that differed from the response to 10^{-7} M GRP alone.

4.3.2 CHOLINERGIC AGONISTS AND ANTAGONISTS

4.3.2.1 CARBACHOL

Carbachol was tested using all three *in vitro* methods. Concentrations from 10^{-9} to 10^{-5} M did not increase gastrin release from dispersed antral mucosal cells in five experiments (Table 3.6). Perifused antropyloric glands showed increased gastrin release to carbachol solutions from 10^{-8} to 10^{-4} M (Fig. 3.4, Table 3.2). The estimated gastrin response of perifused glands above basal was 31% for concentrations of 10^{-7} to 10^{-5} M and 41% for 10^{-4} M.

The effect of carbachol concentrations from 10^{-9} M to 10^{-4} M on gastrin release were determined in at least 17 static incubation experiments and the somatostatin response

Table 4.4 Effect of carbachol on gastrin and somatostatin secretion by ovine antral mucosa. CV = the coefficient of variation calculated from raw data (mean control CV = 13% (gastrin), 26% (somatostatin)); (n) the number of experiments, in each of which there were 16, 24 or 32 control tissue pieces and 8 or 16 test pieces; (*) indicates a significant difference from control at the 5% level (Dunnett method). Tukey analysis of the 17 common experiments revealed that each significant response was different from each other. No somatostatin results differed from one another.

Carbachol concentration	% Gastrin response			% Somatostatin response		
	Rt (mean ± SD)	Mean %CV	n	Rt (mean ± SD)	Mean %CV	n
10^{-9} M	5 ± 15	14	17	-4 ± 21	21	12
10^{-8} M	2 ± 8	13	17	-9 ± 18	26	12
10^{-7} M	3 ± 11	16	17	-12 ± 23	24	12
10^{-6} M	15 ± 16*	16	19	2 ± 30	18	12
10^{-5} M	32 ± 17*	14	19	11 ± 26	22	12
10^{-4} M	41 ± 16*	13	30	19 ± 28	20	24

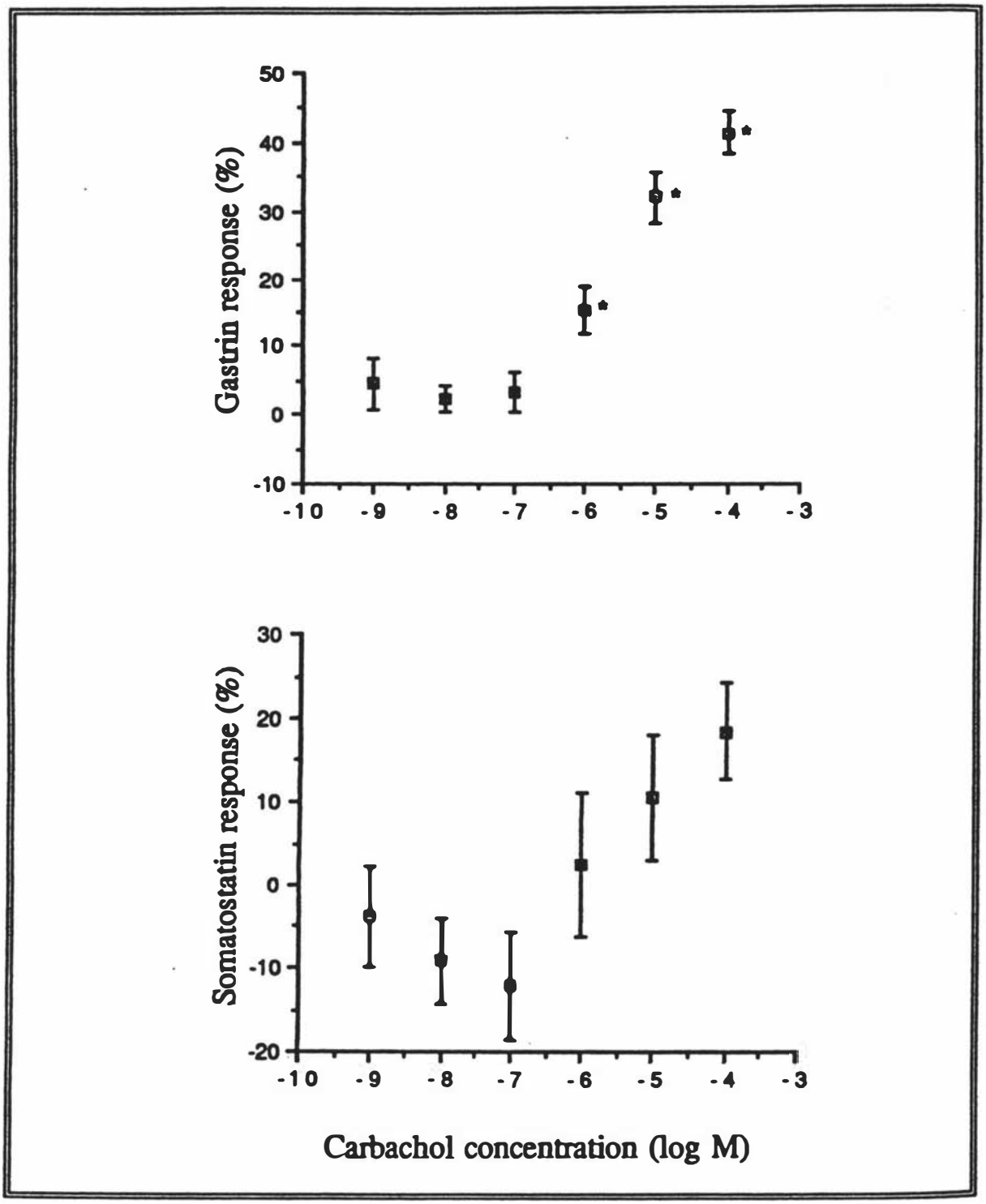

Figure 4.2 Effect of carbachol on gastrin and somatostatin secretion by segments of ovine antral mucosa (mean ± SEM). The gastrin and somatostatin response to treatment with carbachol over the concentration range 10^{-9} to 10^{-4} M was determined using the static incubation method. (*) indicates a significant difference from control (Dunnett method).

Table 4.5 Effect of carbachol on gastrin and somatostatin secretion by ovine antral mucosa in individual experiments. In each experiment (Expt) there were 32 control and 8 test pieces of tissue. Rt is the response (±SD) to treatment; the mean coefficient of variation of control tissue pieces was 13% (gastrin) and 26% (somatostatin); (*) = a significant difference from control (Dunnett method).

Expt	% GASTRIN (G) AND SOMATOSTATIN (SMS) RESPONSE TO TREATMENT WITH CARBACHOL (Rt ± SD)													
	10^{-9} M		10^{-8} M		10^{-7} M		10^{-6} M		10^{-5} M		10^{-4} M		Control	
	G	SMS	G	SMS	G	SMS	G	SMS	G	SMS	G	SMS	G	SMS
1	1 ± 14	-6 ± 5	-2 ± 12	-10 ± 14	3 ± 10	-1 ± 13	17 ± 11*	5 ± 10	22 ± 10*	13 ± 16	56 ± 26*	18 ± 18	0±12	0±14
2	-33 ± 12*	33 ± 28	-8 ± 7	17 ± 24	12 ± 15	-2 ± 16	14 ± 17	-1 ± 15	14 ± 8	4 ± 15	53 ± 19*	5 ± 12	0±13	0±29
3	-25 ± 9*	-23 ± 11*	-12 ± 14	7 ± 27	-16 ± 12*	-2 ± 22	-14 ± 15	-2 ± 16	29± 14*	-5 ± 18	5 ± 13	-3 ± 15	0±12	0±13
4	0 ± 15	4 ± 21	2 ± 24	5 ± 26	5 ± 10	-2 ± 24	4 ± 20	-1 ± 24	13 ± 18	21 ± 23	37 ± 26*	-2 ± 14	0±11	0±31
5	5 ± 9	17 ± 36	-0 ± 16	6 ± 48	-9 ± 15	-10 ± 24	7 ± 23	10 ± 20	24 ± 14*	30 ± 16	38 ± 21*	78 ± 29*	0±14	0±27
6	10 ± 14	12 ± 12	-10 ± 15	-5 ± 18	-11 ± 9	-6 ± 32	-7 ± 13	-5 ± 22	27 ± 11*	20 ± 34	40 ± 17*	21 ± 33*	0±16	0±18
7	-2 ± 12	5 ± 30	12 ± 12	-2 ± 45	5 ± 26	-9 ± 45	9 ± 8	4 ± 39	34 ± 21*	36 ± 53*	55 ± 14*	37 ± 37*	0±15	0±31
8	-12 ± 12	-13 ± 40	-1 ± 17	-27 ± 20	-1 ± 10	9 ± 18	3 ± 20	32 ± 17*	27 ± 18*	25 ± 22	42 ± 10*	24 ± 18	0±15	0±30
9	17 ± 23	-19 ± 22	11 ± 11	-21 ± 26	-18 ± 55	-31 ± 15	-7 ± 12	48 ± 10*	54 ± 20*	28 ± 35	45 ± 20*	17 ± 19	0±17	0±43
10	16 ± 21	8 ± 14	-6 ± 14	-9 ± 11	7 ± 30	14 ± 24	32 ± 14*	41 ± 10*	32 ± 14*	31 ± 28*	66 ± 34*	53 ± 29*	0±17	0±17
11	5 ± 10	-42 ± 9*	3 ± 10	-43 ± 17*	19 ± 13*	-65 ± 8*	29 ± 11*	-51 ± 10*	27 ± 16*	-47 ± 11*	35 ± 7*	-50 ± 19*	0±7	0±23
12	30 ± 15*	-23 ± 9*	17 ± 13	-30 ± 16*	-1 ± 39	-4 ± 17*	18 ± 15	-49 ± 7*	41 ± 45*	-32 ± 14*	27 ± 15*	-16 ± 15*	0±14	0±17
13	11 ± 16	-	13 ± 10	-	9 ± 15	-	54 ± 15*	-	55 ± 32*	-	76 ± 25*	-	0±12	-
14	33 ± 12*	-	11 ± 11	-	16 ± 23	-	51 ± 22*	-	61 ± 27*	-	194 ± 48*	-	0±12	-
15	-4 ± 14	-	3 ± 16	-	16 ± 13	-	11 ± 11	-	45 ± 25*	-	69 ± 14*	-	0±13	-
16	-6 ± 16	-	3 ± 10	-	-20 ± 13	-	-4 ± 16	-	11 ± 12	-	32 ± 3*	-	0±10	-
17	-	-	-9 ± 15	-	-14 ± 14	-	3 ± 14	-	7 ± 10	-	27 ± 22*	-	0±15	-

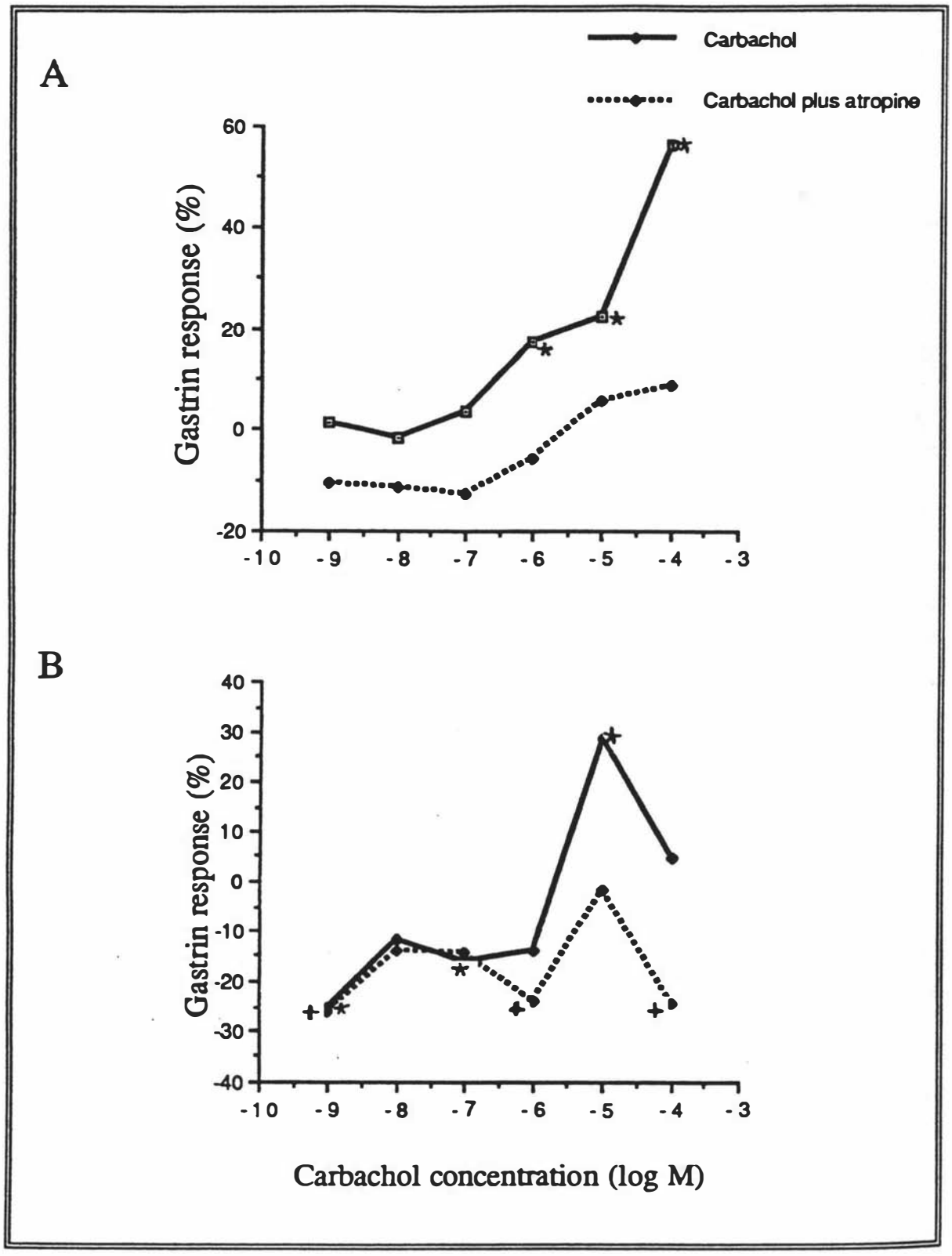

Figure 4.3 Effect of atropine on the gastrin response to carbachol. Two individual experiments in which 10^{-5} M atropine was combined with a range of carbachol concentrations (10^{-9} to 10^{-4} M) are shown. (*) = a significant difference from control for carbachol, (+) = a significant difference from control for carbachol plus atropine (Dunnett method). Note in (A) the gastrin response to 10^{-4} M carbachol was 56% while in (B) it was 5%, but there was a similar reduction in the presence of atropine.

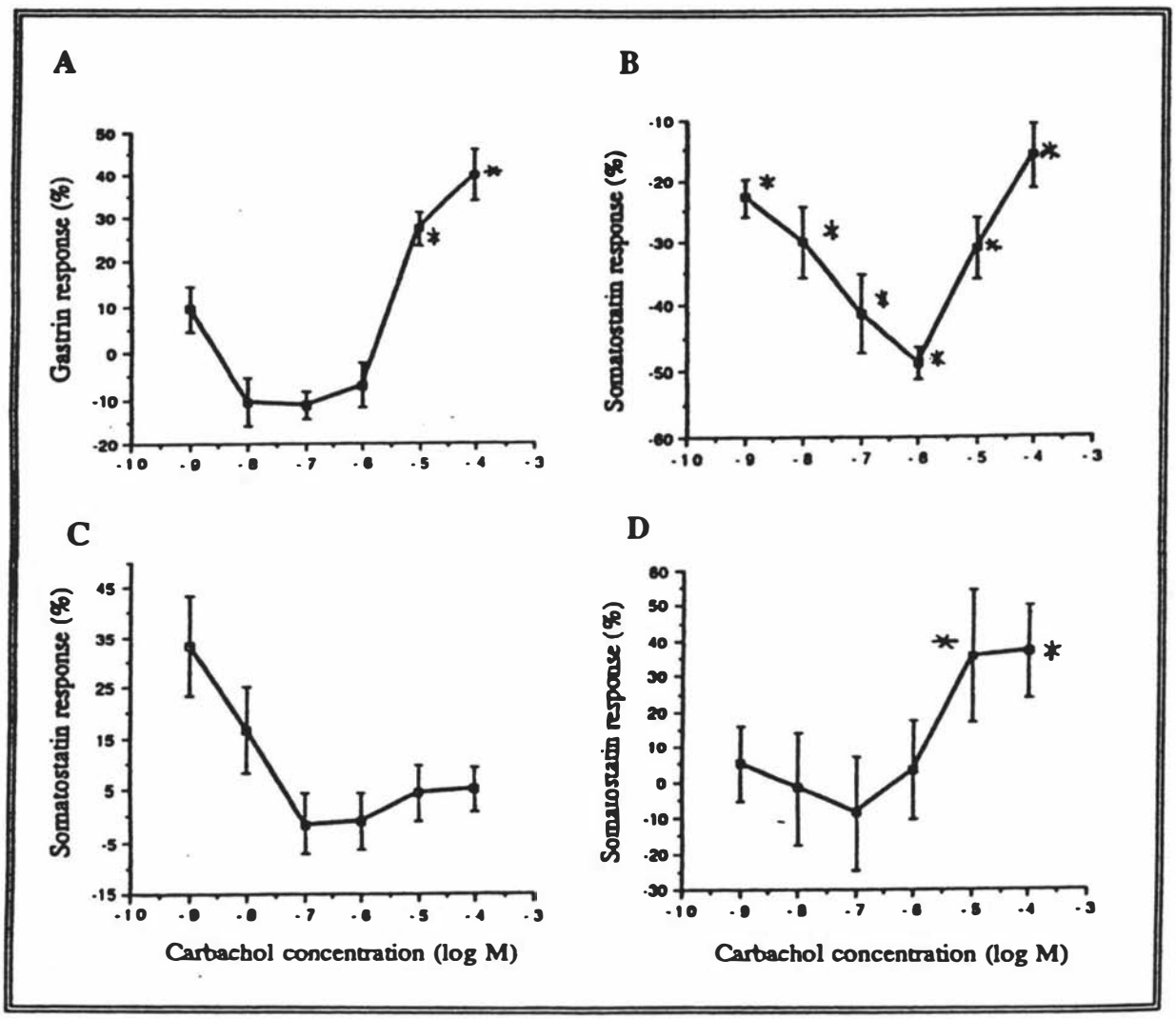

Figure 4.4 Examples of the gastrin and somatostatin response to carbachol from individual experiments (mean ± SEM). (A) gastrin response in which there was a negative response to 10^{-8} and 10^{-7} M; (B) to (D) three recurrent patterns of somatostatin response. (*) indicates a significant difference from control (Dunnett method).

Table 4.6 Effect of carbachol or atropine on the gastrin response (Rt) by ovine antral mucosa. The response to carbachol (CARB) minus the response to atropine (ATR) was calculated; (*) = a significant difference between the two responses (two sample t-test). For the gastrin response to ATR or CARB, (*) = significant difference from control in individual experiments (two sample t-test) and for the mean values (single sample t-test). Mean control coefficient of variation = 15%.

Experiment	% Gastrin response to 10^{-5} M ATR (Rt ± SD)	% Gastrin response to 10^{-4} M CARB (Rt ± SD)	Rt(CARB)-Rt(CARB+ATR)
1	-10 ± 9	40 ± 17	50*
2	-16 ± 18	24 ± 20	40*
3	-18 ± 10*	15 ± 15	33*
4	10 ± 17	33 ± 23	23*
5	-5 ± 13	25 ± 11	30*
6	4 ± 19	27 ± 22	24
7	10 ± 19	45 ± 20	35*
Mean ± SD	-4 ± 12	30 ± 10*	34 ± 10*

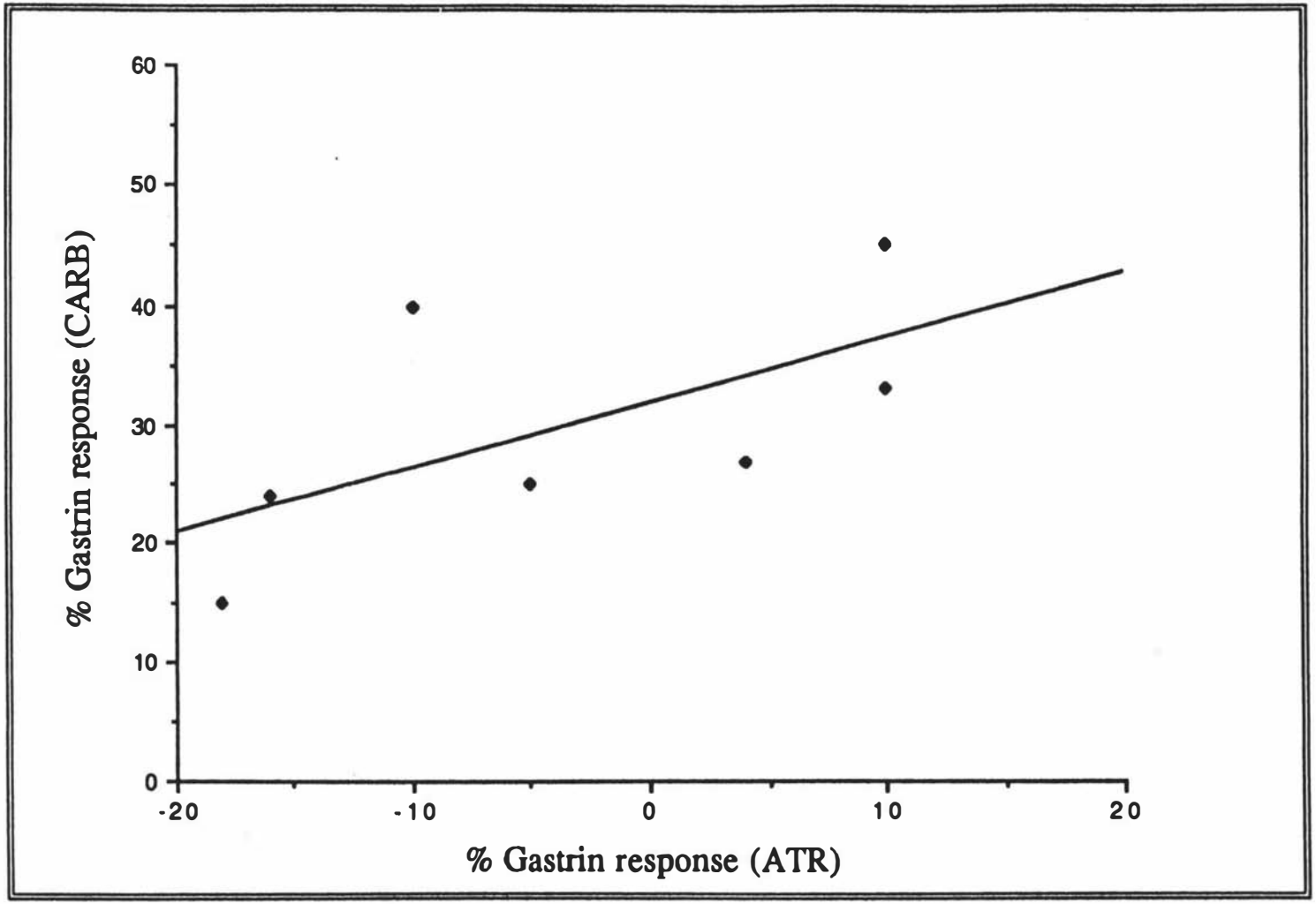

Figure 4.5 The gastrin response to 10^{-4} M carbachol versus the gastrin response to 10^{-5} M atropine within individual experiments. The response to carbachol (CARB) was correlated with the response to atropine (ATR) ($R^2 = 0.4$). This relationship was not significant ($p = 0.13$).

was also determined in at least 12 of these (Table 4.4). Overall, gastrin release was significantly increased by 10^{-6} to 10^{-4} M carbachol, the mean response to 10^{-4} M being 41%, whereas there was no significant effect of any concentration of carbachol on somatostatin release (Fig. 4.2). Since there was considerable variation in the response to carbachol between individual experiments, the gastrin and somatostatin responses for each of the experiments in which the full concentration range was tested (Table 4.5) were examined for obvious groupings. In *most* individual experiments, the gastrin concentration-response curve was not markedly different from that obtained for all experiments (Fig. 4.2). In about half of the experiments, there was a reduced response to 10^{-8} and 10^{-7} M carbachol, as is evident in Expts 5, 6 and 10 (Table 4.5) and illustrated in Fig. 4.4A. In some experiments (3, 9 and 12), the maximum response was obtained with 10^{-5} M (Fig. 4.3B). For somatostatin, a number of quite different responses were observed which appeared to fall into three rough groupings: (1) Expts 1, 2, 3 and 4, which showed little response; (2) Expts 11 and 12, in which somatostatin was significantly inhibited by all concentrations of carbachol; (3) Expts 5, 6, 7, 8, 9, and 10, which had a minimum response at 10^{-8} or 10^{-7} M, followed by an increase to a maximum response to a higher carbachol concentration. An example of each of these three patterns is shown in Fig. 4.4. There appeared to be no consistent correlation between the different gastrin and somatostatin responses in individual experiments: in some cases, the concentration-response relationships were similar (e.g. Expts 5 and 10) while in others they were very different (Expt 11). The inhibition of somatostatin in Expt 11 was associated with consistent stimulation of gastrin secretion. This was also the case in Expt 12, with the exception of the response to 10^{-7} M. There was also no consistent relationship between the absolute level of somatostatin secreted by the tissue in individual experiments and the nature of the somatostatin response or the response of simultaneously secreted gastrin.

4.3.2.2 CARBACHOL, ATROPINE AND HEXAMETHONIUM

Atropine (10^{-5} M) was tested as a treatment in seven experiments and produced a gastrin response varying from an inhibition of 18% to a stimulation of 10% (Table 4.6). The effect of atropine on individual tissues appeared to be related to how responsive that tissue was to the maximum concentration of carbachol: tissue with a small gastrin response to 10^{-4} M carbachol were usually inhibited by atropine and vice versa (Table 4.6 and Fig. 4.5). The difference between the response to atropine and that to carbachol was similar among experiments, while within individual experiments the response to

Table 4.7 The gastrin response (Rt) to 10^{-4} M carbachol and the effect of atropine on on the response to a range of carbachol concentrations by ovine antral mucosa. The effect is the response to carbachol minus the response to carbachol plus atropine. For each experiment, (*) indicates a significant difference between the response to carbachol and the response to carbachol plus atropine in individual experiments (two sample t-test) and from control for the mean effect (single sample t-test). Mean control coefficient of variation = 15%. For experiment 1 to 6, results relate to the same experiments.

Experiment	Rt (carbachol and atropine) - Rt (carbachol)				Rt to 10^{-4} M Carbachol in each experiment
	Carbachol concentration				
	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M	
1	16*	23*	17*	47*	56
2	-18	22*	30*	40*	66
3	-2	10	31*	29*	5
4	2	14	28*	20	27
5	15	23*	11	15	24
6	18	18*	34*	37*	42
7	-	-	61*	-	-
8	-	-	41*	-	-
9	-	-	-	36*	25
10	-	-	-	27*	20
11	-	-	-	22*	15
12	-	-	-	42*	41
Mean ± SD	5 ± 14	18 ± 6*	32 ± 15*	32 ± 11*	32 ± 19*

Table 4.8 Effect of atropine on the somatostatin response (Rt) to a range of carbachol concentrations by ovine antral mucosa. The effect is expressed as the response to carbachol minus the response to carbachol plus atropine.

% somatostatin response to carbachol minus the response to carbachol plus atropine				
Carbachol concentration	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
Number of experiments	6	6	6	10
Mean ± SD	3 ± 31	9 ± 24	5 ± 15	7 ± 27

Table 4.9 Effect of hexamethonium on the gastrin response (Rt) to a range of carbachol concentrations by ovine antral mucosa. The effect is expressed as the response to carbachol minus the response to carbachol plus hexamethonium. CV = the coefficient of variation determined from raw data (mean control CV = 12%). (*) = a significant intraexperimental difference between the Rt_i sets for carbachol verse carbachol plus hexamethonium (two sample t-test).

Experiment	% gastrin response to carbachol minus the response to carbachol plus 10^{-5} M hexamethonium				
	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
1	-4	-22*	-22*	-42*	17
2	-8	-2	10	-2	-21
3	-3	-12	-25*	-18	-1
Mean ± SD	-5 ± 3	-12 ± 10	-13 ± 19	-21 ± 20	-2 ± 19

these two treatments differed significantly. The correlation between the two responses was not significant at the 5% level ($p = 0.13$) and in shown in Fig. 4.5 ($R^2 = 0.4$).

Atropine (10^{-5} M) was used in combination with a range of concentrations of carbachol from 10^{-7} to 10^{-4} M. Atropine reduced the gastrin response to carbachol when compared with that to carbachol alone (Rt (carbachol) - Rt (carbachol plus atropine); Table 4.7). The *absolute* reduction was significant for 10^{-6}, 10^{-5} and 10^{-4} M carbachol - the concentrations of carbachol which significantly increased the secretion of gastrin. Despite the substantial variation in the response elicited by 10^{-4} M carbachol, the difference in the gastrin response to carbachol and carbachol plus atropine was very similar in individual experiments (mean 32%) (Table 4.7 and Fig.4.3). It was also similar in magnitude to the difference between the response to atropine and carbachol of 34% described earlier (Table 4.6). For concentrations of carbachol of 10^{-6} M and above, the response to atropine plus carbachol was not only less than the response to carbachol, but also caused an inhibition of gastrin secretion relative to the controls in many experiments (i.e. the reduction in the response was greater than that stimulated by paired carbachol treatments). The mean reduction in the gastrin response to 10^{-4} M carbachol by the addition of 10^{-5} M atropine (32%) was the same as the mean response to 10^{-4} M carbachol alone (32%) in the relevant experiments. On average, therefore, 100% of the *carbachol-stimulated gastrin response* was inhibited by atropine. Inclusion of 10^{-5} M atropine had no consistent effect on the somatostatin response to carbachol. The mean differences in response are presented in Table 4.8.

The mean gastrin response to hexamethonium as a treatment was not significant (Rt = 4.6 ± 15.4, where n = 6), although a significant increase occurred in one experiment. The combination of hexamethonium with carbachol did not produce a response that differed significantly from that to carbachol over the three experiments in which it was tested (Tukey method), although in almost every case the response was greater (Table 4.9) and within experiments many were significant.

In the one experiment (Expt 3, Table 4.5) in which both atropine and hexamethonium were combined with carbachol (10^{-9} to 10^{-4} M), all treatments reduced the secretion of gastrin, of which two were significant (Dunnett method). However, this experiment was somewhat unusual, in that two concentrations of carbachol on its own produced significant inhibition of gastrin secretion and there was the least response to 10^{-4} M (Rt = 4.7%). Nevertheless, the combination of blockers significantly reduced the response

Table 4.10 Effect of bethanechol on gastrin and somatostatin secretion by ovine antral mucosa. The response to bethanechol (mean Rt ± SD) was derived from 4 experiments (n = 4), for bethanechol (BTH) plus atropine (ATR), n = 3 and for bethanechol plus hexamethonium (HEX), n = 2. Rt(BTH) -Rt(BTH + ATR/HEX) = the difference between the response to BTH and BTH plus either ATR or HEX. CV = the mean coefficient of variation calculated from the raw data (mean control CV = 14% (gastrin) and 17% (somatostatin). The number of tissue pieces for each treatment was 8 and for control 32. (*) = a significant difference (single sample t-test).

BTH concentration	GASTRIN				SOMATOSTATIN			
	% Response to BTH		Rt(BTH) - Rt(BTH+ATR)	Rt(BTH) - Rt(BTH+HEX)	% Response to BTH		Rt(BTH)-Rt(BTH+ATR)	Rt(BTH) - Rt(BTH+HEX)
	Rt (mean ± SD)	Mean %CV			Rt (mean ± SD)	Mean %CV		
10^{-9} M	13 ± 24	17	7 ± 26	-	1 ± 37	21	3 ± 34	-
10^{-8} M	7 ± 10	13	-5 ± 27	-1 ± 15	-2 ± 28	20	13 ± 1*	-13 ± 2
10^{-7} M	9 ± 14	14	6 ± 16	-1 ± 1	-9 ± 23	21	-28 ± 28	-16 ± 4
10^{-6} M	13 ± 20	15	10 ± 10	-13 ± 12	35 ± 14	24	14 ± 32	50 ± 29
10^{-5} M	1 ± 6	16	-2 ± 21	-17 ± 3	-3 ± 28	24	3 ± 17	-24 ± 31
10^{-4} M	15 ± 19	11	2 ± 17	21 ± 2*	-1 ± 24	23	-11 ± 15	-56 ± 45

Table 4.11 Effect of nicotine on gastrin secretion by ovine antral mucosa. CV = the co-efficient of variation of raw data (mean control CV = 11%); mean Rt was determined from three experiments; (*) = a significant difference from control (Tukey method).

Nicotine concentration	10^{-6} M	10^{-5} M	10^{-4} M	5 x 10^{-4} M	5 x 10^{-3} M
Rt (mean ± SD)	-1 ± 9	-1 ± 4	4 ± 15	7 ± 6	42 ± 13*
Mean %CV	14	17	13	17	12

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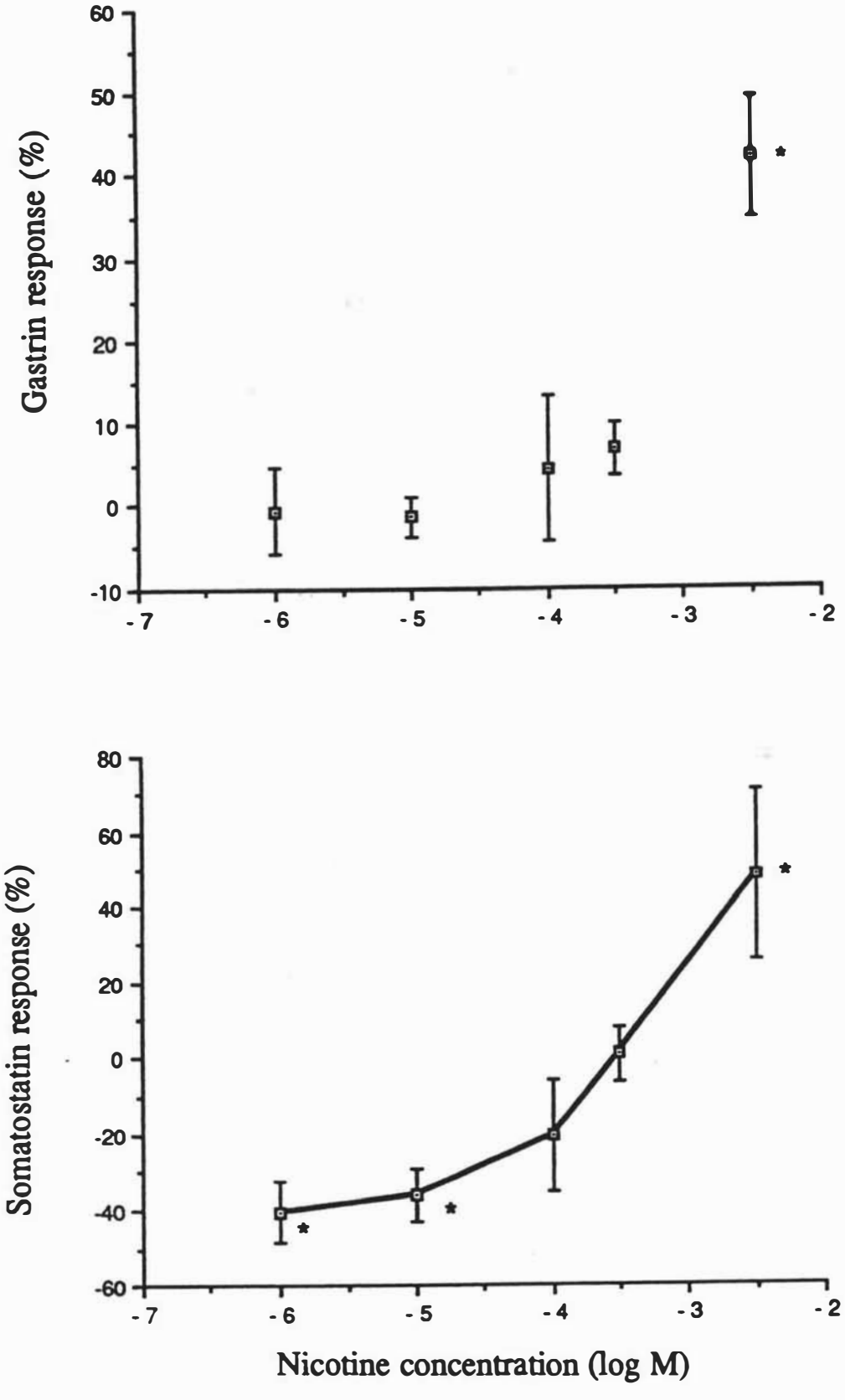

Figure 4.6 Effect of nicotine on the secretion of gastrin and somatostatin by ovine antral mucosa (mean ± SEM). The response to nicotine was determined over the concentration range of 1 x 10^{-6} M to 5 x 10^{-3} M using the static incubation method in three experiments for gastrin and one experiment for somatostatin. (*) indicates a significant difference from control (Dunnett method).

to 10^{-5} and 10^{-4} M carbachol by 38 and 26% respectively, and increased the response to 10^{-9} M carbachol.

4.3.2.3 BETHANECHOL

Bethanechol in concentrations from 10^{-9} to 10^{-4} M did not increase gastrin release from a dispersed antral mucosal cell preparation (Table 3.6). In four experiments using the static incubation method, bethanechol in concentrations from 10^{-9} to 10^{-4} M did not significantly affect the secretion of gastrin or somatostatin at any of the doses tested (Tukey method; Table 4.10). In three of these experiments, all concentrations of bethanechol were tested in combination with 10^{-5} M atropine and no significant effects resulted. In two experiments, all concentrations of bethanechol were combined with 10^{-5} M hexamethonium. Hexamethonium significantly reduced the gastrin response to 10^{-4} M bethanechol (single sample t-test). There was also one significant increase in somatostatin (Table 4.10).

4.3.2.4 NICOTINE

The effect of nicotine in concentrations of 10^{-6}, 10^{-5}, 10^{-4}, 5×10^{-4} and 5×10^{-3} M on gastrin secretion was determined in three experiments. For the 10^{-4} M solution, which was tested in a further three experiments, the somatostatin response was also determined. Nicotine (5×10^{-3} M) stimulated gastrin secretion by 41.9% (Table 4.11). There was no significant response in either gastrin or somatostatin secretion to 10^{-4} M. The somatostatin concentration-response curve to nicotine for the one experiment in which it was determined is shown in Fig. 4.6. In this one experiment, the increase in somatostatin to three nicotine concentrations was significant (Tukey method).

4.3.2.5 ESERINE

In five experiments, 10^{-6} M eserine significantly increased the release of gastrin by 13.4 ± 5.3 % (Tukey method). Within the one experiment in which 10^{-5} M eserine was tested, it increased gastrin secretion by 56%.

4.3.3 VASOACTIVE INTESTINAL PEPTIDE

The effect on the gastrin and somatostatin response to synthetic porcine VIP in concentrations of 4 x 10^{-10} to 4 x 10^{-6} M was tested in three experiments. No concentration of VIP had any significant effect on gastrin secretion, but 4 x 10^{-6} M VIP significantly stimulated somatostatin secretion by 310% (Table 4.12).

4.3.4 GASTRIC INHIBITORY PEPTIDE

The effect on the gastrin and somatostatin response to synthetic porcine GIP in concentrations of 6 x 10^{-10} to 6 x 10^{-7} M was tested in three experiments. GIP had no significant effect on gastrin or somatostatin secretion at all concentrations (Table 4.13), however, there was nevertheless a mean 660% increase in somatostatin in response to 6 x 10^{-7} M. This was very variable over the three experiments (i.e. 94%, 97% and 1800%). The mean somatostatin response at each concentration also suggests GIP may increase somatostatin release in a concentration-dependent manner.

4.3.5 ADRENALINE

In three experiments, adrenaline in concentrations from 10^{-8} to 10^{-4} M had no significant effect on either the gastrin or somatostatin secretion (Table 4.14).

4.3.6 PROSTAGLANDIN -F2α

Prostaglandin-F2α in concentrations from 10^{-10} to 10^{-6} M was tested in one experiment and had no effect on gastrin or somatostatin secretion (Table 4.15).

4.4 DISCUSSION

This investigation has clearly shown that in the static incubation preparation both porcine GRP and the cholinergic agonist, carbachol, stimulate gastrin secretion from segments of ovine antral mucosa in a concentration-dependent manner. This conforms with results obtained in other mammals, in which GRP (or bombesin) is believed to act directly on the G cell (Richelsen *et al.*, 1983; Sugano *et al.*, 1987; Giraud *et al.*, 1987;

Campos *et al.*, 1990) and may also stimulate somatostatin secretion (DuVal *et al.*, 1981; Holst *et al.*, 1987b, 1993; Guo *et al.*, 1988; see Chapter 1, 1.3.4.2.5), although this remains equivocal (e.g. Buchan *et al.*, 1990). Cholinergic agonists are believed to affect gastrin secretion through concentration-dependent somatostatin inhibition (Saffouri *et al.*, 1980; DuVal *et al.*, 1981; Wolfe *et al.*, 1983; Richelsen *et al.*, 1983; Schubert, 1994), VIP and GIP are released by enteric neurons and stimulate somatostatin secretion (Wolfe *et al.*, 1983; Holst *et al.*, 1983; Wolfe & Reel, 1986; Schubert & Hightower, 1989b) and catecholamines may directly stimulate either gastrin or somatostatin release (Hayes *et al.*, 1978; Koop *et al.*, 1983; Yamada *et al.*, 1984; Harty *et al.*, 1988; Buchan, 1991). Results presented in this study suggest that the cholinergic and peptidergic (GRP, VIP, GIP) regulatory mechanisms that have been established for other mammals are also present in the sheep. The was no effect of adrenaline on either gastrin or somatostatin secretion. Despite evidence for their presence in the sheep, however, it was considered that the relative contribution and/or sensitivity to some of these regulatory mechanisms in the sheep may differ from those described in monogastric mammals.

4.4.1 GASTRIN RELEASING PEPTIDE

Porcine GRP stimulated gastrin secretion at the two highest concentrations tested (10^{-7} and 10^{-6} M) whereas there was no response to amphibian bombesin in the static incubation method. In most species, both GRP and bombesin appear to stimulate gastrin secretion with about the same efficacy, although their potencies differ, e.g. canine GRP and bombesin both stimulated gastrin release from enriched canine G cell cultures by over 300%, although bombesin, which had its maximum effect at 10^{-9} M, was the more potent (Sugano *et al.*, 1987). Arterial perfusion of the isolated pig antrum with 10^{-11} M GRP increased gastrin output by about 100% but had no effect on somatostatin secretion (Holst *et al.*, 1987b); as the GRP concentration was increased, the gastrin response decreased and the somatostatin response increased, such that at 10^{-8} M GRP, gastrin was inhibited while somatostatin was increased about eightfold. In the present study, a considerably smaller and non-significant increase (14%) in somatostatin secretion was observed in response to 10^{-8} M GRP. The increase in somatostatin secretion was not associated with a gastrin response and, therefore, could not be attributed to a reciprocal action by gastrin on the D cell (Chapter 1, 1.3.4.2.5). Stimulation of somatostatin secretion in sheep infused with either bombesin or GRP may account for the lack of a

gastrin response reported by some authors (Chapter 1, 1.3.4.2.2), as a significant gastrin response to GRP was observed in sheep immunized against somatostatin (Shulkes *et al.*, 1994). However, the small somatostatin response observed in the present study suggests that this may not be via a direct action on the antral D cell. Most of the somatostatin secreted by the *entire* abomasum comes from the fundus and this may be the source of the somatostatin which restrained the gastrin response to GRP in suggested by that study. It is also possible that the sensitivity of the fundic and antral D cell differ in their sensitivity to GRP in the sheep. Perhaps too, *in vivo* infusion of GRP influences acid secretion and thus increases somatostatin secretion, effectively eliminating a gastrin response. Bloom *et al.* (1983), who infused calves with porcine GRP and produced a circulating GRP concentration of 2 x 10^{-10} M, reported a small but significant rise in plasma somatostatin without a change in gastrin. The function of the GRP-containing neurons that are concentrated in the immediate vicinity of the parietal cell, and their possible affects on acid secretion, has yet to be identified (Walsh, 1989b). Somatostatin infusion, on the other hand, increased gastrin secretion in sheep (Barry *et al.*, 1985; Reynolds *et al.*, 1991). This apparent anomaly may also be secondary to an effect on acid secretion, i.e. somatostatin may inhibit HCl release and thus result in increased gastrin secretion (Chapter 1, 1.3). Certainly, somatostatin release may influence GRP-stimulated gastrin release in *in vivo* sheep preparations, although the source and cause of the somatostatin release need still to be determined. McDonald *et al.* (1988) suggested that ruminants may differ from other mammals in their sensitivity to GRP, particularly the relative sensitivity of the G and D cells. The results of the present study appear to support this: while GRP stimulated significant gastrin release there was no response to concentrations below 10^{-8} M, compared with the pig in which the secretion of gastrin was doubled by 10^{-11} M GRP (Holst *et al.*, 1987b). Thus, although GRP-stimulated gastrin occurs in the sheep, it is a less potent mechanism for gastrin release than in monogastrics. This may explain why doses of bombesin which produced maximal effects on gastrin secretion in the dog (Bertaccini *et al.*, 1974) produced mixed responses in the sheep (McLeay *et al.*, 1989).

In order to determine whether tonic gastrin secretion, or the gastrin response to 10^{-7} M GRP, was restrained by basal or GRP-stimulated somatostatin secretion, somatostatin antiserum was combined with 10^{-7} M GRP in a number of experiments. In four of the five experiments, this was without effect. However, in these experiments there was also no somatostatin response to this concentration of GRP. This suggests that the basal

somatostatin levels were not restraining gastrin secretion *per se*, and therefore, no gastrin response was to be expected. In the fifth experiment, however, where for unexplained[1] reasons GRP significantly stimulated somatostatin release by 330%, somatostatin antiserum converted a decrease in gastrin secretion to an increase that was significant. The difference between the response to GRP and GRP plus antiserum in this experiment was the same as the response to GRP alone in the other experiments, further indicating that somatostatin levels in these experiments were too low to affect appreciably the gastrin response. This one result also suggests that significantly raised somatostatin levels have the ability to block GRP-stimulated gastrin secretion from segments of ovine antral mucosa in the static incubation preparation used. Somatostatin-inhibition of GRP-stimulated gastrin secretion has been demonstrated in other species (e.g. Sugano *et al.*, 1987; Campos *et al.*, 1990) and was implicated in the sheep by Shulkes *et al.* (1994). In retrospect, it would perhaps have been more interesting to combine somatostatin antiserum with 10^{-8} M GRP in this study. Neutralization of somatostatin released at this concentration *may* have afforded expression of an otherwise inhibited gastrin response.

Ovine GRP may differ from porcine GRP in a manner that affects the efficacy and potency of the latter in the sheep. It is possible that such differences may account for the relatively low sensitivity to GRP. Specificity of this kind may also account for the lack of a response to amphibian bombesin in experiments using the static incubation method. The perifusion of bombesin (Chapter 3, see Fig. 3.3) appeared to stimulate gastrin secretion. This apparent discrepancy may reflect differences between the perifusion and static incubation systems, such as the removal of simultaneously released somatostatin in the former. However, bombesin was not associated with a somatostatin response in the static incubation experiments. It is possible that the response observed in the perifusion system was the result of changes in the perifusion medium and not of the perifused secretagogues. Attempts were made to prevent such an effect (see Chapter 3), although the similarity in the magnitude of response to different drug concentrations suggests that this may nevertheless have occurred. Alternatively, as all of the bombesin used in the static incubation experiments was prepared from one 5 mg vial, it is possible that deficiencies in its storage or utilization affected its activity. GRP used in each

1 It was suspected that the somatostatin secretory properties of this medium were not related to GRP. It is possible that bacterial contamination and growth within the medium altered it in a manner that was potently stimulatory to somatostatin secretion. Chapter 6 describes experiments in which the effect of contamination by microorganisms within the test medium were assessed. It was concluded that microorganisms do effect changes in the medium that potently stimulate somatostatin secretion.

experiment was prepared independently.

4.4.2 CHOLINERGIC MECHANISMS

The cholinomimetic carbachol failed to inhibit significantly the *overall* secretion of somatostatin despite stimulating gastrin release at concentrations of 10^{-6}, 10^{-5} and 10^{-4} M. Indeed, within many individual experiments, carbachol stimulated the release of both peptides simultaneously which was contrary to the response expected from work with other species[2]. In monogastric animals, the cholinergic control of gastrin release *appears* simple. Primarily, muscarinic agonists inhibit somatostatin secretion which, in turn, removes tonic restraint on the G cell and affords increased gastrin secretion (Saffouri *et al.*, 1979, 1980; DuVal *et al.*, 1981; Richelsen *et al.*, 1983; Schubert, 1992, 1993, 1994). Tonic restraint of the G cell has been demonstrated many times with antibodies to somatostatin, which, without additional secretagogues, cause increased gastrin secretion (Chiba *et al.*, 1981; Wolfe *et al.*, 1983; Short *et al.*, 1985a; Holst *et al.*, 1992a). Secondly, cholinergic stimulation facilitates gastrin release by the release of GRP which directly stimulates the G cell. It is unclear whether acetylcholine can stimulate the G cell directly, although such an action was considered important in the regulation of gastrin secretion by Schubert & Makhlouf (1992). There appears, however, to be no clear evidence to support such an action (i.e. muscarinic receptors on the G cell) and in many studies (e.g. Holst *et al.*, 1987b, 1993) the gastrin response to electrical or vagal stimulation has been entirely resistant to atropine. The failure of carbachol to stimulate gastrin secretion from dispersed antral cells was possibly due to the absence of receptors on the G cell[3]. Atropine-insensitive vagal and electrical effects are probably mediated through the concomitant release of GRP and VIP (and perhaps also GIP and GABA) from cholinergic and noncholinergic neurons within the enteric plexuses. Thus the effect of parasympathetic agonists, antagonists and of neural stimulation in preparations that include extensive neural elements, is actually far from simple and the gastrin and somatostatin responses to various stimuli may be hard to

2 Exclusion of the four experiments (3, 4, 11 and 12, Table 4.5) in which somatostatin secretion was reduced, converts the remaining 8 to a significant increase in somatostatin release.

3 Failure of carbachol to stimulate gastrin secretion from the dispersed cell preparations (Chapter 3) may indicate that these cells do not posses muscarinic receptors and, therefore, the ability to respond to cholinomimetics directly. Alternatively, as suggested in Chapter 3, disturbance of the cells may have circumvented their responsiveness to stimuli (note there was no response to GRP either). Receptor studies on the ovine G cell, and indeed the G cell in general, would be of considerable interes

predict.

This study clearly indicates that inhibition of somatostatin release is not a prerequisite for cholinergic (carbachol) stimulation of gastrin secretion in the sheep. Indeed, gastrin secretion was stimulated despite significant increases in somatostatin release in one third of the experiments. Carbachol-stimulated gastrin secretion must, therefore, have been mediated by an alternative mechanism, perhaps via enteric neurons retained within the tissue preparation or via a direct action on the G cell[4]. Direct stimulation of muscarinic receptors on the G cell would be consistent with its inhibition by the highly selective antimuscarinic drug atropine and cannot be ruled out in sheep without further investigation. Alternatively, atropine may have competed for muscarinic sites within parasympathetic ganglia or at interneuronal terminations: muscarinic receptors are present to a variable degree on autonomic ganglion cells (Taylor, 1980; Conn & Gebhart, 1989). The almost complete antagonism by atropine and its specificity for muscarinic receptor sites suggests that stimulation of nicotinic sites within the ganglia by carbachol is unlikely to have been of major importance, although carbachol retains considerable nicotinic activity (Taylor, 1980). However, antagonism by atropine does not necessitate that the initial site of action of the drug was at muscarinic receptors. Indeed, the nicotinic agonist DMPP stimulated both gastrin and somatostatin secretion from rat antral segments, responses which were significantly inhibited by atropine (Schubert *et al.*, 1988b), results bearing some resemblance to those elicited by carbachol and blocked by atropine in the present study. Some activation of nicotinic sites by carbachol, therefore, cannot be discounted. It is possible that subsequent to nicotinic activation, muscarinic receptors are involved in interneuronal relays distal to the initial stimulus, but prior to the elicitation of the response. It is also possible that the antagonistic selectivity of atropine was lost at the concentration used in the present study (10^{-5} M compared with 3×10^{-7} M used by Schubert *et al.*). Extensive nicotinic stimulation provides one of the few possible explanations for the failure of bethanechol to affect significantly either gastrin or somatostatin secretion at any of the concentrations tested and the stimulation of somatostatin secretion by higher carbachol concentrations in many individual experiments.

The presence of nicotinic receptors, possibly in autonomic ganglia retained within the

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4 Histological examination of tissue pieces prepared for use in the static incubation method revealed the presence of neural elements on the serosal surface of each segment.

preparation, was verified by the significant response to 5 x 10^{-3} M nicotine. It is likely that activation of these receptors accounts for the gastrin response through either the subsequent release of acetylcholine or GRP, in turn, supporting the earlier suggestion that carbachol may have stimulated nicotinic receptors. Although the prototypical ganglionic blocking drug hexamethonium did not provide any conclusive results, in general its incorporation with carbachol appeared to be associated with increased gastrin secretion, an effect that was significant with some concentrations of carbachol and in some individual experiments. This seems to be in contradiction to the observed effect of nicotine. The effect of hexamethonium on the gastrin response to carbachol, however, may have been confounded by concomitant interactions with the D cell and somatostatin secretion that were not measured. It is interesting that 10^{-4} M nicotine in six experiments was not associated with either a gastrin or somatostatin response. It appears that a concentration of nicotine of greater than 5 x 10^{-4} M was necessary for activation, or possibly blockade, of nicotinic receptors and consequent stimulation of gastrin and somatostatin (Fig. 4.6). Unfortunately nicotine and hexamethonium were not tested in combination.

4.4.3 BASAL CHOLINERGIC ACTIVITY

There appeared to be a preparation specific level of basal/tonic cholinergic discharge. Not only did atropine inhibit carbachol-stimulated gastrin secretion, but in many instances it converted an increase to a decrease, suggesting that there was a component of atropine-sensitive basal activity that was not attributable to the carbachol treatment. In those experiments in which atropine was most inhibitory, there was also the smallest response to carbachol (e.g. Expt 3, Tables 4.6 and 4.7; Fig. 4.5). In other experiments, in which atropine reduced the response to carbachol but did not convert this to a "negative" response, a greater response to carbachol was obtained, such that the absolute difference between the response to carbachol and carbachol plus atropine was relatively constant among all experiments. The response to carbachol or atropine as treatments within the same experiment were not significantly correlated ($p = 0.13$), although this was perhaps a reflection of the low sample number ($n = 7$). The variable response to carbachol (10^{-4} M) and its comparatively constant inhibition by atropine were considered to reflect the degree of *assumed* tonic cholinergic activity. Thus, in retrospect, incorporation of atropine in all experiments as a treatment would have been useful as an index of this activity. The stimulatory effect of the anticholinesterase eserine

provides further evidence of tonic acetylcholine release. Given the presumed tonic cholinergic discharge, atropine as a treatment would be expected to increase somatostatin secretion by eliminating cholinergic inhibition (as reported by Makhlouf *et al.*, 1989). No such effect was observed in this investigation.

The insensitivity of the G cell to cholinergic stimulation in some experiments, perhaps due to tonic discharge, cannot account for the poor response to bethanechol as carbachol was tested in three of the same experiments (10, 12 and 17, Table 4.5) in which 10^{-4} M carbachol elicited a mean 40% increase in gastrin secretion (compared with 23% by bethanechol). This difference is only 17% and a two sample t-test of the respective three values did not separate the two sets of results. It is possible that statistical rejection of bethanechol-stimulated gastrin secretion merely reflects the small sample size (n = 4). Alternatively, although bethanechol, like carbachol, is considered to be relatively specific to the gut and urinary tract, it is possible that it is less potent than carbachol at stimulating muscarinic receptor subtypes involved in mediating the gastrin response. If nicotinic receptor activation is also an important component of the response to carbachol, this too could account for the difference in the gastrin response to carbachol and bethanechol.

4.4.4 SOMATOSTATIN

A curious feature of the experiments described in this study was the simultaneous stimulation of gastrin and somatostatin secretion by carbachol. In general, the secretion of these two peptides appeared to be independent. The most feasible explanation appears to be that the control mechanisms governing gastrin and somatostatin secretion in the static incubation preparation are multifactorial.

As was suggested above, nicotinic or muscarinic stimulation of neural elements of the submucosal plexus, retained on tissue used in this preparation, may have elicited the release of neuropeptides (e.g. GRP). The release of VIP or GIP (the potent stimulatory properties of which this investigation has confirmed) from carbachol-stimulated neural elements may account for the paradoxical increase in somatostatin in many experiments. Alternatively, gastrin may have stimulated somatostatin secretion through a reciprocal paracrine mechanism, as has been described for the rat (Schubert *et al.*, 1991b), although if so, this was not consistent. Using a similar experimental method to the static

incubation technique, in which fragments of rat antral mucosa were incubated in medium for one hour, carbachol (2.5×10^{-6} M) inhibited somatostatin release by about 70% (Wolfe *et al.*, 1983). In the present investigation, however, stimulatory mechanisms must have outweighed direct cholinergic inhibition of somatostatin secretion from the D cell at high carbachol concentrations (10^{-6} to 10^{-4} M) in all but a few experiments (e.g. 11 and 12, Table 4.5). Despite the unusual somatostatin response to carbachol in Expts 11 and 12, the response to other substances tested in them conformed with other results. It is possible that in these experiments (11 and 12), there was particularly good separation of the mucosa from underlying connective tissue and, therefore, relatively few intrinsic neurons, resulting in less of the (*presumed*) indirect stimulatory component which may act through the release of VIP and GIP. In Fig. 4.2, in which the *overall* somatostatin response to carbachol was shown, it appears that as the carbachol concentration was increased from 10^{-9} to 10^{-7} M, somatostatin secretion was increasingly inhibited. As the concentration was further increased, however, this trend changed such that, from 10^{-6} to 10^{-4} M, there was progressively greater stimulation of somatostatin release. This concentration-response curve may indicate that at concentrations of 10^{-6} M carbachol and above, additional mechanisms were activated which stimulated somatostatin release and is consistent with the activation of enteric neurons releasing VIP and GIP at these concentrations. To investigate this proposed mechanism further, VIP or GIP antiserum could be included in the test medium. If antibodies to these peptides altered the response to carbachol only at higher concentrations and converted somatostatin increases to decreases, the proposed mechanism would be confirmed. Variation in the amount of neural tissue associated with tissue is inherent in the preparation of the tissue segments. This variation is likely to have been more important in some experiments than in others, just as the level of basal/tonic cholinergic discharge appears to have varied between experiments and thus affected the sensitivity of the tissue to carbachol in some experiments more than others.

4.4.5 VIP, GIP AND ADRENALINE

Both VIP and GIP substantially increased the secretion of somatostatin. Failure of GIP to significantly affect somatostatin secretion at any of the concentrations at which it was tested probably reflects a combination of the highly conservative Tukey method used to assess the response, the low sample number (n = 3) and deviant values which in all cases were high rather than low, as the response to 6×10^{-7} M GIP was significant

within each experiment individually. The Tukey method was used to assess all of the results because of its high protection against false positives. Despite statistical insignificance, the overall 660% increase in somatostatin by 6 x 10^{-7} M GIP is likely to be of considerable biological significance. The substantial release of somatostatin stimulated by both VIP and GIP was not accompanied by any gastrin response. This is in accord with other studies such as that of Saffouri *et al.* (1984a) and Wolfe & Reel (1986). In the former study, arterial infusion of the isolated, perfused rat stomach with 5 x 10^{-7} M VIP induced a 100% increase in the secretion of somatostatin that was not accompanied by a decrease in gastrin, while when antibodies to somatostatin were perfused on their own they significantly increased gastrin secretion: leading to the suggestion that somatostatin exerts optimal restraint on the G cell. In the latter study, inhibition of carbachol-stimulated gastrin secretion by GIP (10^{-9} to 10^{-7} M) was abolished by somatostatin antibodies, implicating somatostatin as the effector of the inhibition. Arterial infusion of both VIP and GIP stimulates somatostatin secretion from the isolated, perfused rat and pig stomach (Chiba *et al.*, 1980; McIntosh *et al.*, 1981; Holst *et al.*, 1983; 1993). Vagal stimulation causes the release of VIP in the calf and lamb (Bloom *et al.*, 1988; Reid *et al.*, 1988). The secretion of somatostatin in response to VIP release locally, as suggested by the results of this study, may account for the barely detectable increase in plasma gastrin that accompanied vagal stimulation in the sheep by Bladin *et al.* (1983). The present investigation did not demonstrate that the 330% increase in somatostatin by VIP had any effect on gastrin secretion. The effect of VIP in combination with secretagogues (e.g. GRP, carbachol) may have revealed subsequent restraint of gastrin secretion had this been tested.

The lack of a response to adrenaline may indicate that this substance does not have an important action on either the ovine D or G cell, or on components of the elements of the enteric nervous system that are retained within the tissue segments used in the static incubation method.

4.4.6 CONCLUSIONS

This series of investigations suggests that gastrin and somatostatin release from the ovine antrum is regulated by many stimulatory and inhibitory factors. In some respects these appear to be the same as in other mammals: cholinergic agonists and GRP stimulate the release of gastrin; GRP may stimulate somatostatin secretion; VIP and

GIP stimulate somatostatin secretion; somatostatin release (at least at high concentration) can inhibit GRP-stimulated gastrin secretion. The secretory response to many of the substances tested, however, suggests that the sensitivity of the ovine G and D cell to these mechanisms is less than in commonly studied monogastrics such as the dog, pig, human and rat, although such mechanisms do appear to exist. Perhaps the importance of these mechanisms in regulating gastric secretion is less in the sheep, e.g. there was no response to 10^{-4} M adrenaline; GRP did not stimulate gastrin secretion at concentrations below 10^{-7} M; the maximal gastrin response to carbachol was less than that reported in many other studies. Alternatively, the *apparent* lack of sensitivity observed may be a feature of the static incubation method. However, in a study using a comparable static incubation technique, carbachol (10^{-5} M) increased gastrin secretion maximally by 100% from rat antral mucosal segments (Harty & McGuigan, 1980). In the present study, two experiments were also performed using dog tissue. These, however, failed to produce meaningful results using the static incubation method and this was attributed to the high rate of gastrin secretion compared with the sheep: about 20 times more gastrin was secreted than by ovine tissue that was treated similarly. Alterations to the method that would have been necessary in order to study secretion from canine tissue were not pursued. The comparatively low level of secretion by ovine tissue compared with that from the dog, however, may indicate that the sheep is not only less sensitive to stimulation by several regulatory substances, but that basal secretion by the tissue is also lower.

Some results in the present study also appear to conflict with the accepted mammalian pattern, in particular the stimulation rather than inhibition of somatostatin secretion by carbachol. It is possible that, as in the study by Schubert *et al.* (1988b), see 4.4.2, carbachol-stimulated somatostatin release was a reflection of nicotinic activation and that the interplay of subsequent neuropeptide release determines the "net" response observed. In general, there appeared to be little reciprocal correlation between the secretion of somatostatin and gastrin in response to carbachol and in many experiments these were secreted simultaneously. In the two experiments (11 and 12, Table 4.5) in which carbachol significantly inhibited somatostatin secretion there were below average gastrin responses, demonstrating that the level of somatostatin secretion stimulated by carbachol did not inhibit the gastrin response. The anomalous somatostatin response may reflect activation of similar mechanisms (e.g. enteric neurons) to those stimulated by vagal excitation and perhaps may explain the increase in somatostatin secretion that has been observed by others *in vivo*.

The regulatory mechanisms investigated in the present study were related to neurally mediated control of gastrin secretion. In the sheep, neural mechanisms may be less important than in other animals, e.g. responses to food and food teasing are only evident in sheep on restricted diets, while in monogastrics the cephalic phase of digestion is extremely important. If this is indeed the case, perhaps as a result of the pattern of food delivery to the abomasum in ruminants, it may explain the *apparent* lack of sensitivity to, or efficacy of, some substances in the sheep observed in this study and reported by others. It is also possible that the *secretory responsiveness* of ovine tissue is less than in monogastrics, as might be expected in an animal in which the regulatory demands are relatively constant.

The pharmacological studies reported in this Chapter have provided results which suggest that the static incubation method is useful for the study the effect of substances which stimulate gastrin and somatostatin secretion. The method, therefore, provides a useful test system in the search for stimulatory ES products released or contained within *O. circumcincta*.

Chapter 5

EFFECT OF *OSTERTAGIA CIRCUMCINCTA* PRODUCTS ON GASTRIN AND SOMATOSTATIN SECRETION *IN VITRO*

5.1 INTRODUCTION

Infection of parasite-naive sheep with *O. circumcincta* produces hypergastrinaemia, hyperpepsinogenaemia and hypochlorhydria (Chapter 2). It has been suggested that parasite-derived secretions or excretory products may be important in the aetiology of these physiological disturbances by directly stimulating pepsinogen and/or gastrin secretion or inhibiting parietal cell activity (McLeay *et al.*, 1973; Titchen & Anderson, 1977; Eiler *et al.*, 1981; Titchen, 1982; Anderson *et al.*, 1981, 1985; McKellar *et al.*, 1986, 1987, 1990a, 1993).

While the production and release of potentially physiologically important substances by gastrointestinal nematodes has been demonstrated, their significance is less clear (Chapter 1, 1.5.4): acetylcholinesterases may inhibit mucus secretion (Philipp, 1984), proteinases may assist host invasion by the parasite (Matthews, 1982), lectins may modulate the immune response (Klesius, 1993) and many substances have been shown to be antigenic (Rothwell & Love, 1974; Dopheide *et al.*, 1991). Very few attempts, however, have been made to determine the effect of parasite-derived factors on serum pepsinogen, serum gastrin and acid secretion by the abomasum.

McKellar *et al.* (1990a) prepared parasite secretion by incubating adult *O. ostertagi* or *O. circumcincta* in distilled water at 37°C and parasite extract by homogenising adult worms. The secretate stimulated pepsinogen secretion from dispersed bovine and ovine abomasal glands by between 5% and 11%. Similar secretate from *O. ostertagi* had no effect on aminopyrine accumulation by bovine abomasal glands (McKellar *et al.*, 1990b).

In this Chapter, investigations that were designed to determine whether substances released by, or contained in, *O. circumcincta* affect the secretion of gastrin, and in some cases somatostatin, by ovine antral mucosa are reported. These investigations utilized the static incubation method described in Chapter 3.

5.2 MATERIALS AND METHODS

5.2 1 EXPERIMENTAL DESIGN

Exsheathed larvae or adult *O. circumcincta* were incubated in different media to collect their ES products. The media used were: BM, water, saline, BM plus antibiotics, BM without glucose and BM without glucose and BSA. Larvae and adult worms were also disintegrated by ultrasound to produce extract. The effects of these solutions on gastrin secretion, and in some cases somatostatin, by ovine antral mucosa were determined.

5.2.2 EXPERIMENTAL PROCEDURE

A pure strain of *O. circumcincta* was maintained (Appendices 2.2, 2.3 and 2.4). Adult worms were produced by the infection of parasite-free donor sheep or goats. All donor animals were killed by captive bolt followed by exsanguination, the abdomen opened and the abomasum ligated. The abomasum was then removed with its entire contents. The donor animals used for the production of adult worms for *in vitro* experiments are detailed in Appendix 2.13. Adult worms were collected from the abomasal contents and washings by hand or by a novel technique described in Appendix 2.14. Briefly, in this technique, the combined contents and washings were set in 1% agar, from which worms migrated into surrounding saline. Larvae or recovered adult worms were used to prepare the worm-derived solutions described below and reported in detail in Appendix 2.15. These solutions were stored in 4 ml volumes at -20^{0}C and subsequently tested in a ratio of 1:4 with BM using the static incubation method. All solutions were tested on pasture-reared sheep. In addition, some solutions were tested on sheep raised to be parasite-naive (see Appendix 2.1), in combination with somatostatin antiserum, in combination with atropine, on dispersed cells, or after their separation on the basis of molecular size. Larvae-derived solutions were prepared from one suspension of larvae while adult-derived solutions were prepared from five independent worm populations. The adult-derived solutions are referred to as Batches 1, 2, 3, 4 and 5. About 5000

adult worms were used in the preparation of each incubate within each batch.

Larvae: Three million exsheathed larvae were incubated in 50 mls of BM at 37°C for six successive 1 hour periods and then overnight (9 hours). The supernatant from incubates 1 to 3 were pooled together, as were those from incubates 4 to 6. After collection of the overnight incubate, the larvae were sonicated using an ultrasonic disintegrator (MSE Soniprep 150, MSE Scientific Instruments, Manor Royal, England) until no intact larvae were identifiable under the microscope (about 5 minutes) to produce "larval extract".

Batch 1: Worms were collected manually and incubated in BM. The worms underwent four successive 3 hour and then an overnight (9 hour) incubation. The five incubates were pooled before storage. At the conclusion of the incubation series, at which most worms still appeared to be alive, the adult worms were disintegrated to produce extract. Worms were a range of ages although all were adults (only adult worms were collected by hand from the abomasal contents of sheep that had been trickle infected).

Batch 2: Worms were collected following their migration from agar and incubated in BM or distilled water for four successive 3 hour periods and then overnight (9 hour) (BM only). The incubates prepared either in water or BM were pooled before storage. After overnight incubation in BM, the worms were sonicated (extract). Worms were at least 35 days old.

Batch 3: Worms were collected following their migration from agar. These were all placed into BM with added antimicrobials (antibiotics and antifungal) before they were incubated. Worms were either incubated in BM or BM with added antimicrobials for a 6 and then a 9 hour period, or they were sonicated (extract). The worms used to produce extract had not undergone prior incubation. The two incubates for each medium were mixed together and filtered through a series of cellulose acetate membrane filters (Micro Filtration Systems, USA) before storage. Worms were 28 days old.

Batch 4: Worms were collected following their migration from agar and incubated in either distilled water, 0.9% NaCl, BM without glucose, BM without glucose or BSA, or BM without glucose but with antibiotics for four successive 2.5 hour periods and then overnight (9 hours). Extract was prepared from all of the worms. Each incubate was stored separately. Worms were 22 days old and raised in goats.

Batch 5: Worms were collected following their migration from agar and incubated either in BM, BM without glucose, BM with antibiotics, or BM without glucose but with antibiotics for three successive 3 hour periods and then overnight (9 hours). Each incubate was stored separately. Worms were 30 days old.

5.2.2.1 SEPARATION OF WORM PREPARATIONS BY MOLECULAR WEIGHT

Incubate from Batch 1 and extract from Batch 2 were each separated into two size fractions on the basis of molecular weight using a Diaflo filtration system (Amicon, Inc., USA) that retained molecules of 3000 daltons. Due to the limited amount of material separated, the separated fractions from the incubate were tested in only two experiments and the fractions derived from the extract in one.

5.2.2.2 SOMATOSTATIN ANTISERUM

Somatostatin antiserum (monoclonal antibody from mouse ascites; control - keyhole limpet haemocyanin antibody, Dr J H Walsh, UCLA) in final dilutions of 1:500, 1:1000, 1:2500 was tested in combination with a 1:1 mixture of the incubate and extract of Batch 2 in one experiment. The mixed worm preparation was simultaneously tested without added antiserum.

5.2.2.3 ATROPINE

Larval secretions from the second pool of incubates (4 to 6 hours), larval extract, the incubated solution of Batch 1 and the extract of Batch 1 were each tested in combination with 10^{-5} M atropine (A-0257, Sigma Chemical Co., USA) in one experiment.

5.2.2.4 DISPERSED CELLS

Larval secretions (a combination of the first and second pools), larval extract, and the incubate and extract of Batch 1 were each tested on antral and fundic dispersed cell suspensions. These solutions were simultaneously tested using the static incubation method on antral mucosal segments derived from the same sheep.

5.2.3 ESTIMATION OF GASTRIN AND SOMATOSTATIN CONCENTRATION

These were estimated as described in Appendices 1.2 and 1.3. Each experiment was assayed within a single assay to eliminate interassay variation.

5.2.4 STATISTICS

All statistics were performed using the software package MINITAB (Minitab Inc., USA). The data set included Rt values derived from separate experiments. Where related treatments were tested (e.g. solutions from the same batch), the data sets were compared using either the Tukey (equal sample sizes) or Dunnett (unequal sample sizes) multiple comparison method. Where the effect of a treatment (Rt) within an experiment was tested against H_0 and only two variables were involved, two sample t-tests were used. Where multiple treatments from one experiment were compared the Tukey or Dunnett multiple comparison method was used. Differences were considered significant at the 5% level.

5.3 RESULTS

The worm-derived solutions either had no effect on, or inhibited, gastrin secretion, with one exception, and either had no effect on, or stimulated, somatostatin secretion. Consistent features included:

(1) no solution containing antimicrobials/antibiotics had a significant effect on gastrin or somatostatin secretion;

(2) those solutions most inhibitory to gastrin secretion and stimulatory to somatostatin were those in which either the successive incubations had been pooled or they were those that had had the longest incubation times;

(3) all solutions which inhibited gastrin also stimulated somatostatin release;

Table 5.1 Effect of exsheathed *O. circumcincta* L_3 products on gastrin secretion by ovine antral mucosa. The response to treatment (Rt) was determined from 16 control and 8 test tissue pieces in each experiment; n = number of experiments; CV = the coefficient of variation calculated from the raw data (mean control CV = 19%); (*) = a significant difference from control (a = Dunnett method, b = two sample t-test).

Larvae preparation		% Gastrin response		
Incubation	Hours	Rt (mean ± SD)	Mean %CV	n
1	1-3	-4 ± 1	19	3[a]
2	4-6	-36 ± 13*	15	1[b]
Overnight	7-15	-36 ± 6*	25	2[a]
Extract	15	-46 ± 11*	19	3[a]

Table 5.2 Effect of adult *O. circumcincta* products (Batch 2) on gastrin and somatostatin secretion by ovine antral mucosa. The response to treatment (Rt) was determined from 24 control and 8 test tissue pieces in each experiment; n = number of experiments; CV = coefficient of variation calculated from the raw data (mean control CV = 15% (gastrin) and 28% (somatostatin); (*) = a significant difference from control (Dunnett method).

Incubation medium	% Gastrin response			% Somatostatin response		
	Rt (mean ± SD)	Mean %CV	n	Rt (mean ± SD)	Mean %CV	n
Basal medium	-21 ± 16*	17	14	216 ± 49	36	11
Water	17 ± 15	17	3	-	-	-
Extract	-63 ± 15*	30	14	926 ± 532*	50	11

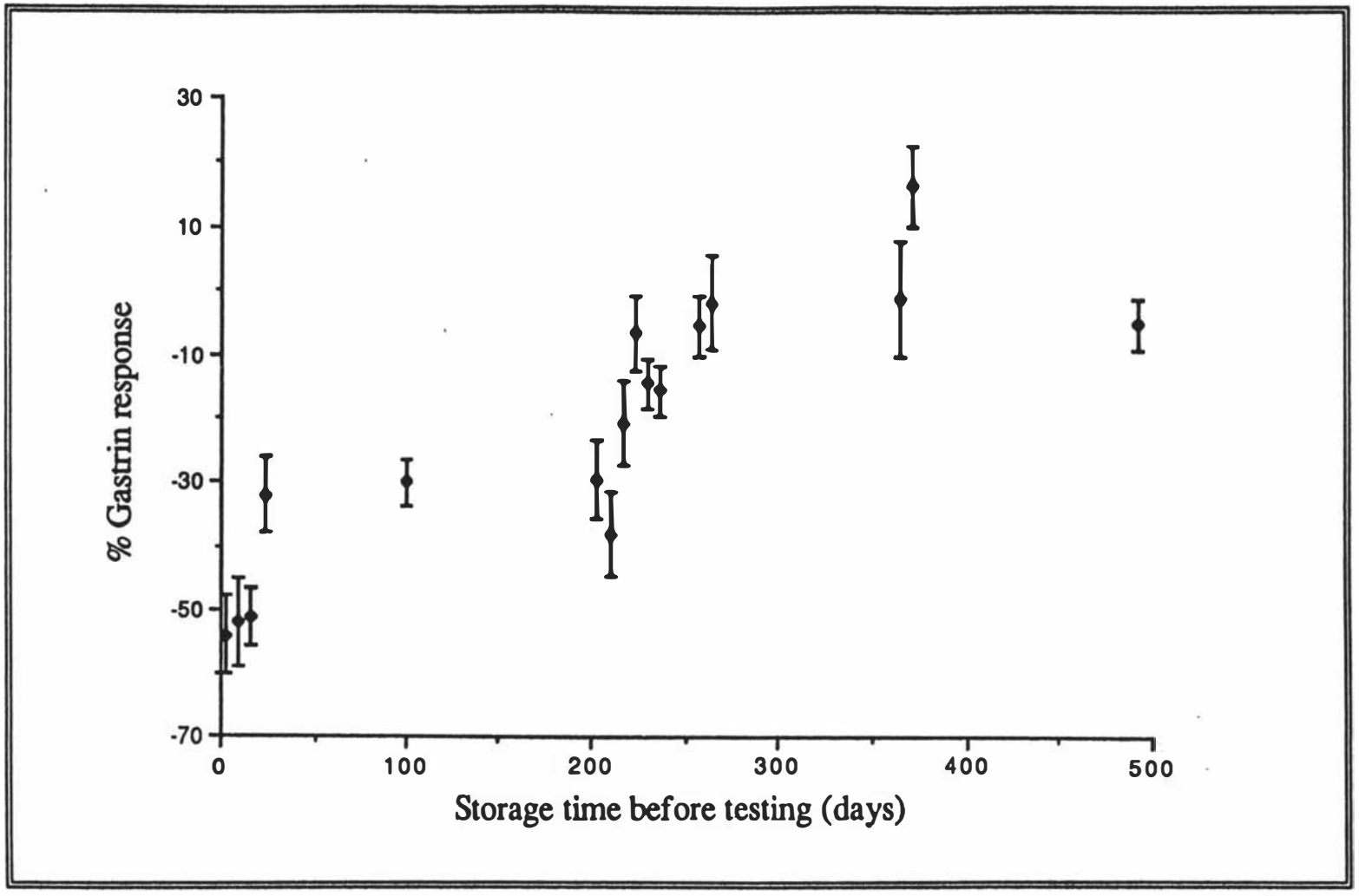

Figure 5.1 Effect of increased storage time on the gastrin inhibitory properties of the Batch 1 *O. circumcincta* incubate. The % gastrin response (Rt ± SEM) by ovine antral mucosa to this solution was determined in 16 experiments over a 20 month period.

(4) significant somatostatin secretion was not necessarily associated with significant inhibition of gastrin release.

The results are detailed below for each individual batch of solutions.

LARVAE-DERIVED SOLUTIONS

Of the three preparations made by incubating exsheathed larvae in BM, only the first pooled collection (1st, 2nd and 3rd hour incubations) had no effect on gastrin secretion, while the 4-6 hour incubate and the overnight incubation significantly inhibited gastrin secretion by 36% (Table 5.1). This inhibition was not significantly different from the 46% inhibition associated with larval extract.

BATCH 1 SOLUTIONS

The effect of the solution prepared by incubating worms in BM on gastrin secretion lessened as the interval between its preparation and testing increased (Fig. 5.1). The solution was tested in three rough groupings, one soon after its preparation (0-100 days), a second after about seven months (200-240 days) and a third from 250-500 days. The inhibition associated with the first and second of these groups (-45% ± 13 and -21 ± 11 respectively (mean ± SD)) were both significant, while no activity was associated with the third group (1% ± 9). No such loss of activity appeared to affect somatostatin release, which this preparation increased by 145% ± 91, although this was not statistically significant. The extract that had been prepared by sonicating the worms after completion of the 21 hours of incubation inhibited gastrin secretion (-57%) and stimulated somatostatin release (1423%).

BATCH 2 SOLUTIONS

The solution derived by incubating worms in water, when mixed with BM (1:4), had 80% the osmolarity of BM. The % gastrin response (Rt = 147%) to this solution (Table 5.2) was no different from that produced by a similar dilution with water alone (Rt = 155%, see Chapter 3, 3.4.6.3). No significant activity was associated with this solution after the osmolarity was corrected by mixing with concentrated BM. The solution derived by incubating worms in BM significantly inhibited gastrin secretion (21%), but less so than the extract. Both the incubate prepared with BM and the extract increased somatostatin release but only the latter was significant.

Table 5.3 Effect of adult *O. circumcincta* products (Batch 3) on gastrin and somatostatin secretion by ovine antral mucosa. The response to treatment (Rt) for gastrin and somatostatin was determined in 4 and 2 experiments respectively, and in each of which there were 32 control and 16 test tissue pieces. CV = the coefficient of variation calculated from the raw data (mean control CV = 15% (gastrin) and 17% (somatostatin). No results were significantly different (Tukey method).

Incubation medium	% Gastrin response		% Somatostatin response	
	Rt (mean ± SD)	Mean %CV	Rt (mean ± SD)	Mean %CV
Basal medium	-2 ± 9	18	-6 ± 20	19
BM + antimicrobials	7 ± 5	17	14 ± 23	23
Extract	4 ± 6	18	122 ± 91	44

Table 5.4 Effect of adult *O. circumcincta* products (Batch 4) on gastrin and somatostatin secretion by ovine antral mucosa. The response to treatment (Rt) was determined from 32 control and 8 or 16 (#) test tissue pieces in each experiment; n = the number of experiments; CV = coefficient of variation calculated from the raw data (mean control CV = 15% (gastrin) and 24% (somatostatin); (*) = a significant difference from control using the Dunnett method, or (t), using the two sample t-test.

Worm preparation		% Gastrin response			% Somatostatin response		
Incubation medium	Incubation time (hrs)	Rt (mean ± SD)	Mean %CV	n	Rt (mean ± SD)	Mean %CV	n
Distilled water	0-2.5	-3 ± 7	12	4	28 ± 30	17	4
	2.5-5	13 ± 30	12	3	-	-	-
	5-7.5	1 ± 12	13	4	136 ± 43*	24	4
	7.5-10	6 ± 14	11	4	48 ± 5	20	2
	10-19	10 ± 6	12	4	71 ± 40	22	4
Saline	0-2.5	17 ± 5	14	4	12 ± 13	17	4
	2.5-5	14 ± 12	11	1#	-	-	-
	5-7.5	8 ± 4	11	4	63 ± 5	26	2
	7.5-10	-2 ± 2	10	2	-	-	-
	10-19	24 ± 23*	9	4	47 ± 3	21	2
Basal medium without BSA or glucose	0-2.5	8 ± 7	13	4	26 ± 17	21	4
	2.5-5	14 ± 14	13	1#	-	-	-
	5-7.5	1 ± 13	12	4	208 ± 8*	33	2
	7.5-10	11 ± 5	12	2	-	-	-
	10-19	1 ± 3	11	4	1290 ± 114*	29	2
Basal medium without glucose	0-2.5	11 ± 3	11	4	31 ± 9	16	4
	2.5-5	43 ± 14t	10	1#	-	-	-
	5-7.5	-1 ± 15	11	4	199 ± 8*	19	2
	7.5-10	12 ± 5	11	2	-	-	-
	10-19	0 ± 4	13	4	1561 ± 168*	21	2
Basal medium without glucose but with antibiotics	0-2.5	7 ± 12	15	4	41 ± 14	23	4
	2.5-5	49 ± 18t	12	1#	-	-	-
	5-7.5	2 ± 7	13	4	159 ± 79*	30	2
	7.5-10	-3 ± 3	9	2	-	-	-
	10-19	15 ± 10	12	4	97 ± 10	24	2
Extract		-25 ± 11*	15	8	4409 ± 1070*	21	6

Table 5.5 Effect of adult *O. circumcincta* products (Batch 5) on gastrin secretion by ovine antral mucosa. The response to treatment (Rt) was determined in 5 experiments, in each of which there were 32 control and 8 test tissue pieces; CV = the coefficient of variation calculated from raw data (mean control CV = 13%); (*) = a significant difference from control (Tukey method).

Worm preparation		% Gastrin response	
Incubation medium	Incubation time (hours)	Rt (mean ± SD)	Mean %CV
Basal medium	1-3	4 ± 5	13
	4-6	9 ± 6	13
	7-9	8 ± 5	11
	10-18	-29 ± 28*	18
Basal medium without glucose	1-3	-3 ± 21	12
	4-6	6 ± 6	12
	7-9	-1 ± 13	12
	10-18	-18 ± 11	15
Basal medium with antibiotics	1-3	8 ± 8	13
	4-6	14 ± 7	13
	7-9	6 ± 9	12
	10-18	-3 ± 2	13
Basal medium with antibiotics but without glucose	1-3	4 ± 7	11
	4-6	7 ± 13	8
	7-9	3 ± 9	12
	10-18	-2 ± 4	11

BATCH 3 SOLUTIONS

There was no significant gastrin or somatostatin response to these preparations which were made using worms that had been pretreated with antimicrobials prior to incubation and which were filtered prior to storage (Table 5.3). The insignificant 7% gastrin response to the solution prepared with BM plus antimicrobials was similar to the effect of the antimicrobials alone. Although not significant, the worm extract increased somatostatin release by 120%.

BATCH 4 SOLUTIONS

The extract significantly inhibited gastrin and stimulated somatostatin secretion. Of the incubates tested more than once (and therefore assessed using the Dunnett method), the overnight incubation in saline significantly increased gastrin secretion by 24% (Table 5.4). In addition, two solutions from the second incubation interval that were tested in only one experiment also stimulated gastrin secretion and most other solutions, although without a significant effect, increased gastrin slightly. A number of solutions significantly increased somatostatin secretion and, although also not significant, there was increased somatostatin release in response to the remaining solutions of this batch. Most of these were significant when compared with the control set using the less conservative t-test.

BATCH 5 SOLUTIONS

One preparation, in which worms were incubated overnight in BM, significantly inhibited gastrin secretion (-29%) (Table 5.5). A second preparation, also incubated overnight in BM (but without glucose), resulted in significantly less gastrin secretion than in those prepared with antibiotic and was not distinguishable from the one that was inhibitory. Whenever the antibiotic mixture was tested as a treatment, it increased gastrin release by 6%, but not significantly. The eight solutions prepared with antibiotics in the medium did not significantly affect gastrin release (mean response 4%).

5.3.1 PARASITE-NAIVE SHEEP

Batch 3 preparations had no effect (as was the case in pasture-reared sheep), while both the incubate and extract from Batch 2 were significantly inhibitory (as in pasture-reared

Table 5.6 Effect of adult *O. circumcincta* products on gastrin secretion by ovine antral mucosal tissue from parasite-naive sheep. The response to treatment (Rt) was determined in 5 experiments, each with 32 control and 16 test tissue pieces; CV = coefficient of variation calculated from raw data (mean control CV = 16%); (*) = a significant difference from control (Tukey method).

Worm preparation		% Gastrin response	
Batch #	Incubation medium or extract	Rt (mean ± SD)	Mean %CV
2	Basal medium	-44 ± 28*	14
2	Extract	-76 ± 8*	27
3	Basal medium	-1 ± 2	14
3	Basal medium + antimicrobials	0 ± 8	17
3	Extract	4 ± 13	17

Table 5.7 Effect of fractions separated on size (3000 daltons) of adult *O. circumcincta* products on gastrin secretion by ovine antral mucosa. Each response to treatment (Rt) relates to one experiment; CV = the coefficient of variation calculated from raw data (mean control CV = 20%); (*) = a significant difference from control (two sample t-test). Note, Batch 1 incubate became less inhibitory with increased storage time.

Worm preparation		% Gastrin response (Rt ± SD)	Number of test tissue pieces in each experiment
Test solution	Size fraction		
Batch 1 incubate 217 days storage	Whole	-21 ± 20*	24
	<3000 daltons	-3 ± 31	24
	>3000 daltons	4 ± 34	24
Batch 1 incubate 257 days storage	Whole	-6 ± 17	8
	<3000 daltons	1 ± 14	8
	>3000 daltons	-6 ± 22	8
Batch 2 Extract	Whole	-83 ± 11*	8
	<3000 daltons	-8 ± 20	8
	>3000 daltons	8 ± 13	8

Table 5.8 Effect of somatostatin antiserum on the gastrin response to an inhibitory solution (Batch 2) derived from *O. circumcincta*. The response to treatment (Rt) is derived from one experiment in which there were 32 control and 16 test tissue pieces. The coefficient of variation calculated from the raw data for control = 13% (gastrin) and 17% (somatostatin); the worm derived solution (WDS) was a combination (1:1) of the incubate and extract from Batch 2; (*) = a significant difference from control (Dunnett method).

Treatment	% Gastrin response (Rt ± SD)
Worm derived solution	-46 ± 13*
WDS and 1:5000 antiserum	-45 ± 13*
WDS and 1:1000 antiserum	-33 ± 13*
WDS and 1:500 antiserum	-7 ± 12
	% Somatostatin response (Rt ± SD)
Worm derived solution	701 ± 206*

Table 5.9 Effect of combining 10^{-5} M atropine with *O. circumcincta* products on gastrin secretion by ovine antral mucosa. The response to treatment (Rt) was derived from one experiment with 16 control and 8 test tissue pieces; the coefficient of variation of the control tissue pieces calculated from raw data was 15%; larval incubate is from the 4-6 hour incubation pool; adult worm incubate was from Batch 2; (1:4) and (2:3) denote the dilution with basal medium when tested; (*) = a significant difference from control (Dunnett method).

Worm preparation	± Atropine	% Gastrin response (Rt ± SD)
Larval incubate	-	-36 ± 13*
	+	-29 ± 20*
Larval extract	-	-38 ± 11*
	+	-64 ± 15*
Adult incubate (1:4)	-	-42 ± 27*
	+	-55 ± 19*
Adult incubate (2:3)	-	-58 ± 17*
	+	-56 ± 19*
Adult extract	-	-75 ± 25*
	+	-71 ± 9*

sheep) (Table 5.6). When Rt sets from either batch were compared with those determined for the same preparations in pasture-reared sheep (Tables 5.2 and 5.3) using two sample t-tests, no differences were found.

5.3.2 SEPARATION OF SOLUTION BY MOLECULAR WEIGHT

The incubation solution (Batch 1) was inhibitory in the first of the two experiments performed (-21%, Table 5.7) but had no effect in the second. This is consistent with the results obtained with this preparation in which activity diminished with increased storage time. The first of the two experiments was from the middle cluster (217 days) in Fig. 5.1 while the second experiment was from the third (257 days). However, in neither experiment did either of the separated fractions affect gastrin secretion, despite the inhibitory activity of the complete solution in the first experiment. Similarly, it was found that the extract from Batch 2, which reliably inhibited gastrin secretion by more than 60% (Table 5.2), when separated into two molecular size fractions, lost its inhibitory properties and neither fraction significantly affected gastrin secretion.

5.3.3 SOMATOSTATIN ANTISERUM

Somatostatin antiserum at dilutions of 1:500 and 1:1000 significantly reduced the effect of the worm preparation (mixture of incubate and extract of Batch 2) on gastrin secretion (Table 5.8). Without antiserum, the worm preparation significantly increased somatostatin (700%) and decreased gastrin (-46%) secretion. The gastrin response to the worm preparation in combination with 1:500 somatostatin antiserum (-7%) was not significantly different from that by control tissue.

5.3.4 ATROPINE

Parasite-derived preparations which inhibited gastrin secretion (Table 5.9) were still inhibitory when combined with 10^{-5} M atropine. The response to these mixtures were not significantly different from those produced by the worm-derived solutions on their own (two sample t-test).

5.3.5 DISPERSED CELLS

All four solutions tested inhibited gastrin secretion by the cell suspension while all

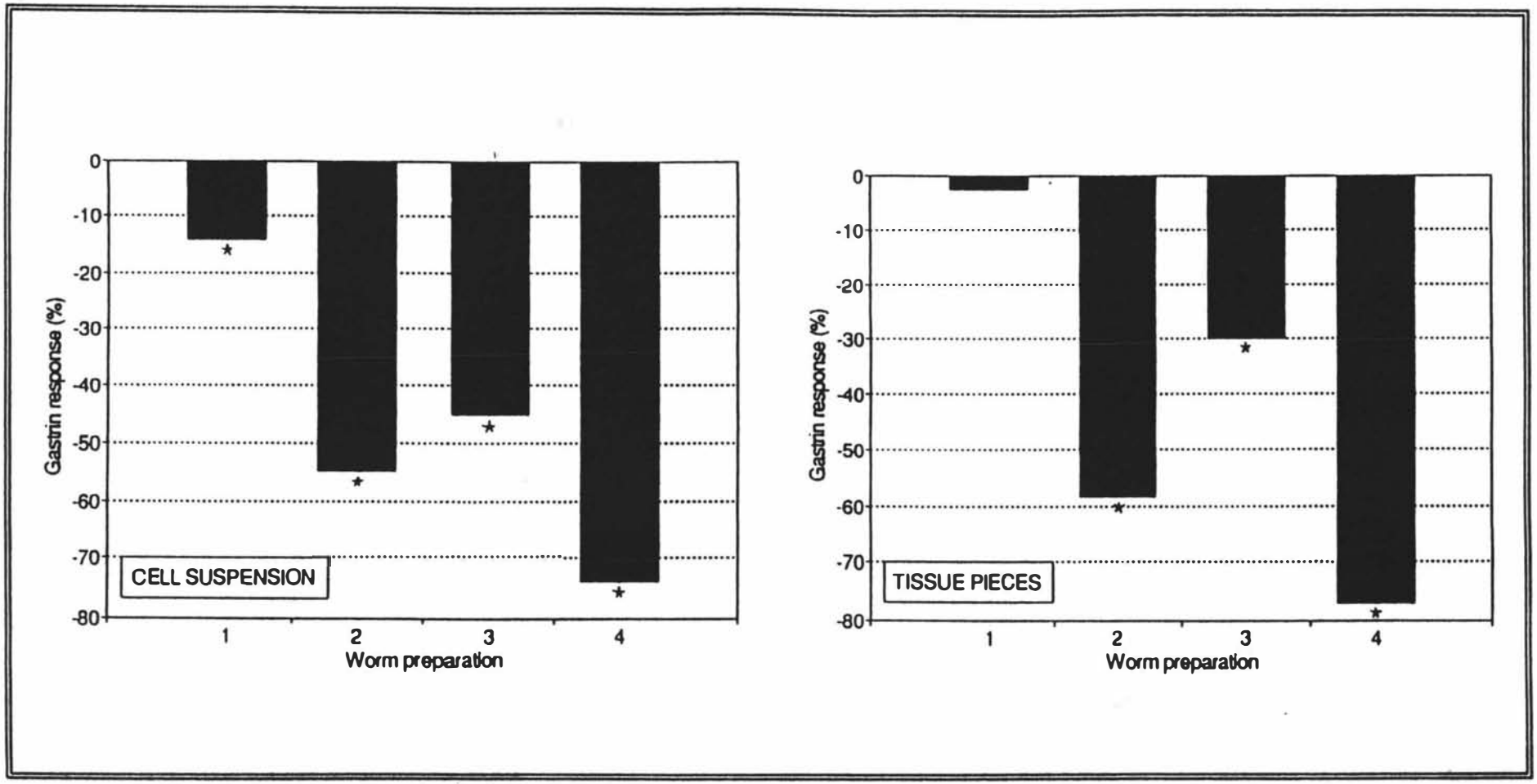

Figure 5.2 Effect of worm products on gastrin secretion by dispersed ovine antral mucosal cells and from segments of antral mucosa. The results of the dispersed cell preparation were previously presented in Fig.3.9. (*) indicates a significant difference from control secretion (Dunnett method). The worm products were derived from *O. circumcincta* larvae and adult worms and were: (1) larval incubate (a combination of first and second pools); (2) larval extract; (3) adult worm incubate (Batch 1); (4) adult worm extract (Batch 1).

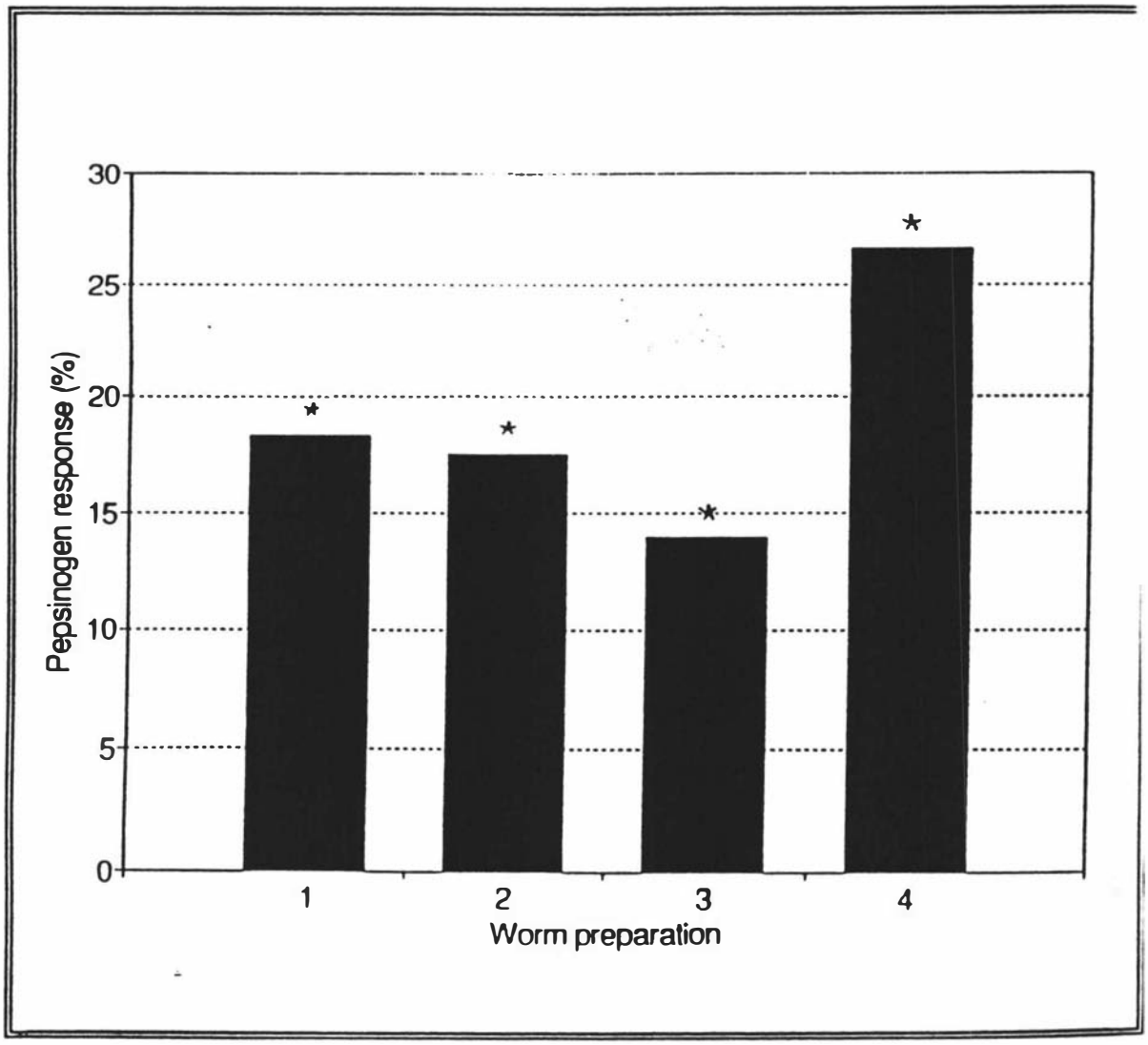

Figure 5.3 Effect of worm products on pepsinogen secretion by dispersed ovine fundic cells. (*) indicates a significant difference from control secretion (Dunnett method). The worm products were derived from *O. circumcincta* larvae and adult worms and were: (1) larval incubate (a combination of first and second pools); (2) larval extract; (3) adult worm incubate (Batch 1); (4) adult worm extract (Batch 1).

except the larval incubate similarly inhibited gastrin secretion in the accompanying static incubation experiment (Fig. 5.2). Two sample t-tests of the respective Rt_i sets revealed that the significant responses to larval and adult extract obtained using either method did not differ from each other, although that to larval and adult secretate did. Pepsinogen release was significantly stimulated by the same four solutions that inhibited gastrin secretion from cells prepared from the same abomasum (Fig. 5.3).

5.4 DISCUSSION

Parasite excretory/secretory products have been suggested as important contributors to the secretory response of the abomasum, but their role has been examined in very few experiments. McKellar and coworkers have tested *in vitro* the effect of *Ostertagia* incubates (in distilled water) on abomasal glands and dispersed fundic cells and have observed only a weak response in pepsinogen secretion and no effect on aminopyrine accumulation. There appears to be no such similar examination of the role of ES products of *O. circumcincta* on the G cell which may contribute to the development of hypergastrinaemia following infection with this parasite. This possibility has been proposed as in some instances serum gastrin levels appeared to rise before the pH of the abomasal contents was raised (Titchen, 1982; Anderson *et al.*, 1985; McKellar *et al.*, 1987). The results of the present investigation, however, appear to suggest that parasite products are inhibitors of gastrin release rather than stimulatory.

Adult *O. circumcincta* were incubated in a range of solutions (water, saline and basal medium), since the mixture of ES products released may depend on the composition of the medium and the time the parasites are exposed to it. The composition of the BM was also varied by omitting glucose or glucose and BSA. Antimicrobial agents were included in some BM solutions. Adult worms were the focus of these investigations as most of the changes, and in particular the increase in serum gastrin, do not develop for some days after larval infection whilst they develop almost immediately after adult transfer (Chapter 2). Many of the incubates had no effect on gastrin release from segments of ovine antral mucosa *in vitro*, a few were stimulatory and a number were strongly inhibitory. This may reflect the presence of more than one biologically active substance in the different solutions, some of which may originate from the parasites, while others may be derived from the abomasal microflora transferred with the worms or from environmental bacteria. The effect on gastrin release did not appear to be

related to previous exposure to parasites, as tissue from sheep raised parasite-naive and that from pasture-reared animals, presumed to have been exposed to field parasitism, responded no differently to incubates (Batches 2 and 3) (Table 5.2, 5.3 and 5.6).

Gastrin release was stimulated only by incubates derived from adult worms (only Batch 4) (Table 5.4) and was significant only for three of the 25 solutions (using the conservative Dunnett multiple comparison method), although almost all of the 25 solutions slightly increased gastrin release. The significance of these observations should be treated with caution since two of the positive results were derived from testing in only one experiment and in each case, the preceding and subsequent incubates had no effect. There were some unique features of Batch 4 solutions: the worms were raised in goats rather than sheep; they were the youngest used (22 days); none of the incubation media contained glucose. The omission of glucose may reduce the production of contaminating microbial products or, alternatively, metabolism by the worms may differ in younger worms and in medium without glucose e.g. McKeand *et al.* (1994b), who compared third stage, fourth stage and adult worm extracts of *D. viviparus*, suggested that acetylcholinesterases are only produced by later stages of this parasite (Chapter 1, 1.5.4).

Many incubates, in contrast, were inhibitory: three of four larval preparations and adult worm-derived solutions from Batches 1 and 2 and one from Batch 5 inhibited gastrin secretion. The common feature among these solutions was the inclusion of overnight incubates (Tables 5.1, 5.2 and 5.5) (overnight larval incubate, Batch 1 pooled incubate, Batch 2 pooled incubate and the Batch 5 overnight incubate (BM)). In addition, the Batch 5 overnight incubation in BM without glucose, although not significant, reduced gastrin secretion by 18%. The exception to this was the 4-6 hour larval incubate which was also inhibitory. Four of the five "extracts" were also inhibitory and in all cases, were prepared from larvae or adults that had previously been incubated overnight, while the extract that had no effect (Batch 3) was made from worms that had not undergone prior incubation and were pretreated with BM containing antimicrobials. Inhibitory properties were clearly associated with prolonged overnight incubation periods and suggested, therefore, that they accumulated in the medium slowly. The slow acquisition of inhibitory properties within the solutions may reflect the slow release of substances by the parasites, that such substances are released by dying worms or, alternatively, the proliferation of contaminating microbes with prolonged incubation at 37^{0}C under favourable conditions.

Examination of the conditions under which the inhibitory properties were acquired supports a microbial origin. First, a long incubation time was necessary. No inhibition developed in any solutions prepared within four hours of commencing incubation which may indicate that this time is too short for appreciable bacterial multiplication to occur. Secondly, no incubates in water or saline were inhibitory (Tables 5.1 and 5.4) which may reflect the lack of bacterial growth in this incomplete medium. Thirdly, in complimentary incubations that differed only by the inclusion of antibiotics in the medium, such as in Batch 5, gastrin inhibitory properties did not develop. As not all of the incubation medium was replaced from one incubation to the next, if bacteria were present in the medium and growing, the effective contaminating dose would increase from one incubation period to the next. The effects of contamination and bacterial growth may, therefore, be most profound in the overnight incubation periods which may have had the greatest initial microbe density as well as the longest incubation time. Some contamination by microorganisms would have been inevitable as, in addition to any seeding of media with organisms associated with the parasites themselves, the incubations were not performed under aseptic conditions.

The strongest support for a microbial origin for the gastrin inhibitor is its failure to develop in the presence of antibiotics. Neither of the pooled incubates, nor the extract of Batch 3 were inhibitory. The Batch 3 incubation in BM differed from those of Batches 1 and 2 in that (a) the worms were exposed to antimicrobials for 30 minutes prior to their incubation and (b) the solution was filtered through a series of cellulose acetate filters prior to storage (see Appendix 2.15 for details). Similarly, the worms from which the extract was made were also pre-soaked in antimicrobials before their disintegration, they had not been incubated and the extract was filtered prior to storage. The remaining group of worms were incubated in the presence of antimicrobials. These results suggest (1) that if the inhibitory properties are associated with bacterial growth, then the pre-incubation in antimicrobials is sufficient to reduce the number or viability of these organisms, thereby preventing their subsequent alteration of the medium; or (2), that if inhibitory properties had developed, they were in some way vulnerable to the multiple filtration process; or (3), that both of these were of some importance.

The failure of either of the fractions separated on molecular size prepared from inhibitory Batch 1 and 2 solutions (Table 5.7) to affect gastrin secretion suggests that the inhibitory properties of these solutions were influenced by the micro-filtration process. It was expected that the inhibitory properties of the parent solutions would have

been associated with either the small (less than 3000 dalton) or large (greater than 3000 dalton) size fraction had they not been affected by the separation process. This loss of activity may indicate that the active properties in the inhibitory solutions are partially labile or adhere to the filter. The possible labile nature of the inhibitory properties may account for the loss of activity that was observed in the Batch 1 incubates with increased storage time (Fig. 5.1). The loss of activity may have occurred through possible failure of the freezer in which the samples were kept to hold its temperature at -20^{0}C.

Some inhibitory products may have originated from worm components released after sonication. Extract prepared from Batch 4 worms at the conclusion of an overnight incubation in a range of media was inhibitory in spite of the lack of inhibition by any of the solutions in which these worms had been incubated immediately prior to their sonification. Microbial contamination, as suggested by inhibitory incubates, would not appear to explain the inhibitory properties of this extract and, therefore, prior contamination alone may not account for all of the activity of extract *per se*. Neither of the overnight incubations in water or saline of this batch (Batch 4) were inhibitory. Indeed, one of these, the overnight incubation in saline, was stimulatory. However, as a high proportion of the worms died during the course of the overnight incubation in water or saline, whereas most were alive in BM, it is possible that substances are released from dead or dying worms that have a net stimulatory effect on gastrin secretion. If so, however, these substances must differ from those released by disintegrated worms. Conversely, if worm components are inhibitory, it would be expected that the sonication products of worms washed in antibiotics (Batch 3) should also be inhibitory to gastrin release.

The mode of action of the gastrin inhibitor may be through stimulation of somatostatin release. The effect of some worm preparations on somatostatin secretion was profound: four solutions produced increases of 1000% or more. Without exception, where evaluated, the worm preparations that inhibited gastrin secretion were accompanied by substantial somatostatin release (although the somatostatin responses to Batch 1 (145%) and Batch 2 (216%) incubates were not statistically significant), suggesting the two may be causally linked. The involvement of somatostatin in the inhibition of gastrin release is supported by the inclusion of somatostatin antiserum in the medium with worm products that stimulated somatostatin release and inhibited gastrin secretion. In this one experiment (Table 5.8), the inhibition of gastrin secretion was reduced progressively as the concentration of antiserum was increased, until, at the highest concentration of

antiserum used (1:500), there was no inhibition of gastrin release.

Not all results support somatostatin as the mediator of the gastrin inhibition. In some cases a large increase in somatostatin secretion was not accompanied by decreased gastrin release (e.g. extract of Batch 3 and four incubates of Batch 4). This is consistent with the reported inability of somatostatin to inhibit basal gastrin release from segments of rat antral mucosa when perifused at a concentration of 2×10^{-7} M (Hayes *et al.*, 1974), a dose higher than the concentrations determined in these investigations. It is evident, however, that in Batch 4, while increases in gastrin secretion were a general feature (although usually not significant), no solution that significantly increased somatostatin had any effect on gastrin release. This may indicate that somatostatin restraint of the G cell is optimal as suggested by Saffouri *et al.*, (1984a), and that, rather than inhibiting gastrin secretion, somatostatin secretion may prevent the G cell from responding to stimulation. In essence, therefore, it is possible that in some instances the incubates may have contained substances stimulatory to the G cell, but that the activity of these was suppressed by simultaneous somatostatin release.

The incubates and extracts probably contain a mixture of substances, derived from both *O. circumcincta* and contaminating microorganisms that are stimulatory and inhibitory to both G and D cells. The net effect of such a "broth" may depend on the relative contribution of the parasites and the microflora. In turn, this may depend on the particular conditions provided by the medium during incubation. Somatostatin may inhibit gastrin secretion at the concentrations released by some solutions, while in others, such inhibition may be counteracted by the presence somatostatin-insensitive stimulators of gastrin secretion, such as amines (Del Valle & Yamada, 1990; Chapter 1, 1.3.4.1.1). Alternatively, rather than stimulatory properties masking somatostatin inhibition of the G cell in some cases, worm-derived G cell stimulants may be masked by potently inhibitory substances released by bacteria. In the absence of inhibitory substances, however, stimulatory properties may still not be expressed because of concomitant somatostatin release. The likelihood that stimulatory substances released by the parasite were masked by bacterial contamination was not supported by the inclusion of antimicrobials in the medium. The loss of gastrin-inhibitory activity (incubate, Batch 1) with increased storage time (Fig. 5.1) did not affect its somatostatin-stimulatory activity. This may indicate that different substances within the incubate affect the D and G cell and that the substance(s) which inhibit gastrin secretion were more vulnerable to the storage conditions than those which effected the somatostatin response.

Digestion of the BSA included in most of the media may have produced a variety of degradation products, including amines, which may influence the secretion of gastrin. Ammonia is produced by organisms such as *Helicobacter pylori* in high concentrations, with which is associated hypergastrinaemia (Yanaka *et al.*, 1993). If present, at the pH used to test the solutions ammonia would have been in both the NH_3 and NH_4^+ forms. Organic acid excretion is a characteristic of parasitic helminths despite the availability of oxygen (Bryant, 1993). Fermentation products are also produced by bacteria and collectively these may affect both G and D cells. Both worms and bacteria may release small amounts of the ubiquitous nucleoside, adenosine which is reported to have dual but contrasting effects on the G cell, mediated via two different A-type receptors (Schepp *et al.*, 1990). Other molecules, such as acetylcholinesterases and VIP (Chapter 1, 1.5.4), may also be released into the medium. As atropine failed to affect the inhibition of gastrin secretion in the experiment in which it was tested (Section 5.3.4), the active component was not considered likely to be a parasympathomimetic.

The inhibitory solutions were also tested on a dispersed fundic cell preparation and proved to be stimulatory to pepsinogen secretion (15-20%), confirming the small increase in pepsinogen secretion by ES preparations reported by McKellar *et al.* (1990a; Chapter 1, 1.5.3.1). The response reported by McKellar *et al.* was "much reduced" by atropine, indicating that their "extremely potent" secretions probably contained a parasympathomimetic. Unfortunately, they do not indicate whether this reduction was significant. In the present study, it has been suggested that the inhibition of gastrin secretion by the solutions tested on the fundic cell suspension may have been due to microbes and not ES products of *Ostertagia.* Stimulation of pepsinogen release may also have been due to substances derived from contaminating microorganisms rather than from *O. circumcincta.* As the worm incubates, presumed to be ES products, made by McKellar *et al.* (1990a,b) also did not include antimicrobials, it is possible that products of microbial origin may have contributed to the pepsinogen response they reported. This may explain why no response to either live adults or larvae, or to extracts of either stage of the parasite was observed. However, they incubated parasites for only 30 minutes and in distilled water - conditions which may not have supported microbial contamination (i.e. inadequate replication time and incomplete medium). Gastrin-inhibitory properties did not develop in any solution prepared within four hours of commencing incubation in the present investigation, nor when worms were incubated in water (Batches 2 and 4). It may also be possible that the pepsinogen response reported by McKellar *et al.* (1990a), although small, was not due to either worms or

contaminants but to an osmolarity effect on the chief cell associated with the 3.3% dilution of their medium with water[1]. The significant effect of a 20% dilution with ES solution prepared in water was abolished when osmolarity changes were compensated for in this investigation. In Chapter 3 (Fig. 3.8), the effect of osmolarity on gastrin but not pepsinogen secretion was reported. If hypo-osmotic solutions have a comparable effect on the chief as was found for the G cell, then the responses reported by McKellar *et al.* (1990a) are to be expected and need not be related to worm ES products. The lack of an aminopyrine response to similar ES products (McKellar *et al.*, 1990b) may reflect correction for osmolarity differences by the similar dilution of controls.

These investigations aimed to assess the proposed contribution of ES products released by *O. circumcincta* to the hypergastrinaemia that may accompany infection of sheep with this parasite. Suggestions made by Anderson *et al.* (1985) and others that worm-derived products may stimulate gastrin secretion in the parasitised sheep were not supported by the present results. Nevertheless, it was shown that the incubation of larval and adult *O. circumcincta* under conditions where microbes may proliferate may generate products which affect the secretion of both gastrin and somatostatin *in vitro*. With one exception, the incubating media either had no affect or were inhibitory to gastrin secretion. The *overall* inhibitory affect did not appear to be related to the test system as almost identical results were obtained when solutions were tested on tissue segments and in the single experiment with dispersed cells (section 5.3.5). This indicates that tissue integrity was not essential for the response and suggests that the active components may act directly on the G cell. Although based on a limited number of results, it appears that the substance(s) which inhibited gastrin secretion may be labile and do not compete with atropine. In all cases, substantial somatostatin release was associated with a decrease or no change in gastrin secretion. As the relationship between the two peptides was variable, it is possible that their secretion was affected independently. As solutions which inhibited gastrin included longer overnight incubation periods and were those in which the medium contained glucose but not antimicrobials, it is suggested that contaminating microorganisms and not worm-derived products may confer the activity. In Chapter 6, investigations aimed at assessing the potential of microbes to produce substances which inhibit gastrin secretion are reported.

1 McKellar *et al.* (1990a) do not report compensating for the dilution by water in this experiment although they did add an equal volume of water to controls when investigating the effect of ES products on aminopyrine accumulation (McKellar *et al.*, 1990b).

Chapter 6

THE EFFECT OF CONTAMINATION BY MICROORGANISMS ON GASTRIN AND SOMATOSTATIN SECRETION *IN VITRO*

6.1 INTRODUCTION

There is a change in, and proliferation of, the bacterial flora within the abomasum when its pH rises following infection with trichostrongylid nematodes (Jennings *et al.*, 1966; Nicholls *et al.*, 1987). Usually the rise in abomasal pH is also accompanied by an increase in serum gastrin. However, as reported in Chapter 2, serum gastrin concentration decreased in some sheep when the abomasal pH was most raised following infection, which effectively reduced the parasite-induced hypergastrinaemia to pre-infection levels. Microbial products generated by the microbial flora of the abomasum during severe hypochlorhydria may be involved in this apparent inhibition of gastrin secretion.

The inhibition of gastrin secretion by a number of worm-derived solutions (reported in Chapter 5) may have been due to products of microbial origin rather than ES products of the parasite. This was suggested as the worm incubates which inhibited gastrin secretion were those most likely to have the greatest bacterial contamination. It was also found that a number of the solutions which inhibited gastrin secretion strongly stimulated somatostatin release.

In this Chapter, investigations are reported which were designed to determine whether microbes of abomasal origin are capable of producing substances *in vitro* that affect the secretion of gastrin, and in some cases somatostatin, by ovine antral mucosa using the static incubation method.

Table 6.1 Two series of solutions (a & b) prepared by the inoculation of Basal Mediu with abomasal contents from two sheep. (+) = inoculated with 0.2 mls, (++) with 0 mls).

Solution number	Antibiotics ±	Inoculated	Incubation time (hours)	Number of experiments	
				Gastrin	Somatostatin
1a	-	+	4	5	2
2a	-	+	8	3	-
3a	-	+	24	5	2
4a	-	+	48	5	2
5a	+	+	4	5	2
6a	+	+	8	3	-
7a	+	+	24	3	-
8a	+	+	48	3	-
9a	-	++	0	3	-
10a	+	++	0	3	-
1b	-	-	2	4	-
2b	-	+	2	4	4
3b	-	-	24	4	-
4b	-	+	24	4	4
5b	-	+	24	4	-
6b	-	+	24	4	4
7b	-	-	48	4	-
8b	-	+	48	4	-
9b	+	-	2	4	4
10b	+	+	2	4	4
11b	+	-	24	4	-
12b	+	+	24	4	4
13b	+	+	24	4	4
14b	+	+	24	4	-
15b	+	-	48	4	-
16b	+	+	48	4	-

6.2 MATERIALS AND METHODS

6.2.1 EXPERIMENTAL DESIGN

Inocula from the abomasal contents of two sheep were used to contaminate BM with or without antibiotics. The inoculated media were then incubated at 37°C for periods between 0 and 48 hours. Additional BM that was not inoculated, but that was exposed to normal environmental contamination, was also incubated. At the end of each incubation, the solutions were stored at -20°C and subsequently tested on segments of ovine antral mucosa using the static incubation method (Section 3.2.2) for activity on gastrin, and in some cases, somatostatin secretion.

6.2.2 TEST SOLUTIONS

Abomasal contents collected from two sheep immediately after death by stunning and exsanguination were left to sediment for 20 minutes. Inocula (0.2 or 0.5 mls) obtained from the supernatant fluid were added to 100 mls basal medium (BM) with or without antibiotics and incubated at 37°C. The antibiotics were 2 ml/L of 5000 U/ml penicillin G sodium and 5000 mcg/ml streptomycin sulfate (Penstrep, GIBCO, Life Technologies, USA), 1 ml/L BM, 200 mg/ml bensylpenicillin sodium BP (Glaxo, NZ) and 100 mg/L kanamycin sulfate (Sigma Chemical Co., USA). Control BM solution was also incubated. The composition of the two series of solutions (a and b) and the length of the incubation period are shown in Table 6.1.

6.2.3 EXPERIMENTAL PROCEDURE

All solutions were mixed with BM in the ratio of 1:4 and tested using the static incubation method (Chapter 3, 3.2.2). The antibiotic mixture was also tested. The tissue from one sheep was used for each experiment and the number of experiments in which each solution was tested is given in the Table 6.1.

6.2.4 ESTIMATION OF GASTRIN AND SOMATOSTATIN CONCENTRATION

These were estimated as described in Appendices 1.1 and 1.2. Interassay variation was not calculated as each experiment was assayed within a single assay.

6.2.5 STATISTICS

All statistics were performed using the statistical software package MINITAB (Minitab Inc., USA). The data set included Rt values each derived from separate experiments. Solutions from each sheep were considered to be related and were compared by the Dunnett (series A) and Tukey (series B) methods. Differences were considered significant at the 5% level.

6.3 RESULTS

The results are summarised in Table 6.2. The solution containing antibiotics always increased gastrin secretion (mean 6%), but this was not significant. The effect of the antibiotic mixture on somatostatin secretion was not tested *per se*, although there was 14% stimulation of somatostatin secretion by solution 9b which was not inoculated and served as the 'least incubated control'.

No solution prepared with antibiotics had a significant effect on either gastrin or somatostatin release. One solution that was not inoculated (solution 7b) and was incubated for 48 hours significantly inhibited gastrin secretion by 62%. Its effect on somatostatin was not evaluated. All inoculated solutions prepared without antibiotics and incubated for more than four hours inhibited gastrin secretion by an average of 60%. In all cases, where determined, these same solutions increased somatostatin secretion substantially (200 to 2000%), although only the responses to solutions 4a and 4b were significant. All solutions that were ineffective on gastrin release were also without effect on somatostatin secretion and vice versa.

6.4 DISCUSSION

The contamination of BM with a small inoculum of abomasal fluid produced changes in the medium that stimulated somatostatin and inhibited gastrin release. These properties were not transferred with the inoculum itself as no inoculated solution containing antibiotics, nor those that were incubated for less than eight hours affected either gastrin or somatostatin secretion. The properties developed with time, and this was prevented by antibiotics and dependent on inoculation. The exception to this was solution 7b, that was not inoculated but was incubated for 48 hours.

Although no specific assessment of microbiological contamination was made, it was assumed that the properties that developed in the contaminated BM were the result of the growth and multiplication of microorganisms within the solutions. The inoculum was considered to seed the solution with a mixture of microorganisms, some of which would grow in the conditions provided. The substantial growth of organisms within the solution was visibly evident as, after 24 hours of incubation, inoculated solutions appeared cloudy and had an aroma typical of bacterial broth cultures. The products generated in solution 7b were assumed to be due to contamination from the unsterile environment in which this work was performed.

Although this investigation did not prove that the changes that developed within the solutions following inoculation and incubation were caused by the growth and multiplication of microorganisms, it has demonstrated clearly that the gastrin-inhibitory and somatostatin-stimulatory properties associated with some solutions in Chapter 5 may not be due *O. circumcincta* and its presumed excretory-secretory products - thus validating the proposed microbial origin of these properties. This may also explain the inability of the worm incubates prepared with antimicrobials to affect the secretion of either peptide. There is little doubt that components of the abomasal microflora would have been transferred with the worms to the various media prior to incubation. The level of contamination was expected to be low as the agar technique used for their collection (Appendix 2.14) provided good separation from the abomasal contents. The effective inoculum would thus have been small and may account for the lack of activity in some solutions.

The reciprocal relationship between somatostatin and gastrin was even more clearly shown in this study than by the worm incubates (Chapter 5). Either this indicates that the microorganisms produce substances which stimulate somatostatin and inhibit gastrin, or that somatostatin mediates the inhibition of gastrin release.

The substances which have these potent affects *in vitro* may also be released by microorganisms in the parasitised sheep when its abomasal pH is raised. Indeed, the possible release of such substances provides an explanation for the lowering of serum gastrin levels when abomasal pH was most raised in many sheep in Chapter 2. It would be interesting to find out which microorganisms produce these substances and the nature of such a powerful inhibitor of gastrin secretion.

Chapter 7

GENERAL DISCUSSION

Parasitism is a form of symbiosis in which one population (or individual) adversely affects another, and yet is dependent upon it. Ostertagiasis can adversely affect the performance of sheep and cattle in intensive agricultural systems. Some individuals are more affected than others, thus while some animals may die, others exhibit an apparent resistance to either the parasite or its detrimental effects. *Ostertagia* is dependent on ruminant hosts for its survival and, therefore, it may not be to the benefit of the parasite to be excessively pathogenic. Indeed, under the natural grazing systems within which these organisms co-evolved, the parasitism may normally be of little consequence as infection rates are likely to be relatively low. Associated with low-level primary infection of young animals, stimuli may be provided to the host which affect the development of the gut and which may even contribute to its normal growth - particularly if the parasitised animal is considered to be the normal animal. High-level infestation, on the other hand, may serve as a natural regulator of population numbers and function to prevent population explosions and potential population crashes.

The natural balance is tipped in favour of the parasite in highly intensive farming systems in temperate areas, such as those typical of the New Zealand pastoral industry. Lambs are exposed to high levels of larval intake at a young age which may compromise their welfare and wellbeing. Even more extreme imbalance may be induced in parasite-naive sheep experimentally infected with high parasite burdens in order to study the pathogenesis of the parasitism. Such heavy infestations, often given as a single dose, may exaggerate the milder effects associated with natural infections. This is useful as it allows effects which may be undetectable in the field to be observed clearly. It is possible that the effects which manifest as pathology in artificial farming or experimental situations are of little consequence to either the host, parasite, or both in natural systems. It is only when performance is assessed in terms of selected production parameters that the effect of mild subclinical parasitism is really appreciated. In natural grazing systems, small increases in abomasal pH may benefit the parasite by

increasing its egg production (Hondo & Bueno, 1982) and the host by increasing gastrin release and promoting hyperplasia of the fundic mucosa (Johnson, 1987). As the level of primary infection increases, larger increases in abomasal pH may occur (as reported in Chapter 2) with which there appears to be no advantage to the host: there may be abnormal proliferation of bacteria (Nicholls *et al.*, 1987) and increases in circulating gastrin levels which may reduce food intake (Fox *et al.*, 1989a,b).

Despite their artificial nature, experimental infections may substantially benefit the researcher. The exaggerated responses such infections provoke may highlight processes occurring within the host, e.g. elevated abomasal pH and hypergastrinaemia were unequivocal in all the parasite-naive sheep infected in this study. The *in vivo* experiments were designed to answer several questions. Do serum gastrin levels increase before the pH of the abomasal contents rises (i.e. does the hypergastrinaemia appear to be independent of hypochlorhydria)? When does hyperpepsinogenaemia develop relative to gastrin and pH changes? Exactly when do these changes occur? Is this affected by dose or route of administration (i.e. intraruminal versus direct transfer of exsheathed larvae into the abomasum)? Are adult worms capable of causing all the pathophysiological changes that follow larval infection? If so, when do these changes occur in comparison with those following larval infection? Is it likely that parasite-derived excretory/secretory products are involved in the pathogenesis of the secretory disturbances? What are the normal ranges of serum pepsinogen, serum gastrin and abomasal pH in the parasite-free sheep? Answers were provided to all these questions in Chapter 2. In part, these were afforded by the intensive sampling regime followed. These answers, however, did not explain the mechanisms involved in many of the responses observed, nor did they always support the conclusions made in previous studies of a similar nature. Both Anderson *et al.* (1985) and McKellar *et al.* (1987) considered there to be an earlier and discordant rise in serum gastrin when compared with the pH of the abomasal contents and thus suggested that gastrin release may be stimulated directly by a parasite-derived factor. The present study, however, suggests that the rise in gastrin cannot be separated from the elevation in abomasal pH after larval or adult infection (at least initially) and thus, following the argument of Anderson *et al.* and McKellar *et al.*, concludes that no such substance need exist. This conclusion was further reinforced by the failure of extensive efforts to produce ES solutions that stimulated gastrin secretion *in vitro*.

DOES SERUM GASTRIN INCREASE INDEPENDENTLY OF ABOMASAL pH?

Although the present investigation concluded that serum gastrin concentration does not increase before the pH of the abomasal contents, it was apparent that later in the infection, abomasal pH returned to the upper limit of (or within) the normal range before either serum pepsinogen or serum gastrin and that during the subsequent trickle infection of some parasitised sheep, while hypergastrinaemia was maintained, the pH did not re-elevate. These observations suggest that factors other than pH may contribute to gastrin release later in infection.

Possible pH independent stimulants to gastrin secretion include activities associated with the parasite or components of the host response to infection. Parasite activities may be either physical or chemical and may affect the D or G cell populations (directly or indirectly), disrupt the mucus layer or stimulate the release of pharmacodynamic substances. Reaction by the host to the physical assault of infection, to parasite-derived antigens and disruption of normal function through the release of pro-inflammatory mediators, changes in blood flow, vascular permeability, cellular infiltration and mucosal repair may all have as yet poorly defined effects on local endocrine cell populations. To date, the relative importance of the parasite *per se* or of the host's reaction to it in the pathophysiological changes associated with ostertagiasis remains unknown.

The prolonged elevation of serum gastrin, relative to abomasal pH, following infection of sheep with larvae (Chapter 2) may be a residual effect from the previously more severe and possibly pH-dependent hypergastrinaemia that occurs earlier in infection. A new level of basal secretion may be established during this time which persists beyond the period of raised pH. Thus, pH independent stimulants to gastrin secretion need not necessarily exist. A new basal secretion rate may also accompany temporary desensitisation to gastrin by the parietal or histamine containing cells following their hyperexposure to it. Anderson *et al.* (1976b) found that, although pH returned to normal within a couple of days of anthelmintic treatment of infected sheep, serum gastrin levels declined less readily. The prolonged elevation of serum gastrin, unlike abomasal pH, suggests that its continued elevation is more closely associated with processes which may persist beyond infection *per se*, such as inflammation or desensitisation.

WHY DOES THE ABOMASAL pH RETURN TO THE NORMAL RANGE THREE WEEKS AFTER A SINGLE DOSE OF LARVAE?

The apparent return of the function of the parietal cells soon after the loss of parasites (as reported by Anderson *et al.*) may be related to the removal of some inhibitory constraint or the rapid recruitment of a new and functional cell population. *Ostertagia* spp. may produce substances that specifically inhibit the parietal cell population or which affect them indirectly (e.g. stimulate the release of substances such as IL-1) and so inhibit their normal function. In order for such substances to continue to inhibit the parietal cell once the worm population has been removed, the effects of such substances would need to be irreversible and long lasting. Klesius (1993) has provided and reviewed evidence which suggests that *Ostertagia* spp. produce substances which are chemotactic to leucocytes and may modulate immunocyte behaviour within the host. The potential of these and/or similar substances to affect the parietal, G, D or chief cell, cannot be discounted. Such substances may also explain the apparent immunological unresponsiveness of lambs to trichostrongylid nematodes.

New parietal cells may be recruited to replace the population affected by infection. Normally parietal cell turnover is slow (Lipkin, 1973; Willems, 1991; Karam, 1993), although this does not preclude rapid cell replacement under abnormal conditions (see Karam & Forte, 1994). In addition to the return of normal abomasal pH following the termination of infection reported by Anderson *et al.* (1976b), the *in vivo* investigations presented here indicate that apparent parietal cell function (as reflected by abomasal pH) may return about three weeks after initial exposure despite the continued presence of the parasite. This may result from the recruitment of a new population of parietal cells during this time that is largely immune to the effects of the parasite. Alternatively, the resident cell population may adapt and become resistant, or the parasite population may stop those activities which affect the parietal cells. A further possibility is that the host tissue stops responding to the physical or chemical activities of the parasite or adapts to these by activating or enhancing its intrinsic defence mechanisms in a way that has effects on parietal cell function.

Adaptation to the parasite and its activities following infection of parasite-naive sheep with *Ostertagia* adults or larvae may be through changes in the composition and quantity of mucus produced by the abomasum e.g. primary infection with adult worms may disrupt the mucus layer as worms establish themselves within the sanctity of this buffer

zone. With such disruption, the protective function the mucus layer subserves may be compromised, thus exposing sensitive interglandular regions of the mucosa to luminal acid, leading to mucosal damage and the possible initiation of processes designed to defend the tissue from such insults by stimulating repair, inhibiting acid secretion and increasing mucus production, perhaps through the release of pro-inflammatory mediators or substances such as EGF and prostaglandins. Substances released locally into the interstitial fluid and mucus may permeate through the mucosa and exert inhibitory effects on the parietal cells. In so doing, the host response may switch off the effector (luminal acid) although not the cause of the initial assault (breach of mucus barrier). This immediate solution may be superseded after a couple of weeks by other processes which may be activated and which may address the cause of the initial insult by stimulating increased mucus production and changes in its composition. Such a scenario is consistent with the timing of the pH disturbances following larval infection and the transfer of adult worms reported in this study i.e. abomasal pH increased on Day 5 when a wave of L_4 emergence might be anticipated and further on Day 10-12 when emergence of immature adults in the larvae infected groups reported in Chpater 2.

It appears likely that the host responds and adapts to the activities of the parasite rather than that these activities change, as the pH disturbance appears to recover despite the continued presence of a considerable adult population in all larval infected sheep. In contrast, the direct transfer of adult worms to naive sheep resulted in an almost immediate pH disturbance. This suggestion (adaption of host tissue to the parasite) holds regardless of the nature of the host response e.g. desensitisation of the parietal cells to substances produced by the worms, increased mucus thickness or recruitment of new parietal cells. Whatever its nature, the adaptation or recovery of the tissue of which restores pH to within the normal range, does not appear to be permanent (at least after a single infection) as the re-infection of previously-exposed sheep in other studies has resulted in further increases in abomasal pH. These increases were, nevertheless, considerably less than after primary infection.

WHAT ROLE DO PARASITE-DERIVED EXCRETORY/SECRETORY PRODUCTS PLAY IN THE PATHOPHYSIOLOGICAL CHANGES?

The *in vitro* studies presented in this thesis concentrated on the G cell although it would be equally important to study the effects of parasite-derived products on other cell types and, in particular, the parietal cell. It has been suggested that *Ostertagia* spp. may

produce substances which stimulate the G cell directly (Titchen, 1982; McKellar *et al.*, 1987). Experiments directly addressing this possibility had not been reported while the effect of *Ostertagia* derived solutions on the secretion of pepsinogen by chief cells and the accumulation of aminopyrine by parietal cells had been investigated (McKellar *et al.*, 1990a,b).

The *in vitro* investigations which used the static incubation method failed to identify (with one exception) solutions prepared with adult *O. circumcincta* that stimulated gastrin secretion despite pharmacological evidence that antral tissue was responsive in this system (Chapter 4). In contrast, a number of solutions potently inhibited gastrin secretion from both tissue segments and dispersed cells. None of these contained antibiotics whereas they were those most likely to be contaminated by bacteria. The inhibitory activity was thus not attributed to *O. circumcincta* but to microorganisms. The contribution of factors other than *O. circumcincta* to the inhibitory activity was confirmed experimentally by inoculating solutions with abomasal contents but without including worms. This raised a further series of questions e.g. what microorganisms produce these substances and what is their nature? Abnormal bacterial proliferation within the abomasum during periods of elevated abomasal pH and the production of substances presumed to be present in the solutions (Chapter 6) may explain the otherwise anomalous decrease in gastrin secretion observed *in vivo* (Chapter 2) over the period when abomasal pH was most raised and when the presumed stimulation of gastrin secretion might be predicted to be maximal. The potency of the contaminated medium to affect the secretion of both gastrin and somatostatin raises questions about the influence that such substances may play in the pathogenesis of ostertagiasis.

Worm incubates that were prepared without antibiotics, and which were found to inhibit gastrin secretion *in vitro*, also stimulated pepsinogen secretion from segments of fundic mucosa. McKellar *et al.* (1990a) reported a small increase in pepsinogen secretion from dispersed bovine and ovine fundic glands in response to *Ostertagia* derived solutions prepared without antibiotics. It is possible, therefore, that the response reported by McKellar *et al.* is also of microbial origin and not related to worms that had been incubated in the solution. This may explain the failure of worm-derived extracts and of live worms to stimulate pepsinogen release in their work.

HOW IS GASTRIN SECRETION CONTROLLED?

Although parasite secretions did not stimulate gastrin secretion, increased serum gastrin levels are a consistent feature of primary infection of sheep with *O. circumcincta.* How is the hypergastrinaemia initiated? Before the effect of the parasitism on the G cell can be identified, the normal regulation of gastrin secretion in the sheep needs to be clarified. Previous studies on ruminants have suggested that the regulatory mechanisms that have been identified in other mammals may differ in the sheep (Barry *et al.*, 1985; McDonald *et al.*, 1988; Reynolds *et al.*, 1991).

The pharmacological studies presented in Chapter 4 which examined the effects of a number of neuropeptides on gastrin secretion *in vitro*, identified a potential role for cholinergic agents, GRP, VIP, and GIP in the regulation of the G cell in the sheep. The role of somatostatin as an important modulator of gastrin secretion, however, remains unclear as anomalous observations remained unexplained. GRP stimulated gastrin secretion while VIP and GIP stimulated somatostatin release. Some solutions (Chapter 5 and 6) potently stimulated somatostatin while inhibiting gastrin secretion. These effects appeared to be related but not dependent. Wherever a solution inhibited gastrin secretion, it also stimulated somatostatin secretion. However, not all solutions that stimulated somatostatin secretion inhibited gastrin secretion, although the effect on somatostatin in some instances was large. These results support the suggestion that the restraint somatostatin is believed to exert on the G cell is optimal, such that, while somatostatin may inhibit G cell stimulation it does not inhibit basal secretion. If this is so, the inhibition of gastrin secretion that was accompanied by increased somatostatin release, may be mediated by independent mechanisms. The possibility that the opposing effect of some solutions on the G and D cell may reflect mediation by different components within the medium released either by *Ostertagia* or microbes is supported by the loss of activity associated with increased storage time of a parasite incubate, which affected only its gastrin inhibitory and not its somatostatin stimulatory properties.

A similar apparent independence between the secretion of somatostatin and that of gastrin was observed in the pharmacological studies reported in Chapter 4. In these investigations, both gastrin and somatostatin secretion were stimulated by carbachol in the majority of experiments. While these results could be explained independently through cholinergic activation of enteric neurons, it was not possible to explain why gastrin secretion increased in the face of increased somatostatin release. It is possible

that the level of somatostatin release required to inhibit either direct or GRP-dependent gastrin secretion in the sheep was greater than the somatostatin response in many experiments. This, in turn, may indicate that the sensitivity of the ovine G cell to somatostatin, or the inhibitory potency of somatostatin *per se* in the sheep, differs from that which is believed to operate in monogastric mammals. It is also possible that this apparent independence or insensitivity is a peculiarity associated with the static incubation method used in these investigations. The use of somatostatin antiserum suggested that the amount of somatostatin released in four of five experiments was not restricting the gastrin response to GRP. In one experiment, however, in which the release of somatostatin was substantially increased (+700%), somatostatin antibodies were effective in increasing the response to GRP. In this experiment it also appeared that somatostatin had (in addition to the GRP-stimulated gastrin secretion) inhibited basal gastrin secretion to some extent. The component of the basal gastrin secretion that was inhibited may have been that associated with the tonic cholinergic activity that is believed to be associated with most experiments using this preparation.

The mere presence of a pathway and the demonstration of its activation *in vitro* do not confirm its physiological importance to the animal. Indeed, the relative contribution of the above mechanisms to the coordinated release of gastrin in the sheep may differ substantially from monogastric species such as the dog, pig, human and rat. In the pig, for example, a GRP-stimulated increase in gastrin secretion comparable to that elicited by 10^{-6} M GRP in the present study was observed in response to a 10^{-11} M solution (Holst *et al.*, 1987b). Nervous control of gastrin secretion may be partially superseded by chemical control in ruminants, in which the abomasum receives digesta of relatively constant composition continuously from the omasum. Such relative constancy may obviate much of the need for the stomach to respond quickly through neural mechanisms to feeding episodes as these have little direct effect on conditions within the abomasum. Feeding rather alters the conditions within the rumen, and so the necessity for responsive secretory processes may have been shifted to this organ. In part, these needs are met by substantial fluctuations in the flow and composition of saliva. The abomasum, on the other hand, must secrete acid, mucus and pepsin continuously and a secretory response by the abomasum to cephalic stimuli in freely grazing animals may be inappropriate. The composition and rate at which digesta is delivered to the abomasum may be the only factors which retain functional importance in the regulation of gastric secretion which suggests that chemical and volume changes (pH and distension) are the variables that are of greatest importance.

EPILOGUE

The overall objective of the present study was to investigate how *O. circumcincta* communicates with the tissues of its ovine host to produce the pathophysiological changes that accompany infection and particularly to assess the role of parasite-derived ES products in effecting these changes in abomasal function. In order to do this, it was and continues to be necessary to identify the normal regulatory mechanisms for gastrin, pepsinogen and gastric acid secretion in the sheep. A method was established that provides a useful method for pharmacological work. Using the static incubation technique the regulation of gastrin secretion in sheep was studied. These investigations are continuing and have now expanded to include a systematic investigation of the ovine chief cell. Efforts are now also being made to ascertain what substances are produced by bacteria which may potently affect both gastrin and somatostatin secretion.

While little of the pathogenesis of the pathophysiological changes was resolved, knowledge was obtained that may assist and guide further investigations. It was found that adult *O. circumcincta* rapidly produce changes in serum pepsinogen, serum gastrin and abomasal pH, although it was not possible to separate the timing of the increase in gastrin from that of pH. ES products were not produced from adult worms which stimulated gastrin secretion. It is possible that parasite-derived chemicals are not involved in the physiological effects of infection on the host, although they are known to act as antigens. What then is responsible for these changes? Should greater emphasis be placed on the host response and less on the parasite? Should greater emphasis be placed on the parietal rather than the G cell? Investigations into the pathophysiological changes associated with infection of sheep with *Ostertagia* and other nematodes are continuing and particular attention is now being directed toward the parietal cell.

Chapter 8

REFERENCES

Allen A (1989) Gastrointestinal mucus. *Handbook of Physiology, section 6: The Gastrointestinal System* **3**:359-381. Edited by S G Schultz, American Physiological Society, Washington D C.

Anderson N, Armour J, Jarrett W F H, Jennings F W, Ritchie J S D & Urquhart G M (1965) A field study of parasitic gastritis in cattle. *The Veterinary Record* **77**:196-204.

Anderson N (1972) Trichostrongylid infections of sheep in a winter rainfall region. I. Epizootiological studies in the western district of Victoria, 1966-1967. *Australian Journal of Agricultural Research* **23**:1113-1129.

Anderson N (1973) Trichostrongylid infections of sheep in a winter rainfall region. II. Epizootiological studies in the western district of Victoria, 1967-68. *Australian Journal of Agricultural Research* **24**:599-611.

Anderson N, Hansky J & Titchen D A (1976a) Hypergastrinaemia during a parasitic gastritis in sheep. *Journal of Physiology* **256**:51-52.

Anderson N, Blake R & Titchen D A (1976b) Effects of a series of infections of *Ostertagia circumcincta* on gastric secretion of the sheep. *Parasitology* **72**:1-12.

Anderson N, Hansky J & Titchen D A (1981) Effects of *Ostertagia circumcincta* infections on plasma gastrin in sheep. *Parasitology* **82**:401-410.

Anderson N, Hansky J & Titchen D A (1985) Effects on plasma pepsinogen, gastrin and pancreatic polypeptide of *Ostertagia* spp. transferred directly into the abomasum of sheep. *International Journal for Parasitology* **2**:159-165.

Anderson N, Reynolds G W & Titchen D A (1988) Changes in gastrointestinal mucosal mass and mucosal and serum gastrin in sheep experimentally infected with *Ostertagia circumcincta*. *International Journal for Parasitology* **18**:325-331.

Armour J, Jarrett W F H, Jennings F W (1966) Experimental *Ostertagia circumcincta* infections in sheep: development and pathogenesis of a single infection. *American Journal of Veterinary Research* **27**:1267-1278.

Armour J, Jennings F W & Urquhart G M (1967a) The possible existence of two strains of *Ostertagia ostertagi*. *The Veterinary Record* **80**:208-209.

Armour J, Jennings F W & Urquhart G M (1967b) The possible existence of two strains of *Ostertagia ostertagi*. *The Veterinary Record* **80**:605-606

Armour J (1970) Bovine ostertagiasis: A review. *The Veterinary Record* **86**:184-190.

Armour J, Bairden K, Duncan J L, Jennings F W & Parkins J J (1979) Observations on ostertagiasis in young cattle over two grazing seasons with special reference to plasma pepsinogen levels. *The Veterinary Record* **105**:500-503.

Armour J & Ogbourne C P (1982) Bovine ostertagiasis: a review and annotated bibliography. *Commonwealth Agricultural Bureaux, England* pp1-93.

Ash R W (1961a) Acid secretion by the abomasum and its relation to the flow of food material in the sheep. *Journal of Physiology* **156**:93-111.

Ash R W (1961b) Stimuli influencing the secretion of acid by the abomasum of sheep. *Journal of Physiology* **157**:185-207.

Attaix D, Meslin J C & Combe E (1984) Intestinal epithelium renewal time in the young lamb. *Nutrition Reports International* **29**:689-697.

Axelson J, Häkanson R, Rosengren E & Sundler F (1988) Hypergastrinaemia induced by acid blockade evokes enterochromaffin-like (ECL) cell hyperplasia. *Cell Tissue Research* **254**:511-516.

Bado A, Cloarec D, Moizo L, Laigneau J-P, Bataille D & Lewin M J M (1993) Neurotensin and oxyntomodulin-(30-37) potentiate PYY regulation of gastric acid and somatostatin secretions. *American Journal of Physiology* **265**:G133-G147.

Baird A W, Main I H M & Pearce J B (1980) Selectivity of action of prostaglandins on acid secretion induced by histamine, gastrin, and nerve stimulation, *in vitro*. *Advances in Prostaglandin and Thromboxane Research* **8**:1543-1545.

Baker S, Gerring E L & Fox M T (1993) Twenty-four hour gastric pH monitoring and blood gastrin concentrations in fasted ponies. *Research in Veterinary Science* **55**:261-264.

Barger I A (1986) Population regulation in trichostrongylids of ruminants. *Parasitology - Pro Vadit. Proceedings of the sixth International Congress of Parasitology* pp531-540. Edited by M T Houdt Australian Academy of Science, Canberra.

Barry T N, Faichney G J & Redekopp C (1985) Gastro-intestinal tract function in sheep infused with somatostatin. *Australian Journal of Biological Science* **38**:393-403.

Becker H D, Reeder D D & Thompson J C (1973) The effect of changes in antral pH on the basal release of gastrin. *Proceeding of the Society of Experimental Biology and Medicine* **143**:238-240.

Befrits R, Uvnäs-Moberg K & Johansson C (1990) Interactions between antral peptides and prostaglandin biosynthesis in gastric acid regulation in man. *Digestion* **45**:9-18.

Bell F R, Titchen D A & Watson D J (1975) The effects of pentagastrin on gastric (abomasal) motility in the unweaned calf. *Journal of Physiology* **251**:165-170.

Bell F R, Titchen D A & Watson D J (1977) The effects of the gastrin analogue, pentagastrin, on the gastric electromyogram and abomasal emptying in the calf. *Research in Veterinary Science* **23**:165-170.

Berghen P (1987) Evaluation of a simplified blood pepsinogen assay. *American Journal of Veterinary Research* **48**:665-669.

Berglindh T, Helander H F & Öbrink K J (1976) Effects of secretagogues on oxygen consumption, aminopyrine accumulation and morphology in isolated gastric glands. *Acta Physiologica Scandanavia* **97**:401-414.

Bertaccini G, Erspamer V, Melchiorri P & Soprenzi N (1974) Gastrin release by bombesin in the dog. *British Journal of Pharmacology* **52**:219-225.

Bladin P H D, Shulkes A, Fletcher D R & Hardy K J (1983) Elevation of plasma gastrin and pancreatic polypeptide by electrical vagal stimulation in sheep: effects of sequential stimulation. *Regulatory Peptides* **6**:89-97.

Blair E L, Greenwell J R, Grund E R, Reed J D & Sanders D J (1975) Gastrin response to meals of different composition in normal subjects. *Gut* **16**:766-773.

Blair, A J, Richardson C T, Walsh J H & Feldman M (1987) Variable contribution of gastrin to gastric acid secretion after a meal in humans. *Gastroenterology* **92**:944-949.

Blanchard J L & Wescott R B (1985) Enhancement of resistance of lambs to *Haemonchus contortus* by previous infection with *Ostertagia circumcincta*. *American Journal of Veterinary Research* **46**:2136-2140.

Bloom S R, Edwards A V & Mitchell S J (1978) Release of vasoactive intestinal peptide from the gastrointestinal tract in response to autonomic stimulation in the calf. *Journal of Physiology* **289**:47.

Bloom S R, Edwards A V & Ghatei M A (1983) Endocrine responses to exogenous bombesin and gastrin releasing peptide in conscious calves. *Journal of Physiology* **344**:37-48.

Bloom S R & Edwards A V (1982) Neuroendocrine responses to stimulation of the peripheral ends of the splanchnic nerves in bursts in the conscious adrenalectomised calf. *Journal of Physiology* **332**:84-85.

Bochna G S, Ring M C, Lassiter D C & Harty R F (1987) Effects of gastrin and bombesin on acetylcholine release from rat antral mucosal/submucosal neurons. *Gastroenterology* **92**:1320.

Bolman R M III, Cooper C W & Wells S A Jr (1978) Somatostatin inhibition and reversal of parathyroid hormone-, calcium-, and acetylcholine-induced gastrin release in the pig. *Endocrinology* **103**:259-266.

Brandsborg O, Brandsborg M & Christensen N J (1975) Plasma adrenaline and serum

gastrin: studies in insulin-induced hypoglycaemia and after adrenaline infusions. *Gastroenterology* **68**:455-460.

Brooks A M & Grossman M I (1970) Effect of secretin and cholecystokinin on pentagastrin-stimulated gastric secretion in man. *Gastroenterology* **59**:114-119.

Brown J C, Buchan A M J, McIntosh C H S & Pederson R A (1989) Gastric inhibitory polypeptide. *Handbook of Physiology, section 6: The Gastrointestinal System* pp403-425. Edited by S G Schultz. American Physiological Society, Washington D C.

Bryant C (1993) Organic acid excretion by helminths. *Parasitology Today* **9**:58-59.

Buchan A M J, Curtis S B & Meloche R M (1990) Release of somatostatin immunoreactivity from human antral D cells in culture. *Gastroenterology* **99**:690-696.

Buchan A M J (1991) Effect of sympathomimetics on gastrin secretion from antral G cells in culture. *Journal of Clinical Investigation* **87**:1382-1386.

Bunnett N W & Harrison F A (1979) Endocrine cells in the alimentary tract of the sheep. *Annales de Recherches Veterinaires* **10**:197-199.

Butterworth A E (1984) Cell-mediated damage to helminths. *Advances in Parasitology* **23**:143-211.

Cabero J L, Li Z-Q & Märdh S (1991) Gastrin potentiates histamine-stimulated aminopyrine accumulation in isolated rat parietal cells. *American Journal of Physiology* **261**:G621-G627.

Cabero J L, Zi Z-Q & Märdh S (1993) Gastrin action on aminopyrine accumulation in isolated pig parietal cells requires cAMP. *Biochimia et Biophysica Acta* **1177**:245-252.

Calingasan N Y, Kitamura N, Yamada J, Osmori Y & Yamashita T (1984) Immunocytochemical study of the gastroenteropancreatic endocrine cells of the sheep. *Acta anatomica* **118**:171-180.

Campos R V, Buchan A M J, Meloche R M, Pederson R A, Kwok Y N & Coy D H (1990) Gastrin secretion from human antral G cells in culture. *Gastroenterology* **99**:36-44.

Carr D H, McLeay L M & Titchen D A (1970) Factors affecting reflex responses of the ruminant stomach. *Physiology of Digestion and Metabolism in the Ruminant* pp35-41. Editor-in-chief A T Phillipson. Oriel Press.

Carr D M & McGuigan J E (1973) The direct measurement of stimulation of antral gastrin release by calcium and by cholinergic agents. *Gastroenterology* **64**:A168/855

Casteleyn PP, Dubrasquet M & Willems G (1977) Opposite effects of gastrin on cell proliferation in the antrum and other parts of the upper-gastrointestinal tract in the rat, *Digestive Diseases* **22**:798-805.

Castro G A, Copeland E M, Dudrick S J & Johnson L R (1976) Serum and antral gastrin levels in rats infected with intestinal parasites. *American Journal of Tropical Medicine and Hygiene* **25**:848-853.

Charleston W A G (1965) Pathogenesis of experimental haemonchosis in sheep, with special reference to the development of resistance. *Journal of Comparative Pathology* **75**:55-67.

Chew C S & Hersey (1982) Gastrin stimulation of isolated gastric glands. *American Journal of Physiology* **242**:G504-G512.

Chew C S & Brown M R (1986) Release of intracellular Ca^{2+} and elevation of inositol triphosphate by secretagogues in parietal and chief cells isolated from rabbit gastric mucosa. *Biochimia et Biophysica Acta* **888**:116-125.

Chew C S (1989) Intracellular activation events for parietal cell hydrochloric acid secretion. *Handbook of Physiology, section 6: The Gastrointestinal System* **3**:255-266. Edited by S G Schultz. American Physiological Society, Washington D C.

Chew C S, Ljungstrom M, Smolka A & Brown M R (1989) Primary culture of secretagogue-responsive parietal cells from rabbit gastric mucosa. *American Journal of Physiology* **256**:G254-G263.

Chew C S (1991) Intracellular mechanisms in control of acid secretion. *Current Opinion in Gastroenterology* **7**:856-862.

Chew C S (1994) Parietal cell culture: new models and directions. *Annual Review of Physiology* **56**:445-461.

Chey W Y, Kim M S, Lee K Y and Chang T M (1981) Secretin is an enterogastrone in the dog. *American Journal of Physiology* **240**:G239-G244.

Chiba T, Taminato T, Kadowaki S, Abe H, Chihara K, Seino Y, Matsukura S & Fujita T (1980) Effects of glucagon, secretin, and vasoactive intestinal polypeptide on gastric somatostatin and gastrin release from isolated perfused rat stomach. *Gastroenterology* **79**:67-71.

Chiba T, Kadowaki S, Taminato T, Chihara K, Seino Y, Matsukura S & Fujita T (1981) Effect of antisomatostatin γ-globulin on gastrin release in rats. *Gastroenterology* **81**:321-326.

Christie M G (1970) The fate of very large doses of *Haemonchus contortus* in the ovine abomasum. *The Journal of Comparative Pathology* **80**:89-100.

Chuang C-N, Tanner M, Chen M C Y, Davidson S & Soll A H (1992) Gastrin induction of histamine release from primary cultures of canine oxyntic mucosal cells. *American Journal of Physiology* **263**:G460-G465.

Chuang C-N, Tanner M, Llyod K C K, Wong H & Soll A H (1993) Endogenous

somatostatin inhibits histamine release from canine gastric mucosal cells in primary culture. *American Journal of Physiology* **265**:G521-G525.

Clark R A F, Gallin J I & Kaplan A P (1975) The selective eosinophil chemotactic activity of histamine. *Journal of experimental Medicine* **142**:1462.

Cobon G S & Woodrow G (1991) Current control measures for parasites of livestock. *Australian Biotechnology* **1**:170-172.

Colturi T J, Unger R H & Feldman M (1984) Role of circulating somatostatin in regulation of gastric acid secretion, gastrin release, and islet cell function. Studies in healthy subjects and duodenal ulcer patients. *Journal of Clinical Investigation* **74**:417-423.

Conn P M & Gebhart G F (1989) *Essentials of Pharmacology.* Davis, Philadelphia.

Connan R M (1991) Type II ostertagiasis in farmed red deer. *The Veterinary Record* **128**:233-235.

Conner J G, Eckersall P D, Wiseman A, Bain R K & Douglas T A (1989) Acute phase response in calves following infection with *Pasteurella haemolytica, Ostertagia ostertagi* and endotoxin administration. *Research in Veterinary Science* **47**:203-207.

Coop R L, Sykes A R & Angus K W (1977) The effect of a daily intake of *Ostertagia circumcincta* larvae on body weight, food intake and concentration of serum constituents in sheep. *Research in Veterinary Science* **23**:76-83.

Costa M & Furness J B (1989) Structure and neurochemical organization of the enteric nervous system. *Handbook of Physiology, section 6: The Gastrointestinal System* **2**:97-109. Edited by S G Schultz. American Physiological Society, Washington D C.

Crean G P, Marshall M W & Rumsey R D E (1969) Parietal cell hyperplasia induced by the administration of pentagastrin (ICI 50,123) to rats. *Gastroenterology* **57**:147-155.

Cryer B, Redfern J S, Goldschmiedt M, Lee E & Feldman M (1992) Effect of aging on gastric and duodenal mucosal prostaglandin concentrations in humans. *Gastroenterology* **102**:1118-1123.

Dakkak A, Fioramonti J & Bueno L (1981) *Haemonchus contortus* third stage larvae in sheep: kinetics of arrival into the abomasum and transformation during rumino-omasal transit. *Research in Veterinary Science* **32**:384-385.

Dakkak A, Fioramonti J & Bueno L (1982) *Haemonchus contortus*: Abomasal transmural potential difference and permeability changes associated with infection in sheep. *Experimental Parasitology* **53**:209-216.

Davidson J B, Murray M & Sutherland I H (1968) *Hyostrongylus rubidus*: a field study of its pathogenesis, diagnosis and treatment. *The Veterinary Record* **83**:582-588.

de Jong A J L, Klamer M, Jansen J B M J & Lamers C B H W (1987) Effect of atropine and somatostatin on bombesin-stimulated plasma gastrin, cholecystokinin and pancreatic polypeptide in man. *Regulatory Peptides* **17**:285-293.

DelValle J & Yamada T (1990) Amino acids and amines stimulate gastrin release from canine antral G-cells via different pathways. *The Journal of Clinical Investigation* **85**:139-143.

DelValle J, Wang L, Grantz I, Schaffer M & Yamada T (1991) Expression and characterization of the cloned canine H_2 receptor: evidence for its linkage to both the cAMP and Ca^{2+} mediated signalling systems. *Gastroenterology* **100**:A636.

DelValle J, Tsunoda Y, Williams J A & Yamada T (1992) Regulation of $[Ca^{2+}]i$ by secretagogue stimulation of canine gastric parietal cells. *American Journal of Physiology* **262**:G420-G426.

DelValle J, Chiba T, Park J & Yamada T (1993) Distinct receptors for cholecystokinin and gastrin on canine fundic D-cells. *American Journal of Physiology* **264**:G811-G815.

de Jong A J L, Klamer M, Jansen J B M J & Lamers C B H W (1987) Effect of atropine and somatostatin on bombesin-stimulated plasma gastrin, cholecystokinin and pancreatic polypeptide in man. *Regulatory Peptides* **17**:285-293.

Denham D A (1969) The development of *Ostertagia circumcincta* in lambs. *Journal of Helminthology* **18**:299-310.

Dial E J, Thompson W J & Rosenfeld G C (1981) Isolated parietal cells: histamine response and pharmacology. *Journal of Pharmacology and Experimental Therapeutics* **219**:585-590.

Dineen J K & Kelly J D (1976) Levels of prostaglandins in the small intestine of rats during primary and secondary infection with *Nippostrongylus brasiliensis*. *International Archives of Allergy Applied Immunology* **51**:429-440.

Dockray G J, Vaillant C & Walsh J H (1979) The neuronal origin of bombesin-like immunoreactivity in the rat gastrointestinal tract. *Neuroscience* **4**:1561-1568.

Domeneghini & Castaldo (1981) The endocrine cells of the bovine cardiac glands. *Basic and Applied Histochemistry* **25**:51-66.

Dopheide T A A, Tachedjian M, Phillips C, Frenkel M J, Wagland B M & Ward C W (1991) Molecular characterization of a protective, 11-kDa excretory-secretory protein from the parasitic stages of *Trichostrongylus colubriformis*. *Molecular and Biochemical Parasitology* **45**:101-108.

Douch P G C, Buchanan L L & Greer K S (1984) Host/parasite interrelationships of trichostrongylid infections in young sheep: effect of induced resistance to *T. colubriformis* on worm burdens acquired in the field. *In: Wallaceville animal Research Centre Annual Report* p195. Ministry of Agriculture and Fisheries, Upper Hutt, New

Zealand.

Duncan J L, Smith W D & Dargie J D (1978) Possible relationship of levels of mucosal IgA and serum IgG to immune unresponsiveness of lambs to *Haemonchus contortus*. *Veterinary Parasitology* **4**:21-27.

Dunsmore J D (1960) Retarded development of Ostertagia species in sheep. *Nature* **186**:986-987.

Dunsmore J D (1961) Effect of whole-body irradiation and cortisone on the development of *Ostertagia* spp. in sheep. *Nature* **192**:139-140.

Dunsmore J D (1963) Effect of the removal of an adult population of *Ostertagia* from sheep on concurrently existing arrested larvae. *Australian Veterinary Journal* **39**:459-463.

Durham P J K & Elliot D C (1975) Experimental *Ostertagia* infection of sheep: pathology following a single high dose of larvae. *New Zealand Veterinary Journal* **23**:193-196.

Durham P J K & Elliott D C (1976) Experimental *Ostertagia* spp. infection of sheep: development of worm populations and lesions resulting from different dose-levels of larvae. *Veterinary Parasitology* **2**:157-166.

DuVal J W, Saffouri B, Wier G C, Walsh J H, Arimura A & Makhlouf G M (1981) Stimulation of gastrin and somatostatin secretion from the isolated rat stomach by bombesin. *American Journal of Physiology* **241**:G242-G247.

Eiler H, Baber W, Lyke W A & Scholtens R (1981) Inhibition of gastric hydrochloric acid secretions in the rat given *Ostertagia ostertagi* (a gastric parasite of cattle) extract. *American Journal of Veterinary Research* **42**:498-502.

Eissele R, Roßkopf B, Koop H, Adler G & Arnold R (1991) Proliferation of endocrine cells in the rat stomach caused by drug-induced achlorhydria. *Gastroenterology* **101**:70-76.

Elliott D C (1974) Experimental *Ostertagia* infection of sheep: worm populations resulting from single larval doses. *New Zealand Journal of Experimental Agriculture* **2**:103-107.

Elliott D C & Durham P J K (1976) Experimental *Ostertagia* spp. infection of sheep: population lesions in previously worm-free and previously exposed animals. *Veterinary Parasitology* **2**:167-175.

Engelhardt W V, Erhlein H J & Hornicke H (1968) Absorption of volatile fatty acids, tritiated water and antipyrine from the abomasum of goats. *Quarterly Journal of experimental Physiology* **53**:282-295.

Enigk K & Dey-Hazra A (1978) Influence of a *Strongyloides* infection on the formation

of gastrin and other hormones in the pig. (English summary). *Zentralblatt Veterinärmedizin* **B25**:697-706.

Esplugues U V, Barrachina M D, Esplugues J (1992) Modulation by peripheral opioids of basal and distension-stimulated gastric acid secretion in the rat. *British Journal of Pharmacology* **106**:33-38.

Eysselein V E, Kovacs T O G, Kleibeuker J H, Maxwell V, Reedy T & Walsh J H (1992) Regulation of gastric acid secretion by gastrin in duodenal ulcer patients and healthy subjects. *Gastroenterology* **102**:1142-11148.

Fahrenkrug J, Haglund U, Jodal M, Lundgren O, Olbe L & Schaffalitzky De Muckadell O B (1978) Nervous release of vasoactive polypeptide in the gastrointestinal tract of cats: possible physiological implications. *Journal of Physiology* **284**:284-305.

Fahrenkrug J (1989) Vasoactive intestinal polypeptide. *Handbook of Physiology, section 6: The Gastrointestinal System* **2**:611-630. Edited by S G Shultz. American Physiological Society, Washington D C.

Feldman M, Richardson C T, Taylor I L & Walsh J H (1979) Effect of atropine on vagal release of gastrin and pancreatic polypeptide. *The Journal of Clinical Investigation* **63**:294-298.

Feldman M & Richardson C T (1986) Role of thought, sight, smell and taste of food on the cephalic phase of gastric acid secretion and gastrin release in humans. *American Journal of Clinical Nutrition* **51**:794-797.

Felley C P, O'Dorisio T M. Howe B, Coy D H, Mantey S A, Pradhan T K, Sutliff V E & Jensen R T (1994) Chief cells possess somatostatin receptors regulated by secretagogues acting through the calcium or cAMP pathway. *American Journal of Physiology* **266**:G789-G798.

Ferguson A & Jarrett E E E (1975) Hypersensitivity reaction in the small intestine. *Gut* **16**:114-117.

Finkelmann F D, Madden K B, Cheever A W, Katona I M, Morris S C, Gately M K, Hubbard B R, Gause W C & Urban J F (1994) Effects of interleukin 12 on immune responses and host protection in mice infected with intestinal nematode parasites. *Journal of Experimental Medicine* **179**:1563-1572.

Fiocchi C, Binion D G & Katz J A (1994) Cytokine production in the human gastrointestinal tract during inflammation. *Current Opinion in Gastroenterology* **10**:639-644.

Fiorucci S & McArthur (1990) Gastrin releasing peptide directly releases pepsinogen from guinea pig chief cells. *American Journal of Physiology* **259**:G760-G766.

Fiorucci S & Morelli A (1993) Motilin and erythromycin stimulate pepsinogen secretion by chief cells isolated from guinea pig stomach. *Gastroenterology* **104**:1030-1036.

Flemström G & Garner A (1989) Secretion of bicarbonate by gastric and duodenal mucosa. *Handbook of Physiology, section 6: The Gastrointestinal System* **2**:309-326. Edited by S G Shultz. American Physiological Society, Washington D C.

Fournet J, Hostein J, Fouque B, Serre F, Gavend M & Bonnet-Eymard J (1977) Action du propranolol sur la sécrétion gastrique stimulée par L'insuline. *Gastroenterology and Clinical Biology* **1**:995-1006.

Forte J G & Soll A (1989) Cell biology of hydrochloric acid secretion. *Handbook of Physiology, section 6: The Gastrointestinal System* 3:207-228. Edited by S G Schultz. American Physiological Society, Washington D C.

Fox M T, Gerrelli D, Pitt S R, Jacobs D E, Hart I C & Simmonds A D (1987) Endocrine effects of a single infection with *Ostertagia ostertagi* in the calf. *International Journal for Parasitology* **17**:1181-1185.

Fox M T, Pitt S R, Gerrelli D, Jacobs D E, Adhikari D R & Goddard P J (1988a) Use of blood gastrin assay in the diagnosis of ovine haemonchiasis. *The Veterinary Record* **122**:136-137.

Fox M T, Pitt S R, Gerrelli D & Jacobs D E (1988b) Effect of diet on gastrin response to *Ostertagia ostertagi* infection in the calf. *International Journal for Parasitology* **18**:547-548.

Fox M T, Gerrelli D, Shivalkar P & Jacobs D E (1989a) Effect of omeprazole treatment on feed intake and blood gastrin and pepsinogen levels in the calf. *Research in Veterinary Science* **46**:280-282.

Fox M T, Gerrelli D, Pitt S R, Jacobs D E, Gill M & Gale D L (1989b) *Ostertagia ostertagi* infection in the calf: effects of a trickle challenge on appetite, digestibility, rate of passage of digesta and liveweight gain. *Research in Veterinary Science* **47**:294-298.

Fox M T (1993) Pathophysiology of infection with *Ostertagia ostertagi* in cattle. *Veterinary Parasitology* **46**:143-158.

Fox M T, Carroll A P, Hughes S A, Uche U E, Jacobs D E & Vaillant C (1993) Gastrin and gastrin-related responses to infection with *Ostertagia ostertagi* in the calf. *Research in Veterinary Science* **54**:384-391.

Frenkel M J, Dopheide T A A, Wagland B M & Ward C W (1992) The isolation, characterization and cloning of a globin-like, host-protective antigen from the excretory-secretory products of *Trichostrongylus colubriformis*. *Molecular and Biochemical Parasitology* **50**:27-36.

Gabella G (1987) The number of neurons in the small intestine of mice, guinea-pigs and sheep. *Neuroscience* **22**:737-752.

Gable T A, O'Dorisio T M, Ellison E C, Fabri P J, Hanson K M & Gower W R (1989) The identification of somatostatin receptors on isolated human antral G cells.

Gastroenterology **96**:A682.

Galli S J, Wershil B K, Bose R, Walker P A & Szabo S (1988) Ethanol-induced acute gastric injury in mast cell-deficient mice and congenic normal mice. Evidence that mast cells can augment the area of damage. *American Journal of Pathology* **128**:131-140.

Gerber J G, Fadul S, Payne N A & Nies A S (1984) Adenosine: a modulator of gastric acid secretion in vivo. *Journal of Pharmacology and Experimental Therapeutics* **231**:109-113.

Gerber J G & Payne N A (1988) Endogenous adenosine modulates gastric acid secretion to histamine in canine parietal cells. *Journal of Pharmacology and Experimental Therapeutics* **235**:190-194.

Gerber J G & Payne N A (1992) The role of gastric secretagogues in regulating gastric histamine release in vivo. *Gastroenterology* **102**:403-408.

Getty R (1975) *Sisson and Grossmans's The anatomy of the domestic animals* W B Saunders Company, Philadelphia, USA.

Gill H S, Husband A J, Watson D L & Gray G D (1994) Antibody-containing cells in the abomasal mucosa of sheep with genetic resistance to *Haemonchus contortus*. *Research in Veterinary Science* **56**:41-47.

Giraud A S, Soll A H, Cuttitta F & Walsh J H (1987) Bombesin stimulation of gastrin release from canine gastrin cells in primary culture. *American Journal of Physiology* **252**:G413-G420.

Glaucert A M, Butterworth A E, Sturrock R F & Houba (1978) The mechanism of antibody-dependent, eosinophil-mediated damage to schistosomula of *Schistosoma mansoni in vitro:* a study by phase-contrast and electron microscopy. *Journal of Cell Science* **34**:173-192.

Goldenring J, Tsunoda Y, Stoch S, Coffey R & Modlin I (1993) Transforming growth factor-alpha (TGF-α) inhibition of parietal cell secretion: structural requirements for activity. *Regulatory Peptides* **43**:36-47.

Goodlad R A, Lee C Y, Levin S & Wright N A (1991) Effects of the prostaglandin analogue misoprostol on cell proliferation in the canine small intestine. *Experimental Physiology* **76**:561-566.

Goossens J, van Reempts J & van Wauwe J P (1987) Cytoprotective effects of sodium cromoglycate on rat stomach mucosa. *British Journal of Pharmacology* **91**:165-169.

Greenberg G R, Pokol-Daniel S & Fung L (1992) Influence of gastric acid on circulating somatostatin-14 and -28 released after insulin-induced hypoglycaemia in conscious dogs. *Endocrinology* **131**:1527-1533.

Gregory R A (1962) *Secretory Mechanisms of the Gastro-Intestinal Tract.* Edward

Arnold, London.

Griffiths G & Pritchard D I (1994) Purification and biochemical characterization of acetylcholinesterase (AChE) from the excretory/secretory products of *Trichostrongylus colubriformis*. *Parasitology* **108**:579-586.

Grossman M I & Konturek S J (1974) Inhibition of acid secretion in the dog by metinamide, a histamine antagonist acting on H_2 receptors. *Gastroenterology* **68**:517-521.

Guo Y-S, Thompson J C & Singh P (1988) Role of Ca^{2+} in bombesin-stimulated release of gastrin and somatostatin from isolated perfused rat stomach. *American Journal of Physiology* **255**:G627-G632.

Guo Y S, Singh P & Thompson J C (1990) Role of gastrin in bombesin-stimulated somatostatin release. *Gastroenterology* **99**:1297-1302.

Gurnsey M P (1985) *Endocrine cells in the gastrointestinal tract of sheep*, PhD thesis, Massey University, Palmerston North, New Zealand.

Guzman S, Lonovics J, Chayvialle J A, Hejtmancik K E, Rayford P L & Thompson J C (1980) Effects of gastrin on circulating levels of somatostatin, pancreatic polypeptide, and vasoactive intestinal peptide in dogs. *Endocrinology* **107**:231-236.

Habel R E (1956) A study of the innervation of the ruminant stomach. *Cornell Veterinarian* **46**:555-633.

Häkanson R, Bottcher G, Ekbald E, Panula P, Simonsson, Dohlsten M, Hallberg T & Sundler F (1986) Histamine in endocrine cells in the stomach. *Histochemistry* **86**:5-17.

Hansky J & Cain M D (1969) Radioimmunoassay of gastrin in human serum. *Lancet* **2**:1388-1390.

Harrison F A & Hill K J (1962) Digestive secretions and the flow of digesta along the duodenum of the sheep. *Journal of Physiology* **162**:225-243.

Harty R F & McGuigan (1980) Effects of carbachol and atropine on gastrin secretion and synthesis in rat antral organ culture. *Gastroenterology* **78**:925-930.

Harty R F & Franklin P A (1986) Cholinergic mediation of τ-aminobutyric acid-induced gastrin and somatostatin release from rat antrum. *Gastroenterology* **91**:1221-1226.

Harty R F, Maico D G & McGuigan J E (1988) Comparison of adrenergic and cholinergic receptor-mediated stimulation of gastrin release from rat antral fragments. *Peptides* **9**:463-468.

Harty R F & Ren J (1992) Gastrointestinal hormones, growth factors, and neurotransmitters. *Current Opinion in Gastroenterology* **8**:927-933.

Hayes J R, Ardill J, Kennedy T L, Shanks R G & Buchanan K D (1972) Stimulation of gastrin release by catecholamines. *The Lancet* pp819-821.

Hayes J R, Johnson D G, Koerker D & Williams R H (1975) Inhibition of gastrin secretion by somatostatin *in vitro*. *Endocrinology* **96**:1374-1376.

Hayes J R & Williams R H (1975) The effect on gastrin secretion of agents which increase the intracellular concentration of 3',5'-Adenosine monophosphate. *Endocrinology* **97**:1210-1214.

Hayes J R, Ardill J, Shanks R G & Buchanan K D (1978) Effect of catecholamines on gastrin release. *Metabolism: Clinical and Experimental* **27**:385-391.

Helander H F (1981) Cells of the gastric mucosa. *International Review of Cytology.* **70**:217-289.

Herfarth H H & Sartor R B (1994) Cytokine regulation of experimental intestinal inflammation. *Current Opinion in Gastroenterology* **10**:625-632.

Hersey S J, May D & Schyberg (1983) Stimulation of pepsinogen release from isolated gastric glands by cholecystokinin-like peptides. *American Journal of Physiology* **244**:G192-G197.

Hersey S J (1989) Cellular basis of pepsinogen secretion. *Handbook of Physiology, section 6: The Gastrointestinal System* **3**:267-278. Edited by S G Schultz. American Physiological Society, Washington D C.

Hilderson H, Berghen P, Vercruysse J, Dorny P & Braem L (1989) Diagnostic value of pepsinogen for clinical ostertagiosis. *The Veterinary Record* **125**:376-377.

Hill K J (1955) Continuous gastric secretion in the ruminant. *Quarterly Journal of Experimental Physiology* **154**:115-132.

Hill K J (1960) Abomasal secretion in the sheep. *Journal of Physiology* **154**:115-132.

Hill K J (1968) Abomasal function. *Handbook of Physiology, section 6: Alimentary Canal* **5**:2745-2759. Edited by C F Code, American Physiology Society, Washington D C.

Hirschowitz B I & Hutchinson H (1977) Evidence for a histamine H_2 receptor that inhibits pepsin secretion in the dog. *American Journal of Physiology* **233**:E225-E228.

Hirschowitz B I & Gibson R G (1978) Effect of cimetidine on stimulated gastrin secretion and serum gastrin in the dog. *American Journal of Gastroenterology* **70**:437-447.

Hirschowitz B I & Molina E (1983) Relation of gastric acid secretion and pepsin secretion to serum gastrin levels in dogs given bombesin and gastrin-17. *American Journal of Physiology* **244**:G546-G551.

Hirschowitz B I (1989) Neural and hormonal control of gastric secretion. *Handbook of Physiology, section 6: The Gastrointestinal System* **2**:127-159. Edited by S G Shultz. American Physiological Society, Washington D C.

Hirschowitz B I (1991) Control of pepsinogen secretion. *Current Opinion in Gastroenterology* **7**:863-869.

Hirst B H (1989) The gastric mucosal barrier. *Handbook of Physiology, section 6: The Gastrointestinal System* **3**:279-308. Edited by S G Schultz. American Physiological Society, Washington D C.

Hollande F, Gusdinar T, Bali J-P & Magous R (1993) Neurohormonal regulation of histamine release from isolated rabbit fundic mucosal cells. *Agents Actions* **38**:149-157.

Hollande F, Bali J-P & Magous R (1994) Neurohormonal regulation of histamine release in isolated rabbit fundic mucosal cells. *American Journal of Physiology* **266**:G395-G402.

Holmes P H & MacLean J M (1971) The pathophysiology of ovine ostertagiasis: a study of the changes in plasma protein metabolism following single infections. *Research in Veterinary Science* **12**:265-271.

Holst J J, Jensen S L, Knuhtsen S, Nielsen O V & Rehfeld J F (1983) Effect of vagus, gastric inhibitory polypeptide, and HCl on gastrin and somatostatin release from perfused pig antrum. *American Journal of Physiology* **244**:G515-G522.

Holst J J, Knuhtsen S, Orskov C, Skak-Nielsen T, Poulsen S S, Jensen S L & Nielsen O V (1987a) GRP nerves in pig antrum: role of GRP in vagal control of gastrin secretion. *American Journal of Physiology* **253**:G643-G649.

Holst J J, Knuhtsen, Orskov C, Skak-Nielsen T, Poulsen S S & Nielsen O V (1987b) GRP-producing nerves control antral somatostatin and gastrin secretion in pigs. *American Journal of Physiology* **253**:G767-G774.

Holst J J, Jorgensen P N, Rasmussen T N & Schmidy P (1992a) Somatostatin restraint of gastrin secretion in pigs revealed by monoclonal antibody immunoneutralization. *American Journal of Physiology* **263**:G908-G912.

Holst J J, Skak-Nielsen T, Ørskov C & Poulsen S S (1992b) Vagal control of the release of somatostatin, vasoactive intestinal polypeptide, gastrin-releasing peptide, and HCl from porcine non-antral stomach. *Scandinavian Journal of Gastroenterology* **27**:677-685.

Holst J J, Rasmussen T N, Schmidt P & Poulsen S S (1993) Transmitters in the control of gastrin and acid secretion in the pig stomach. *Gastrin* pp243-258. Edited by J H Walsh, Raven Press Ltd., New York.

Horak I G & Clark R (1964) The pathological physiology of *Ostertagia circumcincta* infestation. *Onderstepoort Journal of Veterinary Research* **31**:163-176.

Hotez P J & Cerami A (1983) Secretion of a proteolytic anticoagulant by *Ancylostoma* hookworms. *Journal of Experimental Medicine* **157**:1594-1603.

Hsu W H & Cooper C W (1977) Serum gastrin in the rat: cholinergic and adrenergic effects. *Proceedings of the Society for Experimental Biology and Medicine* **154**:401-406.

Hungate R E (1966) *The Rumen and its Microbes.* New York: Academy Press.

Hungate R E (1968) Ruminal fermentation. *Handbook of Physiology, section 6: Alimentary Canal* 5:2725-2745. Edited by C F Code, American Physiological Society, Washington D C.

Hunter A R & MacKenzie G (1982) The pathogenesis of a single challenge dose of *Haemonchus contortus* in lambs under six months of age. *Journal of Helminthology* **56**:135-144.

Hurst S & Collins S M (1993) Interleukin-1ß modulation of norepinephrine release from rat myenteric nerves. *American Journal of Physiology* **264**:G30-G35.

Hutton K, Bailey F J & Annison E F (1971) Measurement of the bacterial nitrogen entering the duodenum of the ruminant using diamino-pimelic acid as a marker. *British Journal of Nutrition* **25**:165-173.

Jarrousse C, Carles-Bonnet C, Niel H, Sabatier R, Audousset-Puech M, Blache P, Kervran A, Martinez J & Bataille D (1993) Inhibition of gastric acid secretion by oxyntomodulin and its 19-37 fragment in the conscious rat. *American Journal of Physiology* **264**:G816-G823.

Jennings F W, Armour J, Lawson D D & Roberts R (1966) Experimental *Ostertagia ostertagi* infections in calves: studies with abomasal cannulas. *American Journal of Veterinary Research* **27**:1249.

Jessen K R (1981) GABA in the enteric nervous system - a neurotransmitter function? *Molecular and Cellular Biochemistry* **38**:69-76.

Johnson L R, Aures D & Yuen L (1969) Pentagastrin-induced stimulation of protein synthesis in the gastrointestinal tract. *American Journal of Physiology* **217**:251-254.

Johnson L R & Guthrie P D (1980) Stimulation of rat oxyntic gland mucosal growth by epidermal growth factor. *American Journal of Physiology* **238**:G45-G49.

Johnson L R (1987) Regulation of gastrointestinal growth. *Physiology of the Gastrointestinal Tract 2nd Edition* pp301-333. Edited by L R Johnson. Raven Press, New York.

Karam S M (1993) Dynamics of epithelial cells in the corpus of the mouse stomach. IV. Bidirectional migration of parietal cells ending in their gradual degeneration and loss. *The Anatomical Record* **236**:314-332.

Karam S M & Forte J G (1994) Inhibiting gastric H^+ -K^+ -ATPase activity by omeprazole promotes degeneration and production of parietal cells. *American Journal of Physiology* **266**:G745-G758.

Karanu F N, Rurangirwa F R, McGuire T C & Jasmer D P (1993) *Haemonchus contortus*: identification of proteases with diverse characteristics in adult worm excretory-secretory products. *Experimental Parasitology* **77**:362-371.

Kay A B (1985) Eosinophils as effector cells in immunity and hypersensitivity disorders. *Clinical and Experimental Immunology* **62**:1-12.

Keith K A, Duncan M C, Murray M, Bairden K & Tait A (1990) Stage-specific cuticular proteins of *Ostertagia circumcincta* and *Ostertagia ostertagi*. *International Journal for Parasitology* **20**:1037-1045.

Kimberg D V (1974) Cyclic nucleotides and their role in gastrointestinal secretion. *Gastroenterology* **67**:1023-1064.

Kitamura N, Yamada J, Calingasan N Y & Yamashita T (1985) Histologic and immunocytochemical study of endocrine cells in the gastrointestinal tract of the cow and calf. *American Journal of Veterinary Research* **46**:1381-1386.

Klesius P H, Haynes T B & Cross D A (1985) Chemotactic factors for eosinophils in soluble extracts of L_3 stages of *Ostertagia ostertagi*. *International Journal for Parasitology* **15**:517-522.

Klesius P H (1991) A lectin-like eosinophil chemotactic factor in the secretory/excretory substances of a parasitic nematode. *Proceedings of the American Association of Veterinary Parasitology* p61.

Klesius P H (1993) Regulation of immunity to *Ostertagia ostertagi*. *Veterinary Parasitology* **46**:63-79.

Kleveland P M, Haugen S E, Sandvik S & Waldum H L (1986) The effect of pentagastrin on the gastric secretion by the totally isolated vascularly perfused rat stomach. *Gastroenterology* **21**:379-384.

Knox D P & Jones D G (1990) Studies on the presence and release of proteolytic enzymes (proteinases) in gastro-intestinal nematodes of ruminants. *International Journal for Parasitology* **20**:243-249.

Knuhtsen S, Holst J J, Knigge U, Olesen M & Nielsen O V (1984) Radioimmunoassay, pharmacokinetics and neuronal release of gastrin-releasing peptide in anaesthetized pigs. *Gastroenterology* **87**:372-378.

Koelz H R, Hersey S J, Sachs G & Chew C S (1982) Pepsinogen release from isolated gastric glands. *American Journal of Physiology* **243**:G218-G225.

Konturek S J, Biernat J, Kwiecien N & Oleksy J (1975) Effect of glucagon on meal-

induced gastric secretion in man. *Gastroenterology* **68**:448-454.

Konturek S J, Cieszkowski M, Jaworek J, Konturek J, Brzozowski T & Gregory H (1984) Effects of epidermal growth factor on gastrointestinal secretions. *American Journal of Physiology* **246**:G580-G586.

Konturek S J, Bielanski W & Solomon T E (1990) Effects of an antral mucosectomy, L-364,718 and atropine on cephalic phase of gastric and pancreatic secretion in dogs. *Gastroenterology* **98**:47-55.

Koop H, Behrens I, Bothe E, Koschwitz H, McIntosh C H, Pederson R A, Arnold R & Creutzfeldt W (1983) Adrenergic control of rat gastric somatostatin and gastrin release. *Scandinavian Journal of Gastroenterology* **18**:65-71.

Kovacs T O G, Walsh J H, Maxwell V, Wong H C, Azuma T & Katt E (1989) Gastrin is a major mediator of the gastric phase of acid secretion in dogs: Proof by monoclonal antibody neutralization. *Gastroenterology* **97**:1406-1413.

Kronborg O, Pederson T, Stadil F & Rehfeld J F (1974) The effect of beta-adrenergic blockade upon gastric acid secretion during hypoglycaemia before and after vagotomy. *Scandinavian Journal of Gastroenterology* **9**:173-176.

Lamas S, Michel T, Brenner B M & Marsden P A (1991) Nitric oxide synthesis in endothelial cells: evidence for a pathway inducible by TNF-α. *American Journal of Physiology* **261**:C634-C641.

Larsson L-I, Goltermann N, De Magistris L, Rehfeld J F & Schwartz T W (1979) Somatostatin cell processes as pathways for paracrine secretion. *Science* **28**:1393-1394.

Lawton D E B & Simpson H V (1993) A method for assessing the effect of *Ostertagia circumcincta* products on the release of gastrin by ovine antral mucosa *in vitro*. *Proceedings of the Physiological Society of New Zealand* **13**:19.

Lehy T & Willems G (1976) Population kinetics of antral gastrin cells in the mouse. *Gastroenterology* **71**:614-619.

Lehy T, Accary J P, Labeille D & Dubrasquet M (1983) Chronic administration of bombesin stimulates antral gastrin cell proliferation in the rat. *Gastroenterology* **84**:914-919.

Leid R W (1987) Parasite defence mechanisms for evasion of host attack, a review. *Veterinary Parasitology* **25**:147-162.

Lewin M J M (1992) The somatostatin receptor in the GI tract. *Annual Reviews in Physiology* **54**:455-468.

Li D-S, Raybould H E, Quintero E & Guth P H (1992) Calcitonin gene-related peptide mediates the gastric hyperaemic response to acid back-diffusion. *Gastroenterology* **102**:1124-1128.

Lichtenberger L M, Forssmann W G & Ito S (1980) Functional responsiveness of an isolated and enriched fraction of rodent gastrin cells. *Gastroenterology* **79**:447-459.

Lightowlers M W & Richard M D (1988) Excretory-secretory products of helminth parasites : effects on the host immune responses. *Parasitology* **96**:S123-S166.

Lipkin M (1973) Proliferation and differentiation of gastrin cells. *Physiological Reviews* **53**:891-915.

Ljungstrom M & Chew C S (1991) Calcium oscillations and morphological transformations in single cultured gastric parietal cells. *American Journal of Physiology* **260**:G67-G78.

Lloyd K C K, Raybould H E, Tache Y & Walsh J H (1992a) Role of gastrin, histamine, and acetylcholine in the gastric phase of acid secretion in anaesthetized rats. *American Journal of Physiology* **262**:G747-G755.

Lloyd K C K, Raybould H E & Walsh J H (1992b) Cholecystokinin inhibits gastric acid secretion through type "A" cholecystokinin receptors and somatostatin in rats. *American Journal of Physiology* **263**:G287-G292.

Lowry P J (1974) A sensitive method for the detection of corticotrophin releasing factor using a perfused pituitary cell column. *Journal of endocrinology* **62**:163-164.

Lugea A, Salas A, Guarner F, Azpiroz F & Malagelada J R (1992) Duodenal mucosal resistance to intraluminal acid in the rat: role of adaptive cytoprotection. *Gastroenterology* **102**:1129-1135.

MacDonald T T & Spencer J (1988) Evidence that activated mucosal T cells play a role in the pathogenesis of enteropathy in human small intestine. *Journal of Experimental Medicine* **167**:1341-1349.

Main C, Blennerhassett P & Collins S M (1993) Human recombinant interleukin-1ß suppresses acetylcholine release from rat myenteric plexus. *Gastroenterology* **104**:1648-1654.

Makhlouf G M, Grider J R & Schubert M L (1989) Identification of physiological function of gut peptides. *Handbook of Physiology, section 6, The Gastrointestinal system.* **2**:123-131. Edited by S G Shultz. American Physiological Society, Maryland.

Manson-Smith D F, Bruce R G & Parrott D M V (1979) Villous atrophy and expulsion of intestinal *Trichinella spiralis* are mediated by T cells. *Cellular Immunology* **47**:285.

Mapes C J & Coop R L (1970) The interaction of infections of *Haemonchus contortus & Nematodirus battus* in lambs. *Journal of comparative Pathology* **80**:123-136.

Mapes C J & Coop R L (1973) On the relationship between abomasal electrolytes and some population parameters of the nematodes *Haemonchus contortus* and *Nematodirus battus*. *Parasitology* **66**:95-100.

Mårdh S, Norberg L, Ljungstrom M, Wollert S, Nyren O & Gustavsson S (1985) A method for *in vitro* studies on acid formation in human parietal cells. Stimulation by histamine, pentagastrin and carbachol. *Acta Physiologica Scand.* **123**:349-354.

Mårdh S, Song Y-H, Carlsson C & Bjorkman T (1987) Mechanisms of stimulation of acid production in parietal cells isolated from the pig gastric mucosa. *Acta Physiologica Scandinavia* **131**:589-598.

Martin M G, Wu S V, Ohning G, Wong H & Walsh J H (1994) Parenterally or enterally administered anti-somatostatin antibody induces increased gastrin in suckling rats. *American Journal of Physiology* **266**:G417-G424.

Martinez-Cuesta, Barrachina M, Pique J, Whittle B & Esplugues J (1992) The role of nitric oxide and platelet-activating factor in the inhibition by endotoxin of pentagastrin-stimulated gastric acid secretion. *European Journal of Pharmacology* **218**:351-354.

Martzen M R, McMullen B A, Smith N E, Fujikawa K & Peanasky R J (1990) Primary structure of the major pepsin inhibitor from the intestinal parasitic nematode *Ascaris suum. Biochemistry* **29**:7366-7372.

Matsumoto H, Dickinson K E J, Shirakawa T, Komiyana K & Hirschowitz B I (1987) Synergism between cellular messengers and agonist combinations in pepsinogen secretion. *American Journal of Physiology* **253**:G557-G565.

Matthews B E (1982) Skin penetration by *Necator americanus* larvae. *Zeitschrift für Parasitenkunde* **68**:81-86.

Mayer E A, Elashoff J, Mutt V & Walsh J H (1982) Reassessment of gastric acid inhibition by cholecystokinin and gastric inhibitory polypeptide in dogs. *Gastroenterology* **83**:1047-1050.

McCormack S A, Johnson L R (1991) Role of polyamines in gastrointestinal mucosal growth. *American Journal of Physiology* **260**:G795-G806.

McDonald T J, Houghton P, Challid J R G & Hramiak I M (1988) The effect of gastrin-releasing peptide on the endocrine pancreas. *Annals of the New York Academy of Sciences* **547**:242-254.

McGillivery D J, Yong W K, Riffkin G G & Adler B (1989) Extraction and identification of a 31,000 mol.wt glycoprotein antigen of *Ostertagia circumcincta* by sera from resistant sheep. *International Journal for Parasitology* **19**:473-478.

McGillivery D J, Yong W K, Riffkin G G & Adler B (1990) The distribution and localization of the stage-specific GP31 antigen from infective *Ostertagia circumcincta* larvae. *International Journal for Parasitology* **20**:87-93.

McHugh J K, Weingarten H P, Thompson R C & Collins S M (1992) Interleukin-1ß mediates the anorexia and weight loss in experimental colitis in the rat. *Gastroenterology* **102**:A663.

McHugh J K, Weingarten H P & Collins S M (1993) Anorexia in experimental colitis is mediated by interleukin-1 at both a peripheral and central level. *Gastroenterology* **104**:A251.

McIntosh C H S, Pederson R A, Koop H & Brown J C (1981) Gastric inhibitory polypeptide-stimulated secretion of somatostatin-like immunoreactivity from the stomach: inhibition by acetylcholine or vagal stimulation. *Canadian Journal of Physiology and Pharmacology* **59**:468-472.

McIntosh C H S, Dadger A & Kowk Y N (1992) Cholinergic stimulation of neuropeptide Y secretion from the isolated perfused rat stomach. *Regulatory Peptides* **39**:83-94.

McKeand J B, Knox D P, Duncan J L & Kennedy M W (1994a) Genetic control of the antibody repertoire against excretory/secretory products and acetylcholinesterases of *Dictyocaulus viviparus*. *Parasite Immunology* **16**:251-260.

McKeand J B, Knox D P, Duncan J L & Kennedy M W (1994b) The immunogenicity of the acetylcholinesterases of the cattle lungworm *Dictyocaulus viviparus*. *International Journal for Parasitology* **24**:501-510.

McKellar Q, Duncan J L, Armour J & McWilliam P (1986) Response to transplanted adult *Ostertagia ostertagi* in calves. *Research in Veterinary Science* **40**:367-371.

McKellar Q & Bogan J A (1987) Effect of sodium cromoglycate on the response to *Ostertagia circumcincta* in adult sheep. *Research in Veterinary Science* **43**:157-159.

McKellar Q A, Duncan J L, Armour J, Lindsay F E F & McWilliam (1987) Further studies on the response to transplanted adult *Ostertagia ostertagi* in calves. *Research in Veterinary Science* **42**:29-34.

McKellar Q A, Mostofa M & Eckersall P D (1990a) Stimulated pepsinogen secretion from dispersed abomasal glands exposed to *Ostertagia* species secretion. *Research in Veterinary Science* **48**:6-11.

McKellar Q A, Mostofa M & Eckersall P D (1990b) Effect of *Ostertagia ostertagi* secretions and various putative secretagogues and inhibitors on aminopyrine accumulation in dispersed bovine abomasal gland cells. *Research in Veterinary Science* **49**:323-326.

McKellar Q A (1993) Interactions of *Ostertagia* species with their bovine and ovine hosts. *International Journal of Parasitology* **23**:451-462.

McLeay L M & Titchen D A (1970) Abomasal secretory responses to teasing with food and feeding in the sheep. *Journal of Physiology* **206**:605-628.

McLeay L M, Anderson N, Bingley J B & Titchen D A (1973) Effects on abomasal function of *Ostertagia circumcincta* infections in sheep. *Parasitology* **66**:241-257.

McLeay L M & Titchen D A (1974) Effects of the amount and type of food eaten on secretion from fundic abomasal pouches of sheep. *British Journal of Nutrition* **32**:375-387.

McLeay L M & Titchen D A (1975) Gastric, antral and fundic pouch secretion in sheep. *Journal of Physiology* **248**:595-612.

McLeay L M & Titchen D A (1977a) Acid and pepsin secretion of separated gastric pouches during perfusion of antral pouches with cholinergic stimulating and blocking agents and lignocaine. *Journal of Physiology* **264**:215-227.

McLeay L M & Titchen D A (1977b) Inhibition of hydrocholric acid and pepsin secretion from gastric pouches by antral pouch acidification in sheep. *Journal of Physiology* **273**:707-716.

McLeay L M & Bell F R (1980) Effect of cholecystokinin, secretin, glucagon, and insulin on gastric emptying and acid secretion in the calf. *American Journal of Veterinary Research* **41**:1590-1594.

McLeay L M & Fitzgerald J M (1982) Inhibition of ovine gastric acid secretion by intraduodenal long-chain fatty acids. *American Journal of Physiology* **243**:G127-G133.

McLeay L M, Oliver M H, Thomas L W, Simpson H V, Reynolds G W & Carr D H (1989) Effects of bombesin on gastrin release and electromyographic activity of the gastrointestinal tract in sheep. *Australian Journal of Agricultural Science* **2**:195-196.

Meijer J L, Jansen J B M J, Crobach L F S J, Biemond I & Lamers C B H W (1993) Inhibition of omeprazole induced hypergastrinaemia by SMS 201-995, a long acting somatostatin analogue in man. *Gut* **34**:1186-1190.

Michel J F (1974) Arrested development of nematodes and some related phenomena. *Advances in Parasitology* **12**:279-366.

Miller H R P (1993) Immunopathology of gastrointestinal nematode infestation and expulsion. *Current Opinion in Gastroenterology* **9**:986-993.

Mostofa M & McKellar (1989) Effect of an antimuscarinic drug on the plasma pepsinogen activity of sheep infected with *Ostertagia circumcincta*. *Research in Veterinary Science* **47**:208-211.

Muller M J, Defize J & Hunt R H (1990) Control of pepsinogen synthesis and secretion. *Gastroenterology Clinics of North America* **19**:27-40.

Mungan Z, Ozmen V, Ertan A & Arimura A (1992) Pituitary adenylate cyclase activating polypeptide-27 (PACAP-27) inhibits pentagastrin-stimulated gastric acid secretion in conscious rats. *Regulatory Peptides* **38**:199-206.

Murray M (1969) Structural changes in bovine ostertagiasis associated with increased permeability of the bowel wall to macromolecules. *Gastroenterology* **56**:763-772.

Murray M (1970) The fine structure of bovine gastric epithelia. *Research in Veterinary Science* **11**:411-416.

Murray M, Jennings F W & Armour J (1970) Bovine ostertagiasis: Structure, function and mode of differentiation of the bovine gastric mucosa and kinetics of the worm loss. *Research in Veterinary Science* **11**:417.

Mylrea P J & Hotson I K (1969) Serum pepsinogen activity and the diagnosis of bovine ostertagiasis. *British Veterinary Journal* **125**:379-387.

Nakamura M, Oda M, Kanenko K, Akaiwa Y, Tsukads N, Komatsu H & Tsuchiya M (1988) Autoradiographic demonstration of gastrin-releasing peptide-binding sites in the rat gastric mucosa. *Gastroenterology* **94**:968-976.

Nakata H, Matsui T, Ito M, Taniguchi T, Naribayashi Y, Arima N, Nakamura A, Kinoshita Y, Chihara K, Hosoda S & Chiba T (1992) Cloning and characterization of gastrin receptors from ECL carcinoid tumour of *Mastomys natalensis*. *Biochemica et Biophysica Research Communication* **187**:1151-1157.

Nawa Y (1979) Increased permeability of gut mucosa in rats infected with *Nippostrongylus brasiliensis*. *International Journal for Parasitology* **9**:251.

Nicholls C D, Lee D L, Adrian T E, Bloom S R & Care A D (1985) Endocrine effects of *Haemonchus contortus* infection in lambs. *Regulatory Peptides* **13**:85.

Nicholls C D, Hayes P R & Lee D L (1987) Physiological and microbiological changes in the abomasum of sheep infected with large doses of *Haemonchus contortus*. *Journal of Comparative Pathology* **97**:299-308.

Nicholls C D, Lee D L, Adrian T E, Bloom S R & Care A D (1988) Hypergastrinaemia of sheep infected with *Haemonchus contortus*. *Research in Veterinary Science* **45**:124-126.

Nicholson T (1982) The mode of action of intravenous pentagastrin injections on forestomach motility of sheep. *Quarterly Journal of Experimental Physiology* **67**: 537-542.

Nishi S, Seino Y, Takemura J, Ishida H, Seno M, Chiba T, Yanaihara C, Yanaihara N & Imura H (1985) Vagal regulation of GRP, gastric somatostatin, and gastrin secretion in vitro. *American Journal of Physiology* **248**:E425-E431.

Nishizaki Y, Guth P H, Kim G, Wayland H & Kaunitz J D (1994) Pentagastrin enhances gastric mucosal defences in vivo: luminal acid-dependent and independent effects. *American Journal of Physiology* **267**:G94-G104.

Ogilvie B M & Jones V E (1971) *Nippostrongylus brasiliensis:* A review of immunity and the host/parasite relationship in the rat. *Experimental Parasitology* **29**:138-177.

Ogilvie B M, Rothwell T L W, Bremner K C, Schnitzerling H J, Nolan J & Keith R K

(1973) Acetylcholinesterase secretion by parasitic nematodes-I. Evidence for secretion of the enzyme by a number of species. *International Journal for Parasitology* **3**:589-597.

O'Halloran D J, Nikou G C, Kreymann B, Ghatei M A & Bloom S R (1990) Glucagon-like peptide-1 (7-36)-NH2: a physiological inhibitor of gastric acid secretion in man. *Journal of Endocrinology* **126**:169-173.

Olesen M, Holst J J, Sottimano C, Nielsen O V (1987) Autonomic nervous control of fundic secretion of somatostatin and antral secretion of gastrin and somatostatin in pigs. *Digestion* **36**:24-35.

Onapito S J, Donawick W J & Merritt A M (1978) Effects of gastrin on emptying and composition of digesta of the omasum of sheep. *American Journal of Veterinary Research* **39**:1455-1458.

Opperman C H & Chang S (1992) Nematode acetylcholinesterases: molecular forms and their potential role in nematode behaviour. *Parasitology Today* **8**:406-411.

Park J, Chiba T & Yamada T (1987) Mechanisms for direct inhibition of canine gastric parietal cells by somatostatin. *Journal of Biological Chemistry* **262**:14190-14195.

Park J, Chiba T, Yokotani K, Del Valle J & Yamada T (1989) Somatostatin receptors on canine fundic D-cells: evidence for autocrine regulation of gastric somatostatin. *American Journal of Physiology* **257**:G235-G241.

Parkins J J, Bairden K & Armour J (1982a) *Ostertagia ostertagi* in calves: a growth nitrogen balance and digestibility study conducted during winter feeding following thiabendazole anthelmintic therapy. *Journal of Comparative Pathology* **92**:219-227.

Parkins J J, Bairden K & Armour J (1982b) *Ostertagia ostertagi* in calves: growth nitrogen balance and digestibility study conducted during winter feeding following different fenbendazole therapy programmes. *Research in Veterinary Science* **14**:21-28.

Payne N A & Gerber J G (1992) Differential effects of somatostatin and prostaglandins on gastric histamine release to pentagastrin. *The Journal of Pharmacology and Experimental Therapeutics* **263**:520-526.

Pearce E & Appleton J (1992) Production of immunomodulatory neuropeptides by *Schistosoma mansoni*. *Parasitology Today* **8**:353-354.

Penston J & Wormsley K G (1987) Achlorhydria: hypergastrinaemia: carcinoids - a flawed hypothesis? *Gut* **28**:488-505.

Pfeiffer A, Rochlitz H, Noelke B, Tacke R, Moser U, Mutschler E & Lambrecht G (1990) Muscarinic receptors mediating acid secretion in isolated rat gastric parietal cells are of the glandular M_3-type. *Gastroenterology* **98**:218-222.

Philipp M (1984) Acetylcholinesterase secreted by intestinal nematodes: A

reinterpretation of its putative role of 'biochemical holdfast'. *Transactions of the Royal Society for Tropical Medicine and Hygiene* **78**:138-139.

Pique J M, Esplugues J V & Whittle B J R (1992) Endogenous nitric oxide as a mediator of gastric mucosal vasodilation during acid secretion. *Gastroenterology* **102**:168-174.

Pitt S R, Fox M T, Gerrelli D & Jacobs D E (1988) Blood gastrin and pepsinogen responses to subclinical infection with *Ostertagia ostertag*i in adult dairy cattle. *Research in Veterinary Science* **45**:130-131.

Polk W H, Dempsey P J, Russell W E, Brown P I, Beauchamp R D, Barnard J A & Coffey R J (1992) Increased production of transforming growth factor-α following acute gastric injury. *Gastroenterology* **102**:1467-1474.

Powell D W (1984) Barrier function of epithelia. *American Journal of Physiology* **241**:G275-G288.

Price B A, Jaffe B M & Zinner M J (1985) Effect of exogenous somatostatin infusion on gastrointestinal blood flow and hormones in the conscious dog. *Gastroenterology* **88**:80-85.

Pritchard D I (1993) Immunity to helminths:is too much IgE parasite- rather than host-protective? *Parasite Immunology* **15**:5-9.

Pritchard D I, Lawrence C E, Appleby P, Gibb I A & Glover K (1994) Immunosuppressive proteins secreted by the gastrointestinal nematode parasite *Heligmosoides polygyrus*. *International Journal for Parasitology* **24**:495-500.

Qian J-M, Rowley W H & Jensen R T (1993) Gastrin and CCK activate phospholipase C and stimulate pepsinogen release by interacting with two distinct receptors. *American Journal of Physiology* **264**:G718-G727.

Ramos E, Esplugues J & Esplugues J V (1992) Gastric acid secretory responses induced by peptone are mediated by capsaicin-sensitive sensory afferent neurons. *American Journal of Physiology* **262**:G835-G839.

Raufman J-P, Kasbekar D K, Jensen R T & Gardner J D (1983) Potentiation of pepsinogen secretion from dispersed glands from rat stomach. *American Journal of Physiology* **245**:G525-G530.

Raufman J-P, Sutliff V E, Kasbekar D K, Jensen R T & Gardner J D (1984) Pepsinogen secretion from dispersed chief cells from guinea pig stomach. *American Journal of Physiology* **247**:G95-G104.

Raufman J-P & Singh L (1991) Actions of peptide YY and neuropeptide Y on chief cells from guinea pig stomach. *American Journal of Physiology* **260**:G820-G825.

Raufman J-P (1992a) Regulation of pepsinogen secretion. *Current Opinion in*

Gastroenterology **8**:907-910.

Raufman J-P (1992b) Gastric chief cells: receptors and signal transduction mechanisms. *Gastroenterology* **102**:699-710.

Read M A, Read D M, Kapuscinski M & Shulkes A (1992) Achlorhydria induced changes in gastrin, somatostatin, H^+/K^+-ATPase carbonic anhydrase in the sheep. *Regulatory Peptides* **40**:13-27.

Redfern J S, Lin H J, McArthur K E, Prince M D & Feldman M (1991) Gastric acid and pepsin hypersecretion in conscious rabbits. *American Journal of Physiology* **261**:G295-G304.

Rees W D W, Rhodes J, Williams B C, Owen E & Mewcombe G (1978) Effect of mast cell degranulation on gastric mucosal damage produced by sodium taurochlorate in the rat. *Gastroenterology* **74**:492-495.

Reid A M, Shulkes A & Titchen D A (1988) Effects of the vagus nerves on gastric motility and release of vasoactive intestinal polypeptide in the anaesthetized lamb. *Journal of Physiology* **396**:11-24.

Reid J F S & Armour J (1975) Seasonal variations in the gastro-intestinal nematode populations of Scottish hill sheep. *Research in Veterinary Science* **18**:307-313.

Reyl F, Silve C & Lewin M J M (1979) Somatostatin receptors on isolated gastric cells. *Hormone receptors in digestion and nutrition* pp391-400. Edited by G Rosselin, P Fromageot and S Bonfils. Elsevier, Amsterdam.

Reynolds G W & Titchen D A (1977) Inhibition of gastric acid and pepsin secretion in sheep on first eating. *Proceedings of the International Union of Physiological Sciences* **13**:1862P.

Reynolds G W, Stiffe G E, Hansky J & Titchen D A (1978) Serum gastrin levels and abomasal secretion in response to feeding in sheep on restricted food intakes. *Proceedings of the Australian Physiological and Pharmacological Society* **9**:18P.

Reynolds G W, Hansky J & Titchen D A (1980) Effects of vagotomy on serum gastrin levels and gastric secretion during insulin hypoglycaemia in the sheep. *New Zealand Medical Journal* **10**:444.

Reynolds G W, Simpson H V & Carr D H (1989) Stimulation of gastrin release by the intra-abomasal infusion of rumen fluid in sheep. *Australian Journal of Agricultural Science* **2**:185-186.

Reynolds G W, Simpson H V, Carr D H & McLeay L M (1991) Gastrin: its molecular forms and secretion in sheep. *Physiological Aspects of Digestion and Metabolism in Ruminants: Proceedings of the Seventh International Symposium on Ruminant Physiology* Chapter 4:63-87. Edited by T Tsuda, Y Sasaki & R Kawashima. Academic Press, Inc., California.

Rhee J C, Chang T M, Lee K Y, Jo Y H & Chey W Y (1991) Mechanism of oleic acid-induced inhibition on gastric acid secretion in rats. *American Journal of Physiology* **260**:G564-G570.

Rhoads M L (1984) Secretory cholinesterases of nematodes: possible functions in the host-parasite relationship. *Tropical Veterinarian* **2**:3-10.

Richelsen B, Rehfeld J F & Larsson L-I (1983) Antral gland cell column: a method for studying release of gastric hormones. *American Journal of Physiology* **245**:G463-G469.

Rioux K P & Wallace J L (1994) Mast cell activation augments gastric mucosal injury through a leukotriene-dependent mechanism. *American Journal of Physiology* **266**:G863-869.

Ritchie J D S, Anderson N, Armour J, Jarrett W F H, Jennings F W & Urquhart G M (1966) Experimental *Ostertagia ostertagi* infections in calves: parasitology and pathogenesis of a single infection. *American Journal of Veterinary Research* **27**:659-667.

Rizzotti M, Domeneghini C & Castaldo L (1980a) The endocrine cells of the proper gastric glands of adult ox. *Basic and Applied Histochemistry* **24**:79-93.

Rizzotti M, Domeneghini C & Castaldo L (1980b) The endocrine cells of the pyloric glands of adult ox. *Basic and Applied Histochemistry* **24**:33-52.

Robert A, Nezamis J E, Lancaster C & Hanchar A J (1979) Cytoprotection by prostaglandins in rats. Prevention of gastric necrosis produced by alcohol, HCl, NaOH, hypertonic NaCl, and thermal injury. *Gastroenterology* **77**:433-443.

Robert A, Olafsson A S, Lancaster C & Zhang W (1991) Interleukin-1 is cytoprotective, antisecretory, stimulates PGE_2 synthesis by the stomach, and retards gastric emptying. *Life Science* **48**:123-134.

Roche S, Bali J P, Galleyrand J C & Magous R (1991) Characterization of a gastrin-type receptor on rabbit gastric parietal cells using L365,260 and L364,719. *American Journal of Physiology* **260**:G182-G188.

Rogers W P & Sommerville R I (1960) The physiology of the second ecdysis of parasitic nematodes. *Parasitology* **50**:329-348.

Rogers W P & Sommerville R I (1963) The infective stage of nematode parasites and its significance in parasitism. *Advances in Parasitology* **1**:109-177.

Rogers W P & Sommerville R I (1968) The infectious process, and its relation to the development of early parasitic stages of nematodes. *Advances in Parasitology* **6**:327-348.

Ross J G (1963) Experimental infections of calves with the nematode parasite *Ostertagia ostertagi*. *The Veterinary Record* **75**:129-131.

Rothwell T L W & Love R J (1974) Vaccination against the nematode *Trichostrongylus colubriformis*-I. Vaccination of guinea-pigs with worm homogenates and soluble products released during *in vitro* maintenance. *International Journal for Parasitology* **4**:293-299.

Rothwell T L W (1989) Immune expulsion of parasitic nematodes from the alimentary tract. *International Journal for Parasitology* **19**:139-168.

Rubin W (1972) Endocrine cells in the normal human stomach. *Gastroenterology.* **62**:784-800.

Ruckebusch Y (1971) The effect of pentagastrin on the motility of ruminant stomach. *Experimentia* **27**:1185-1186.

Ruoss S J, Hartmann T & Caughey G H (1991) Mast cell tryptase is a mitogen for cultured fibroblasts. *The Journal of Clinical Investigation* **88**:493-499.

Ryberg B, Carlsson E, Carlsson K, Hakanson R, Lundell L, Mattsson H & Sundler F (1990) Effects of partial resection of acid-secreting mucosa on plasma gastrin and enterochromaffin-like cells in the rat stomach. *Digestion* **45**:102-108.

Sachs G, Kaunitz J, Mendlein J & Wallmark B (1989) Biochemistry of gastric acid secretion: H^+-K^+-ATPase. *Handbook of Physiology, Section 6: The Gastrointestinal System* **2**:229-253. Edited by S G Shultz, American Physiological Society, Washington D C.

Sachs G (1990) Therapeutic control of acid secretion: pharmacology of the parietal cell. *Current Opinion in Gastroenterology* **6**:859-866.

Saffouri B, Weir G, Bitar K & Makhlouf G (1979) Stimulation of gastrin secretion from the perfused rat stomach by somatostatin antiserum. *Life Sciences* **25**:1749-1754.

Saffouri B, Weir G C, Bitar K N & Makhlouf G M (1980) Gastrin and somatostatin secretion by perfused rat stomach: functional linkage of antral peptides. *American Journal of Physiology* **238**:G495-G501.

Saffouri B, DuVal J W, Arimura A & Makhlouf G M (1984a) Effects of vasoactive intestinal peptide and secretin on gastrin and somatostatin secretion in the perfused rat stomach. *Gastroenterology* **86**:839-842.

Saffouri B, DuVal J W, Arimura A & Makhlouf G M (1984b) Stimulation of gastrin secretion in vitro by intraluminal chemicals: regulation by intramural cholinergic and noncholinergic neurons. *Gastroenterology* **87**:557-561.

Sanders M J, Amirian A, Ayalon A & Soll A H (1983) Regulation of pepsinogen release from canine chief cells in primary monolayer culture. *American Journal of Physiology* **245**:G641-G646.

Sandvik A K & Waldum H L (1988) The effect of misoprostol on base-line and

stimulated acid secretion and on gastrin and histamine release in the totally isolated, vascularly perfused rat stomach. *Scandinavian Journal of Gastroenterology* **23**:696-700.

Sandvik A K, Brenna E & Waldum H L (1993) Calcium mediates gastrin-induced gastric histamine release in the rat. *American Journal of Physiology* **264**:G51-G56.

Saperas E S, Yang H, Rivier C & Taché Y (1990) Central action of recombinant interleukin-1 to inhibit acid secretion in rats. *Gastroenterology* **99**:1599-1606.

Saperas E S, Cominelli F & Taché Y (1992) Potent inhibition of gastric acid secretion by intravenous interleukin-1ß and -1α in rats. *Peptides* **13**:221-226.

Savin K W, Dopheide T A A, Frenkel M J, Wagland B M, Grant W N & Ward C W (1990) Characterization, cloning and host-protective activity of a 30-kilodalton glycoprotein secreted by the parasitic stages of *Trichostrongylus colubriformis*. *Molecular and Biochemical Parasitology* **41**:167-176.

Schallig H D P H, Leeuwen M A W, Hendrikx W M L & van Leeuwen M A W (1994) Immune responses of texel sheep to excretory/secretory products of adult *Haemonchus contortus*. *Parasitology* **108**:351-357.

Schepp W, Soll A H & Walsh J H (1990) Dual modulation by adenosine of gastrin release from canine G-cells in primary culture. *American Journal of Physiology* **259**:G556-G563.

Schepp W, Schmitler J, Dehne K, Schusdziarra V & Classen M (1992) Pertussis toxin-sensitive and pertussis toxin-insensitive inhibition of parietal cell response to GLP-1 and histamine. *American Journal of Physiology* **262**:G660-G668.

Schepp W, Schmitler J, Dehne K, Schusdziarra V & Classen M (1992) Pertussis toxin-sensitive and pertussis toxin-insensitive inhibition of parietal cell response to GLP-1 and histamine. *American Journal of Physiology* **262**:G660-G668.

Schepp W, Chan C B, Giraud A S, Avedian D, Chen M C Y, Chew P, Walsh J H & Soll A H (1994) Effects of prostaglandins on gastrin release from canine antral mucosal cells in primary culture. *American Journal of Physiology* **266**:G194-G200.

Schmidtler J, Schepp W, Janczewska I, Weigert N, Furlinger C, Schusdziarra V & Classen M (1991) GLP-1-(7-36) amide, -(1-37), and -(1-36) amide: potent cAMP-dependent stimuli of rat parietal cell function. *American Journal of Physiology* **260**:G940-G950.

Schmidtler J, Rosenthal W, Offermanns S, Schusdziarra V, Classen M & Schepp W (1992) Pertussis toxin reverses prostaglandin E_2- and somatostatin-induced inhibition of the rat parietal cell H^+-production. *Cell Signal* **4**:321-329.

Schepp W, Chan C B, Giraud A S, Avedian D, Chen M C Y, Chew P, Walsh J H & Soll A H (1994) Effects of prostaglandins on gastrin release from canine antral mucosal cells in primary culture. *American Journal of Physiology* **266**:G194-G200.

Schmidtler J, Dehne K, Offermanns S, Rosenthal W, Classen M & Schepp (1994) Stimulation of rat parietal cell function by histamine and GLP-1-(7-36) amide is mediated by $G_2\alpha$. *American Journal of Physiology* **266**:G775-G782.

Schubert M L, Bitar K N & Makhlouf G M (1982a) Regulation of gastrin and somatostatin secretion by cholinergic and non cholinergic intramural neurons. *Gastroenterology* **82**:A1172.

Schubert M L, Bitar K N & Makhlouf G M (1982b) Regulation of gastrin and somatostatin secretion by cholinergic and noncholinergic intramural neurons. *American Journal of Physiology* **243**:G442-G447.

Schubert M L, Saffouri B, Walsh J H & Makhlouf G M (1985) Inhibition of neurally mediated gastrin secretion by bombesin antiserum. *American Journal of Physiology* **248**:G456-G462.

Schubert M L, Edwards N F & Makhlouf G M (1988a) Regulation of gastric somatostatin secretion in the mouse by luminal acidity: a local feedback mechanism. *Gastroenterology* **94**:317-322.

Schubert M L, Saffouri B & Makhlouf G M (1988b) Identical patterns of somatostatin secretion from isolated antrum and fundus of rat stomach. *American Journal of Physiology* **254**:G20-G24.

Schubert M L & Hightower J (1989b) Release of gastric somatostatin during distension is mediated by gastric VIP neurons: a mechanical reflex for inhibition of gastrin. *Gastroenterology* **96**:A455.

Schubert M L, Hightower J & Coy D H (1990) Participation of cholinergic and bombesin neurons of the fundus in acid secretion stimulated by luminal peptone. *Gastroenterology* **98**:A121.

Schubert M L (1991) Stomach and duodenum (editorial overview). *Current Opinion in Gastroenterology* **7**:839-841.

Schubert M L, Jong M J & Makhlouf G M (1991b) Bombesin/GRP-stimulated somatostatin secretion is mediated by gastrin in the antrum and intrinsic neurons in the fundus. *American Journal of Physiology* **261**:G885-G889.

Schubert M L (1992) Control of gastric acid secretion. *Current Opinion in Gastroenterology* **8**:895-906.

Schubert M L, Coy D H & Makhlouf G M (1992) Peptone stimulates gastrin from the stomach by activating bombesin/GRP and cholinergic neurons. *American Journal of Physiology* **262**:G685-G689.

Schubert M L & Makhlouf G M (1992) Neural, hormonal, and paracrine regulation of gastrin and acid secretion. *The Yale Journal of Biology and Medicine* **65**:553-560.

Schubert M L (1993) Control of exocrine and endocrine secretion from the stomach. *Current Opinion in Gastroenterology* **9**:889-901.

Schubert M L & Makhlouf G M (1993) Gastrin secretion induced by distension is regulated by cholinergic and vasoactive peptide neurons in rats. *Gastroenterology* **104**:834-839.

Schubert M L (1994) Regulation of gastric secretion. *Current Opinion in Gastroenterology* **10**:575-588.

Schusdziarra V, Harris V, Conlon J M, Arimura A & Unger R (1978) Pancreatic and gastric somatostatin release in response to intragastric and intraduodenal nutrients and HCl in the dog. *The Journal of Clinical Investigation* **62**:509-518.

Seal A, Yamada T, Debas H, Hollinshead J, Osadchey B, Aponte G & Walsh J (1982) Somatostatin-14 and -28: clearance and potency on gastric function in dogs. *American Journal of Physiology* **243**:G97-G102.

Seaton D S, Jackson F, Smith W D & Angus K W (1989) Development of immunity to incoming radiolabelled larvae in lambs continuously infected with *Ostertagia circumcincta*. *Research in Veterinary Science* **46**:241-246.

Seidler U, Beinborn M & Sewing K-F (1989) Inhibition of acid formation in rabbit parietal cells by prostaglandins is mediated by the prostaglandin E2 receptor. *Gastroenterology* **96**:314-320.

Selman I E, Armour J, Jennings F W & Reid F W (1977) Interpretation of the plasma pepsinogen test. *The Veterinary Record* **100**:249-250.

Shaw G P, Hatt J F, Anderson N G & Hanson P J (1987) Action of epidermal growth factor on acid secretion by rat isolated parietal cells. *Biochemistry Journal* **244**:699-704.

Sher R & Glover A (1976) Isolation of human eosinophils and their lymphocyte-like rosetting properties. *Immunology* **31**:337-341.

Shirakawa T & Hirschowitz B I (1984) Bombesin-induced pepsinogen secretion from frog esophagus peptic glands in vitro. *Gastroenterology* **86**:1250.

Shorrock C J & Rees W D W (1992) Mucosal adaptation to indomethacin induced gastric damage in man: studies on morphology, blood flow, and prostaglandin E_2 metabolism. *Gut* **33**:164-169.

Short G M, Doyle J W & Wolfe M M (1985a) Effect of antibodies to somatostatin on acid secretion and gastrin release by the isolated perfused rat stomach. *Gastroenterology* **88**:984-988.

Short G M, Reel G M, Doyle J W & Wolfe M M (1985b) Effect of GRP on B-adrenergic-stimulated gastrin and somatostatin release in the isolated rat stomach. *American Journal of Physiology* **246**:G197-202.

Shulkes A & Hardy K J (1980) Effect of bombesin on pancreatic polypeptide secretion in the sheep. *Proceedings of The Australian Physiological and Pharmacological Society* **11**:12.

Shulkes A & Hardy K J (1982) Effect of somatostatin on basal concentrations of gastrin and pancreatic polypeptide in the fetal sheep. *Biology of the Neonate* **42**:249-256.

Shulkes A & Read M A (1991) Regulation of somatostatin by gastrin- and acid-dependent mechanisms. *Endocrinology* **129**:2329-2334.

Shulkes A, Westbrook S and Hardy K (1994) Somatostatin inhibition of GRP stimulated gastrin secretion unveiled by active immunization against somatostatin. *Gastroenterology* **106**:A840.

Simpson H V, Reynolds G W & Carr D H (1993) Low tissue gastrin content in the ovine distal duodenum is associated with increased percentage of G34. *Comparative Biochemistry and Physiology* **104A**:461-468.

Sjodin L, Dahlen H & Viitanen E (1992) Binding of epidermal growth factor to receptors in preparations of enriched porcine parietal cells and inhibition of aminopyrine uptake. *Scandinavian Journal of Gastroenterology* **27**:495-500.

Smith W D, Jackson F, Jackson E & Williams J (1983a) Local immunity and *Ostertagia circumcincta*: changes in the gastric lymph of sheep after a primary infection. *Journal of Comparative Pathology* **93**:471-478.

Smith W D, Jackson F, Jackson E & Williams J (1983b) Local immunity and *Ostertagia circumcincta:* changes in the gastric lymph of immune sheep after a challenge infection. *Journal of Comparative Pathology* **93**:479-488.

Smith W D, Jackson F, Jackson E, Williams J & Miller H R P (1984) Manifestations of resistance to ovine ostertagiasis associated with immunological responses in the gastric lymph. *Journal of Comparative Pathology* **94**:591-601.

Smith W D, Jackson F, Jackson E & Williams J (1985a) Age immunity to *Ostertagia circumcincta*: comparison of the local immune responses of 4½-and 10-month-old lambs. *Journal of Comparative Pathology* **95**:235-245.

Smith W D, Jackson F, Jackson E & Williams J (1985b) Ovine ostertagiasis: a natural host parasite interaction for studying protective local immune responses in the gut. *Immunology of Sheep* pp483-498. Edited by B Morris & M Miyasaka. Editones <Roche>, Switzerland.

Smith W D, Jackson F, Jackson E, Graham R, Williams J, Willadsen S M & Fehilly C B (1986) Transfer of immunity to *Ostertagia circumcincta* and IgA memory between identical sheep by lymphocytes collected from gastric lymph. *Research in Veterinary Science* **41**:300-306.

Smith W D, Jackson F, Graham R, Jackson E & Williams J (1987) Mucosal IgA

production and lymph cell traffic following prolonged low level infections of *Ostertagia circumcincta* in sheep. *Research in Veterinary Science* **43**:320-326.

Smith W D (1988) Mechanisms of immunity to gastrointestinal nematodes of sheep. *Increasing small ruminant productivity in semi-arid areas* pp275-286. Edited by E F Thompson & F S Thomson. ICARDA, Netherlands.

Snider T G, Williams J C, Karns P A, Markovits J E & Romaire T L (1988a) High concentration of serum gastrin immunoreactivity and abomasal mucosal hyperplasia in calves infected with *Ostertagia ostertagi* and/or *Trichostrongylus axei*. *American Journal of Veterinary Research* **49**:2101-2104.

Snider T G, Willliams J C, Knox J W, Marbury K S, Crowder C H & Willis E R (1988b) Sequential histopathologic changes of Type I, pre Type II and Type II ostertagiasis in cattle. *Veterinary Parasitology* **27**:169-179.

Soll A H (1978) The actions of secretagogues on oxygen uptake by isolated mammalian parietal cells. *The Journal of Clinical Investigation* **61**:370-380.

Soll A H & Walsh J H (1979) Regulation of gastric acid secretion. *Annual Reviews in Physiology* **41**:35-53.

Soll A H & Wollin A (1979) Histamine and cyclic AMP in isolated canine parietal cells. *American Journal of Physiology* **237**:E444-E450.

Soll A H (1980) Secretagogue stimulation of [^{14}C]aminopyrine accumulation by isolated canine parietal cells. *American Journal of Physiology* **238**:G366-G375.

Soll A H, Amirian D A, Thomas L P, Reedy T J & Elashoff J D (1984a) Gastrin receptors on isolated canine parietal cells. *Journal of Clinical Investigation* **73**:1434-1447.

Soll A H, Yamada T, Park J & Thomas L P (1984b) Release of somatostatin-like immunoreactivity from canine fundic mucosal cells in primary culture. *American Journal of Physiology* **247**:G558-G566.

Soll A H, Chen C Y, Amirian D A, Toomey M & Alvarez R (1986) Prostanoid inhibition of canine parietal cells. *American Journal of Medicine* **81**,Supplement 2A:5-11.

Sommerville R I (1954) The histotrophic phase of the nematode parasite, *Ostertagia circumcincta*. *Australian Journal of Agricultural Research* **5**:130-140.

Sommerville R I (1956) The histology of the ovine abomasum, and the relation of the globule leucocyte to nematode infestations. *Australian Veterinary Journal* pp237-240.

Sommerville R I (1957) The exsheathing mechanism of nematode infective larvae. *Experimental Parasitology* **6**:18-30.

Sommerville R I (1963) Differential growth of *Ostertagia* spp. in the sheep's abomasum. *The Journal of Parasitology* **49**:698-699.

Soulsby E J L (1965) *Textbook of Veterinary Clinical Parasitology, Volume 1, Helminths*. Blackwell Scientific Publications, Oxford.

Soulsby E J L (1982) *Helminths, Arthropods and Protozoa of Domesticated Animals*. Bailliere Tindall, London.

Stafford K J, West D M & Pomroy W E (1994) Nematode worm egg output by ewes. *New Zealand Veterinary Journal* **42**:30-32.

Stanovnik L & Logonder-Mlinsek M (1993) Is mast cell histamine involved in the regulation of acid secretion in the guinea pig stomach? *Agents Actions* **38**,Supplement:C298-C300.

Stechschulte D J Jr, Morris D C, Jilka R L, Stechschulte D J & Dileepan K N (1990) Impaired gastric acid secretion in mast cell-deficient mice. *American Journal of Physiology* **259**:G41-G47.

Stringfellow F & Madden P A (1979) Effects of *Ostertagia ostertagi* on pepsinogen granules of chief cells from calf abomasa correlated with selected plasma and abomasal proteins. *Proceedings of the Helminthological Society of Washington* **46**:233-239.

Strunz U T, Walsh J H & Grossman M I (1978) Stimulation of gastrin release in dogs by individual amino acids. *Proceedings of the Society for Experimental Biology and Medicine* **157**: 440-441.

Sugano K, Park J, Soll A & Yamada T (1986) Phorbol esters stimulate somatostatin release from cultured cells. *American Journal of Physiology* **250**:G686-G690.

Sugano K, Park J, Soll A H & Yamada T (1987) Stimulation of gastrin release by bombesin and canine gastrin-releasing peptides. *The Journal of Clinical Investigation* **79**:935-942.

Svetic A, Madden K B, Zhou X D, Lu P, Katona I M , Finkelman F D, Urban A F & Gause W C (1993) A primary intestinal helminthic infection rapidly induces a gut-associated elevation of Th2-associated cytokines and IL-3. *Journal of Immunology* **150**:3434-3441.

Sykes A R & Coop R L (1977) Intake and utilization of food by growing sheep with abomasal damage caused by daily dosing with *Ostertagia circumcincta* larvae. *Journal of Agricultural Science* **88**:671-677.

Tabuchi Y & Furuhama K (1994) Inhibitory effect of DS-4574, a mast cell stabilizer with peptidoleukotriene antagonism, on gastric acid secretion in rats. *European Journal of Pharmacology* **225**:229-234.

Taché Y, Lesiege D & Goto Y (1986) Neural pathways involved in intracisternal

bombesin-induced inhibition of gastric secretion in rats. *Digestive Disease Science* **31**:412-417.

Taché Y (1991) Central mechanisms in control of acid secretion. *Current Opinion in Gastroenterology* **7**:842-848.

Takeuchi K, Ohuchi T & Okabe S (1994) Endogenous nitric oxide in gastric alkaline response in the rat stomach after damage. *Gastroenterology* **106**:367-374.

Tang L H, Miller M D, Goldenring J R, Modlin I M & Hersey S J (1993) Partial agonism by gastrin for a cholecystokinin receptor mediating pepsinogen secretion. *American Journal of Physiology* **265**:G865-G872.

Tarnawski A, Stachura J, Durbin T, Sarfeh I J & Gergely H (1992) Increased expression of epidermal growth factor receptor during gastric ulcer healing in rats. *Gastroenterology* **102**:695-698.

Taylor I N, Bryne W J, Christie D L, Ament M E & Walsh J H (1982) Effect of individual L-amino acids on gastric acid and serum gastrin and pancreatic polypeptide release in humans. *Gastroenterology* **83**:273-278.

Taylor L M, Parkins J J, Armour J, Holmes P H, Bairden K, Ibarra-Silva A M, Salman S K & McWilliam P N (1989) Pathophysiological and parasitological studies on *Ostertagia ostertagi* infections in calves. *Research in Veterinary Science* **46**:218-225.

Taylor P (1980) *Ganglionic Stimulating and Blocking Agents.* Goodman and Gilman's The Pharmacological Basis of Therapeutics, 6th edition. Edited by A G Gilman, L Goodman & A Gilman; MacMillan, New York.

Threlkeld W L (1934) The life history of *Ostertagia circumcincta. Technical Bulletin 52 Virginia Polytechnic Institute* pp1-23.

Titchen D A & Anderson N (1977) Aspects of the physio-pathology of parasitic gastritis in the sheep. *Australian Veterinary Journal* **53**:369-373.

Titchen D A (1982) Hormonal and physiological changes in helminth infestations. *Biology and control of endoparasites* pp257-275. McMaster Laboratory 50th Anniversary Symposium.

Titchen D A & Reid A M (1988) Putative roles of peptides in the genesis and control of parasitic diseases. *Aspects of Digestive Physiology in Ruminants* pp217-238. Edited by A Dobson and M J Dobson. Cornell University Press.

Todd A C, Arbogast F M, Wyant Z N, Stone W M & Elam G W (1951) On the blood picture of sheep exposed to the medium stomach worms. *Veterinary Medicine* pp136-142.

Uete T, Wasa M & Shimogami A (1969) A simplified method for the determination of pepsinogen in blood and urine. *Clinical Chemistry* **15**:42-55.

Uvnäs-Mobberg K, Järhult J & Alino S (1984) Neurogenic control of release of gastrin and somatostatin. *Scandinavian Journal of Gastroenterology* **19**:131-136.

Van Bruchem J (1977) Abomasal secretion and motility in sheep. *Verslag Landbouwkunde Onderstepoort* **868**:1-140.

Van Bruchem J & Van 'T Klooster A T (1980) Effect of protein on abomasal secretion of acid in sheep. *British Journal of Nutrition* 44:307-312.

Varner A A, Modlin I M & Walsh J H (1981) High potency of bombesin for stimulation of human gastrin release and gastric acid secretion. *Regulatory Peptides* **1**:289-296.

Vergara-Estaras P, Harrison F A & Brown D (1990) The localization of somatostatin-like immunoreactivity in the alimentary tract of the sheep with observations on the effect of an infection with the parasite *Haemonchus contortus*. *Experimental Physiology* **75**:779-789.

Vigna S R, Mantyh C R, Giraud A S, Soll A H, Walsh J H & Mantyh P W (1987) Localization of specific binding sites for bombesin in the canine gastrointestinal tract. *Gastroenterology* **93**:1287-1295.

Von Brand T (1973) Proteases. In: *Biochemistry of Parasites*. pp268-275. Academic Press, New York.

Wakelin D (1978) Immunity to intestinal parasites. *Nature* **273**:617-620.

Wakelin D (1992) Immunogenetic and evolutionary influences on the host-parasite relationship. *Developmental and Comparative Immunology* **16**:345-353.

Wallace J L, Cucala M, Mugridge & Parente L (1991) Secretagogue-specific effects of interleukin-1 on gastric acid secretion. *American Journal of Physiology* **261**:G559-G564.

Wallace J L & Bell C J (1992) Gastroduodenal mucosal defense. *Current Opinion in Gastroenterology* **8**:911-917.

Walsh J H (1981) Gastrointestinal hormones and peptides. *Physiology of the Gastrointestinal Tract* pp59-144, edited by L R Johnson, Raven, New York.

Walsh J H (1984) Gastric Secretion. In: *The role of the gastrointestinal tract in nutrient delivery* pp107-118. Edited by
M Greene & H L Greene, Academic Press.

Walsh J H (1989a) Bombesin-like peptides. *Handbook of Physiology, section 6: The Gastrointestinal System* pp587-605. Edited by S G Shultz, American Physiological Society, Washington D C.

Walsh J H (1989b) Why does the gastric oxyntic gland mucosa contain gastrin-releasing peptide nerve-fibres? (editorial) *Gastroenterology* **97**:793-797.

Wang L, Lucey M R, Fras A M, Wilson E J & Del Valle J (1993) Epidermal growth factor and transforming growth factor-α directly inhibit parietal cell function through a similar mechanism. *The Journal of Pharmacology and Experimental Therapeutics* **265**:308-313.

Wathuta E M (1986) The distribution of vasoactive intestinal polypeptide-like, substance P-like and Bombesin-like immunoreactivity in the digestive system of the sheep. *Quarterly Journal of Experimental Physiology* **71**:615-631.

Wershil B K (1992) Immune mediators and cytokines in gastrointestinal inflammation. *Current Opinion in Gastroenterology* **8**:975-982.

White R L, Rossiter C D, Hornby P J, Harmon J W, Kasbekar D K & Gillis R A (1991) Excitation of neurons in the medullary raphe increase gastric acid and pepsin production in cats. *American Journal of Physiology* **260**:G91-G96.

Whittle B J R & Vane J R (1987) Prostanoids as regulators of gastrointestinal function. *Physiology of the Gastrointestinal Tract* **1**:143-180. Edited by L R Johnson. Raven, New York.

Wilkes J M, Kajimura M, Scott D R, Hersey S J & Sachs G (1991) Muscarinic responses of gastric parietal cells. *The Journal of Membrane Biology* **122**:97-110.

Willems G, Vansteenkiste Y & Limbosch J M (1972) Stimulating effect of gastrin on cell proliferation kinetics in canine fundic mucosa. *Gastroenterology.* **62**:583-589.

Willems G (1991) Neurohormonal regulation of gastric mucosal growth. *Growth of the Gastrointestinal Tract: Gastrointestinal Hormones and Growth Factors* pp131-142. Edited by J Morisset and T E Solomon. CRC Press, USA.

Williams J C, Roberts E D & Todd J M (1974) *Ostertagia ostertagi*: establishment of patent infections in calves by intravenous inoculation. *International Journal for Parasitology* **4**:55-61.

Williams S E & Turnberg L A (1980) Retardation of acid diffusion by pig gastric mucus: a potential role in mucosal protection. *Gastroenterology* **79**:299-304.

Wolfe M M, Reel G M & McGuigan J E (1983) Inhibition of gastrin release by secretin is mediated by somatostatin in cultured rat antral mucosa. *Journal of Clinical Investigation* **72**:1586-1593.

Wolfe M M, Jain D K, Reel G M & McGuigan J E (1984) Effects of carbachol on gastrin and somatostatin release in rat antral tissue culture. *Gastroenterology* **87**:86-93.

Wolfe M M & Reel G M (1986) Inhibition of gastrin release by gastric inhibitory peptide mediated by somatostatin. *American Journal of Physiology* **250**:G331-G335.

Wright N A, Pike C & Elia G (1990) Induction of a novel epidermal growth factor-secreting cell lineage by mucosal ulceration in human gastrointestinal stem cells. *Nature*

343:82-85.

Yakoob A, Holmes P H & Armour J (1983) Pathophysiology of gastrointestinal trichostrongyles in sheep: plasma losses and changes in plasma pepsinogen levels associated with parasite challenge of immune animals. *Research in Veterinary Science* **34**:305-309.

Yamada T, Soll A H, Park J & Elashoff J (1984) Autonomic regulation of somatostatin release: studies with primary cultures of canine fundic mucosal cells. *American Journal of Physiology* **247**:G567-G573.

Yamashika Y, Tabuchi Y & Kokue E (1994) Antisecretory effect of DS-4574, a mast cell stabilizer with peptidoleukotriene antagonism, on gastric acid secretion in the pig. *Agents Actions* **41**:17-20.

Yanaka A, Muto H, Ito S & Silen W (1993) Effects of ammonium ion and ammonia on function and morphology of in vitro frog gastric mucosa. *American Journal of Physiology* **265**:G277-G288.

Yiango Y, Christofides N D, Blank M A, Yanaihara N, Tatemoto K, Bishop A E, Polak J M & Bloom S R (1985) Molecular forms of peptide histidine isoleucine-like immunoreactivity in the gastrointestinal tract. *Gastroenterology* **89**:516-524.

Yoneda M & Taché (1992) Central thyrotropin-releasing factor analog prevents ethanol-induced gastric damage through prostaglandins in rats. *Gastroenterology* **102**:1568-1574.

Zachary I, Woll P J & Rozengurt E (1987) A role for neuropeptides in the control of cell proliferation. *Developmental Biology* **124**:295-30

Zeiger R S, Yurdin D L & Colten H R (1976) Histamine metabolism. II. Cellular and subcellular localization of the catabolic enzymes, histaminase and histamine methyl transferase, in human leukocytes. *Journal of Allergy and Clinical Immunology* **58**:172-179.

Appendix 1

ASSAYS

1.1 PEPSINOGEN ASSAY

Pepsinogen concentration was determined by converting the proenzyme to pepsin with HCl and assaying the amount of product (tyrosine) liberated from substrate (serum proteins or BSA in BM). To increase the amount of substrate in the BM to about the same as in serum, 1% BSA was included in the incubation fluid (1.25 ml of 0.6 M HCl).

0.25 ml of sample was mixed with 1.25 ml of 0.06 M HCl in duplicate in 5 ml plastic tubes. To one of each duplicate, 1 ml of 10% TCA was added immediately while the other was incubated for three hours at 37^{0}C before TCA was added. Both duplicates were centrifuged at 3500 rpm for 15 minutes. It was found to be important to allow the mixture to stand for at least 10 minutes after the addition of TCA before centrifugation. However, immediately prior to centrifugation it was necessary to vortex the mixture momentarily in order to ensure that the precipitate sedimented completely. From each duplicate, 1.0 ml of supernatant was taken and added to 2.0 ml of 0.5 M NaOH. A further 0.5 ml of Folin-Ciocateus phenol reagent was added to each of the duplicates. After 6 minutes of colour development (oxidation) absorbence was measured at 700nm using a spectrophotometer. The amount of tyrosine present was equated with absorbence by use of a standard curve. The difference in free tyrosine concentration between incubated and non-incubated samples was attributed to pepsinogen derived pepsin activity. Sample pepsinogen concentration was expressed as mU tyr/L serum (1 µmole tyr/litre/minute x 1000; 1 IE = 1 mmol tyr). One IE/L (international enzyme unit/L) is defined as the quantity of enzyme that will per L that will release 1 umol of substrate per minute at optimal pH and temperature.

The standard curve formed a linear relationship. It was derived by reading the absorbance in solutions of similar composition to the alkalinised sample derivates after addition of the phenol reagent. The standards, however, had known concentrations of tyrosine. The minimum standard (0) was made by adding 1 ml of H_20 to 2 ml of 0.5 M NaOH. The maximum standard (100 uM) was made by adding 0.4 ml TCA, 0.1 ml H_2O and 0.5 ml of tyrosine stock solution to 2 ml of 0.5 M NaOH. As the sample volume (of 0.25 ml) is only 10% of the final test volume from which the 1 ml of supernatant is drawn,

$$\text{uM tyr} = (OD_{test} - OD_{control}) \div (OD_{total} - OD_{blank}) \times 0.1$$

This concentration is then expressed as mU tyr by the equation

$$\text{mU/L} = \text{uM} \div 180 \text{ (length of incubation)} \times 1000 \text{ (mls/litre)}$$

1.2 GASTRIN RADIOIMMUNOASSAY

This was a modification of the method of Hansky & Cain (1969).

Assay Buffer: 0.02 M Veronal buffer pH 8.6 containing per litre:
4.12 g Na barbiturate
0.744 g barbitone
5 g bovine serum albumin
100 mg thiomerosal
10 mg neomycin

Tracer: Synthetic ns human G-17 (Research Plus, Bayanne N.J. U.S.A.) was labelled with I^{125} using the chloramine T method. The label was purified on a Sephadex G10 column followed by separation on a DEAE cellulose column with a NaCl gradient from 0 to 1 M. The tracer for the assay contained 1200-1600 cpm per 500 µl.

Antiserum: Ab 74 (the kind gift of Dr Hansky) which binds equally with human, porcine and ovine sulphated and non-sulphated G14, G17 and G34 was used in a final dilution of 1:100,000 with 1:400 normal rabbit serum (NRS). The solution for the assay contained 1:250 NRS and 1:40000 Ab.

Standards: Synthetic ns human G-17 was made up in assay buffer in concentrations of 0, 2, 5 10, 20, 50, 100 and 200 pM.

Second antibody: Serum from sheep immunized with rabbit gamma globulin was used as the precipitating antibody. This was standardized against Donkey anti-rabbit globulin (IDS, England).

Assay procedure: Assays were conducted in triplicate. Assay tubes contained:
(i) Total - 500 µl tracer
(ii) NSB - 100 µl buffer, 400 µl NRS without Ab, 500 µl tracer
(iii) Standards - 100 µl standard solution, 400 µl Ab, 500 µl tracer
(iv) Samples - 100 µl sample, 400 µl Ab, 500 µl tracer

The tubes were incubated for 2 days at 4°C. 200 µl second Ab was added to all tubes except (i) and incubated for a further 3 days at 4°C. Tubes were centrifuged at 3000 rpm for 30 minutes, the supernatant discarded and the pellet counted for 5 minutes in a gamma counter. Gastrin concentrations were determined from a standard curve and expressed as pM.

1.3 SOMATOSTATIN RADIOIMMUNOASSAY

Assay Buffer: pH 7.4 containing per litre:
4.9 g Na barbital
0.32 g Na acetate
5 g bovine serum albumin
100 mg thiomerosal
10 ml Trasylol

Tracer: ^{1}Tyr-somatostatin (Peninsular Laboratories, California, U.S.A.) was labelled with I^{125} using the chloramine T method. On the day of the assay, the label was purified on CM-cellulose (CM 52) column with ammonium acetate buffer gradient from 0.002 to 0.2 M NaCl. The tracer for the assay contained 3500 cpm per 100 µl.

Antiserum: Monoclonal antibody Soma 03 (the kind gift of Prof C.H. McIntosh) was used in a final dilution of 1:4,000,000. (The solution for the assay contained 1:100,000 Ab).

Standards: Somatostatin was dissolved in 0.1 N acetic acid containing 0.05% BSA to give a final concentration of 5 µg/50 µl and stored lyophilized. For the assay, 100 µl distilled water followed by 400 µl assay buffer were added to give a concentration of 10 µg/ml. Sequential dilutions with buffer gave standards of concentrations of 0, 31.25, 62.5, 125, 250, 500, 1000, 2000, 4000 and 8000 pg/ml.

Assay procedure: Assays were conducted in triplicate. Glass tubes were used.
(i) Total - 100 µl tracer
(ii) NSB - 300 µl buffer, 100 µl tracer
(iii) Standards - 100 µl standard solution, 100 µl buffer, 100 µl Ab, 100 µl tracer
(iv) Samples - 100 µl each of sample, buffer, Ab and tracer.

The tubes were incubated for 3 days at 4^{0}C.

Separation of bound and free label:

Dextran T 70 was dissolved in 0.05 M phosphate buffer pH 7.5 then activated charcoal (Norit 1.25 g/100 ml) and hormone free plasma (100 µl/100 ml) added and the mixture stirred for 1 hour. One ml aliquots were added to each tube except (i), mixed, allowed to stand for 10-15 minutes then centrifuged at 3000 rpm for 30 minutes. The supernatant was decanted, the pellet dried and counted for 3 minutes in a gamma counter. Somatostatin concentrations were expressed as pg/ml.

Appendix 2

PARASITOLOGY

2.1 PROCEDURE FOR RAISING PARASITE NAIVE SHEEP

Experiment 2 Pregnant ewes were brought indoors approximately 1 week prior to lambing and held on clean grating. They were treated with ivermectin (Ivomec liquid for sheep and goats, MSD, AGVET, NZ) and had a slow release anthelmintic capsule placed in their rumens. Upon lambing, lambs were separated from their dams and hand reared on milk replacer for the first 30 days of life.

Experiment 3 Pregnant ewes were left on pasture to lamb. Lambs were removed from their dams within 1 hour of birth and housed indoors. The lambs were reared on milk replacer for 30 days. From 30 days onwards the lambs were allowed access to replacement meal. Weaning was at 60 days, at which time meal, lucerne nuts and lucerne chaff were made freely available.

2.2 DONOR SHEEP

Rams less than 1 year old were obtained off pasture. on acquisition they were drenched with double doses of ivermectin (0.8 mg/kg; Ivomec liquid for sheep and goats, MSD, AGVET, NZ) and Leviben (16 mg/kg; Young's Animal Health Ltd, NZ). Faecal floats were performed 1 and 2 weeks after drenching to ensure these sheep were free of all nematode parasites. Animals were then infected with a pure strain of *O. circumcincta* larvae by ruminal intubation and kept in a metabolism crate. Infections were monitored by faecal examination.

2.3 PROCEDURE USED TO CULTURE LARVAE

Donor sheep were infected with approximately 50,000 larvae. When the infection became patent faeces were collected daily with a faecal bag. Faeces were mixed with approximately half their volume of vermiculite and sufficient deionised water to produce a moist yet friable crumb. The resulting mixture was incubated in a plastic tray covered by a sheet of glass for 7 days at 27°C. The moisture level of the mixture was checked occasionally to ensure it did not dehydrate. After 7 days the larvae were recovered using a modified Baermann technique.

2.4 PROCEDURE USED TO RECOVER LARVAE

Larvae were recovered from cultured faeces using a modified Baermann technique. An inverted funnel with a short length of rubber tubing attached to its spout was used. The tubing was clamped using artery forceps and the funnel almost filled with deionised water. A large sieve was lined with tissue paper (Snowtex). The sieve was filled with the larval culture mix (after 7 days incubation) and placed over the surface of the funnel so that the mixture was immersed in water. After 24 hours 50 mls of fluid containing

most of the larvae that had migrated from the faecal mix was tapped off from the bottom of the funnel. The larvae were rinsed by mixing the 50 mls with a litre of deionised water in a measuring cylinder and allowing the larvae to sediment for 4 hours. Surface water was removed by aspiration. Rinsing was repeated 2 or 3 times. Larvae were bottled in 50 mls deionised water and stored at 10°C.

2.5 PROCEDURE FOR COUNTING LARVAE AND ASSESSING VIABILITY

Counting: 10 replicate dilutions of stock suspension were made. One ml of each dilution was placed in a well on a glass slide. A few drops of iodine were added to kill and colourise the larvae. A coverslip was placed over the meniscus of fluid in the well. Two minutes were allowed for the dead larvae to settle. Larvae were counted using a microscope and their number in the stock suspension estimated by multiplying the average of the 10 counts by the dilution factor.

Viability: A drop of larval suspension was placed on a slide and covered with a glass slip. 100 larvae were observed and counted using a microscope and the number that wriggled was taken as the percentage viable.

2.6 IDENTIFICATION OF LARVAE

Taxonomic determination of larvae can be performed using a variety of criteria. Some possible contaminants such as *Nematodirus* spp. were excluded as they do not hatch within the 7 days over which faeces were incubated. Others could be distinguished from Trichostrongylid larvae on gross morphology. Differentiation of Trichostrongylid spp. was made on the basis of length (> 720 µm total length) and tail morphology using the Ministry of Agriculture, Fisheries and Food's "Manual of Veterinary Parasitological Laboratory Techniques" (reference book 418).

For confirmation of larval identity and culture purity, the parasite burden in donor sheep that were used to produce the eggs, and thus larvae, was examined when they were destroyed. The parasites present in animals infected with the larvae provided retrospective information on culture purity.

2.7 POSTMORTEM PARASITOLOGY

The postmortem parasitological examination of sheep that were used as experimental animals or donors involved counting two 5% volumes of total contents from the proximal 6 m of intestine or from the abomasum. Caecum, colon and other parts of the gastrointestinal tract were examined for the presence of *Trichuris*, *Oesophagostomum*, *Chabertia*, *Moniezia* and other parasites.

2.8 PROCEDURE USED TO EXSHEATH LARVAE AND TRANSFER THEM TO RECIPIENT SHEEP

An equal volume of larvae stock suspension was mixed with 0.2% sodium hypochlorite and placed in a water bath at 37°C for 15 minutes. Confirmation of exsheathment was by microscopic examination. The mixture was filtered using a 43 µm millipore filter (Micro Filtration Systems, USA). The larvae, which were retained on the filter, were rinsed 5 times with deionised water. Larvae were rinsed off the filter paper with a small

volume of deionised water and counted (Appendix 2.5). The required volume of larval suspension was transferred by syringe into the abomasum *per cannula*, followed by a 10 ml water chaser.

2.9 PROCEDURE USED TO DETERMINE THE PRESENCE OF NEMATODE EGGS IN FAECES (faecal floats)

Faeces were collected per rectum. Two grams of faeces were macerated and mixed with saturated NaCl solution (approximately 30 mls). The mixture was poured into a vial so as to form a meniscus at its surface, over which a cover slip was placed. After 15 minutes the cover slip was carefully uplifted (with a deft upward motion) so as to take with it the surface layer of mixture, placed on a microscope slide and examined under 10 x objective. The absence of any nematode eggs after the examination of 2 faecal floats on two successive days was considered to indicate the absence of nematode parasites and a negative result.

2.10 PROCEDURE FOR COUNTING THE NUMBER OF EGGS PER GRAM FAECES

Faecal egg counts were performed using a modified McMaster technique (Stafford *et al.*, 1994). Fresh faeces were collected per rectum, of which 2 grams were mixed with 28 ml of saturated NaCl in a sieve in a bowl. Using a teaspoon the faeces were worked through the sieve and the faecal residue discarded. While mixing the contents of the bowl a sample was taken and immediately transferred to one chamber of a counting slide. Mixing and sampling were repeated to fill the other chamber. The slide was kept level at all times. After 2 minutes the eggs within the grids of both chambers were counted using a microscope. Each egg counted represents 50 e.p.g.

2.11 PRODUCTION, COLLECTION AND TRANSFER OF ADULT WORMS

Adult worms for transfer were obtained by the single infection of 8 four month old donor sheep (Appendix 2.2) with 100 000 larvae each. Four weeks after infection the sheep were killed by captive bolt and exsanguination and the contents **only** of their abomasa collected. The contents were pooled and concentrated by a repeated process of sedimentation in a 2 L measuring cylinder, itself standing in a water bath at 37^{0}C. Once the contents had been reduced to a volume of approximately 1 L, they were gently mixed and five 10 ml samples were taken for worm counting. These samples were replaced after counting was complete. The concentrate was again gently mixed immediately prior to its transfer and the abomasa of recipient animals drained by releasing the cannulae for about 2 minutes. Each of the recipient animals was given a volume of 280 mls of concentrated abomasal contents, given as two 140 ml volumes 30 minutes apart, and followed by 10 mls of water within 4 hours of the slaughter of donors. A lavage syringe which had a wide bore nozzle was used to inject the concentrate via the cannula so as to minimise damage to the worms. It is estimated that each sheep received 15 000 adult worms. Exact counting of adult worms was confounded by their tendency to form clumps. Although larvae were not specifically searched for it was noted during counting that, although immature adults were present, no larval stages (L4) were apparent.

2.12 *NEMATODIRUS* EGGS FOUND IN EXPERIMENT 3 SHEEP (Chapter 2)

In sheep 15-32 respectively: 0,2,2,0,0,4,1,0,3,4,1,3,2,4,0,0,0,1.
An average of 1.5 eggs were detected per faecal float.

2.13 PRODUCTION OF ADULT WORMS FOR INCUBATIONS *IN VITRO*.

Batch 1: 3 donor sheep that had been infected thrice weekly with 10 000 larvae for 28 days were killed 35 days after initial infection.

Batch 2: 1 donor sheep that had been infected and used to collect eggs for larval cycling was killed 35 days after last infection with 50 000 larvae.

Batch 3: 2 donor sheep were killed 28 days after infection with 100 000 larvae.

Batch 4: 3 female goats were killed 22 days after infection with 50 000 larvae.

Batch 5: 3 sheep infected with 100 000 larvae were killed 30 days after infection.

2.14 METHOD FOR THE RETRIEVAL OF CLEAN AND VIGOROUS WORMS

Ligated abomasa were removed from donor animals (Appendix 2.13) at the time of their death (captive bolt and exsanguination). Abomasa were opened along their greater curvature and the contents collected. The luminal surface of each abomasum was washed with 0.9% NaCl and the washings retained. Once clean, the abomasum was submerged in saline and agitated by hand for 5 minutes before it was discarded. The contents, washings and agitant were mixed together and allowed to settle in a large measuring cylinder. Surface fluid from which worms had sedimented was removed in order to reduce the volume of worm mixture. Three percent agar (Bacto Agar, DIFCO Laboratories, USA) was prepared using a microwave and allowed to cool. When it was between 40 and 50^{0}C, and prior to its setting, it was rapidly mixed with twice its volume of worm mixture (final concentration 1% agar). Once thoroughly mixed and before setting the worm-agar mixture was poured into sealed sieves. Prior to slaughter of the sheep a number of large kitchen sieves were sealed by covering the outer convex wire surface with gladwrap plastic. It was possible to pull the gladwrap sheet sufficiently tightly over the sieve so as to prevent any pockets of fluid collecting between it and the gladwrap. Once the worm-agar mixture had set in the sealed sieves, the gladwrap was removed from the outside and the sieve was placed in a bowl of saline at 37^{0}C. The saline was of a depth that flooded the surface of the agar block within the sieve. Worms immediately migrated from the agar block into the saline where they proceeded to form clumps. About 90% of those worms that migrated from the agar had done so in the first hour. Clumps of worms were easily picked out of the saline for further work. These worms were remarkably free of abomasal debris and, as they had just wriggled free of the agar gel, were all active.

2.15 PREPARATION OF WORM DERIVED SOLUTIONS

Larvae. 3 x 10^6 larvae were exsheathed (Appendix 2.8) and washed off the filter paper with BM. The mixture was made up to 50 mls in a measuring cylinder and incubated at 37^{0}C in a water bath. The larvae gradually sedimented to the bottom of the cylinder. Every hour 40 mls was removed from the top of the mixture using a syringe with a silastic tube attached to its nozzle and the 40 mls replaced by fresh BM. Care was taken not to disturb or suck up larvae from the bottom. The 40 ml of supernatant was centrifuged at 2000 rpm for 10 minutes to remove suspended larvae. The supernatants from each of three consecutive hourly incubations were pooled, mixed and stored at -20^{0}C in 4.0 ml aliquots. After 6 successive hourly incubations the larvae were incubated overnight (9 hours) in 50 mls of medium. Following removal of the supernatant from the overnight incubation the larvae were sonicated using an ultrasonic disintegrator (MSE Soniprep 150, MSE Scientific Instruments, Manor Royal, England) for about 5

minutes and until no complete larvae were identifiable under the microscope. This "extract" was suspended in 100 mls of BM and stored.

Batch 1. 6000 adult worms were collected manually and placed in BM. The worms were then transferred to 50 mls of fresh BM in a measuring cylinder and incubated in a water bath at 37°C. Every 3 hours the solution was changed (supernatant removed and replaced, centrifuged, and further supernatant separated). After 4 such incubations a final overnight incubation followed (9 hours). The supernatant from each incubation was retained at 4°C until, after completion of the overnight incubation, all the supernatants were pooled together and mixed before storage. The remaining adult worms all appeared to be alive. These were then sonicated and the resultant extract suspended in 100 mls of BM.

Batch 2 (migration from agar). Clumps of worms were placed in BM or distilled water. The worms were given 4 successive 3 hour incubations in BM or water at 37°C. After this time many of the worms incubated in water appeared lifeless and their incubation was stopped. The supernatants were combined and stored. Incubation of the worms in BM was continued for a further period overnight (9 hours). The supernatants from each incubation which had been retained at 4°C were pooled and mixed before storage. The adult worms from the BM incubation all appeared to be alive. These were sonicated and the resultant extract suspended in 100 mls of BM.

Batch 3 (migration from agar). Clumps of worms were placed in BM with antimicrobials. The antimicrobials included: 5000 U/ml penicillin G sodium and 5000 mcg/ml streptomycin sulfate (Penstrep, GIBCO, Life Technologies, USA) at 2 ml/L BM; 200 mg/ml benzylpenicillin sodium BP (Glaxo, NZ) at 1 ml/L BM; kanomycin sulfate (Sigma Chemical Co.,USA) at 100 mg/L BM; Fungizone (E R Squibb & Sons, Inc., USA) at 1 ml/L PM. After 30 minutes the worms were divided into 3 groups. One was incubated in BM, one in BM with antimicrobials and one was retained for sonification and production of extract without incubation. The sonicated worms were resuspended in 100 mls of BM. The sonicated-extract mixture was sequentially drawn through 0.8, 0.45 and 0.2 µm cellulose acetate membrane filters (Micro Filtration Systems, USA) by suction before storage. The incubated groups underwent 2 periods of incubation: firstly for 6 hours, followed by overnight (9 hours). Both were in 150 ml volumes at 37°C. Supernatant from the first incubations was retained at 4°C until subsequent combination with supernatant from the second. Before storage these solutions were filtered as described for the extract. Excess fluid was removed from the remaining worms by filtration under suction and the worms weighed. Each group consisted of about 0.2g of adult worms.

Batch 4 (migration from agar). Five groups of worms were incubated in: distilled water, 0.9% NaCl, BM without glucose, BM without glucose or 0.25% BSA, BM without glucose but with antibiotics. This antibiotic mixture differs from the antimicrobial mixture (above) by the exclusion of fungizone. Each group was incubated in 50 mls for four successive 2.5 hour periods at 37°C, followed by a 9 hour overnight incubation. The supernatant from each incubation period was stored and tested separately for each group after centrifugation. Worms were taken from the three solutions based on BM after the overnight incubation and sonicated to produce extract.

Batch 5 (migration from agar). Clumps of worms were divided into 4 groups. These

were incubated in: BM, BM without the usual 0.1% glucose, BM plus antibiotics, BM plus antibiotics but without glucose. Each group was incubated in 50 mls for three successive 3 hour periods at 37^{0}C, followed by a 9 hour overnight incubation. Supernatant from each incubation was stored and tested separately after centrifugation.

CORRIGENDA

Page	Line	
iii	7	not "and carbachol release" but "and gastrin release"
ix	34	not "AETIOLPGY" but "AETIOLOGY"
xiv	7	not "thier" but "their"
xxii	25	not "effects" but "effect"
34	23	not "affects" but "effects"
41	16	Eh = oxidation-reduction potential
54	11	not "or" but "of"
56	1	not "proprionate" but "propionate"
63	5	not "(experiment 1 (Expt 1))" but "Expt 1"
67	27	not "were" but "was"
67	28	not "worm" but "worms"
75	20	not "*Monesia*" but "*Moniezia*"
80	4	not "were" but "was"
86	26	not "change the" but "change in the"
89	14	not "effected" but "affected"
125	28	not "were" but "was"
131	6	not "GRP in suggested" but "GRP suggested"
140	12	not "study the" but "study of the"
153	22	not "presence somatostatin-insensitive" but presence of somatostatin-insensitive"
159	14	not "due *O.circumcincta*" but "due to *O.circumcincta*"
	27	not "affects" but "effects"
169	15	not "*Journal of Physiology*" but "*Journal of Parasitology*"
169	22	not "**2**:159-165" but "**15**:159-165"
176	5	not "Ostertagia" but "*Ostertagia*"
189	18	not "*Australian Journal of Agricultural Science*" but "*Asian-Australasian Journal of Animal Sciences*"
193	31	not "*Australian Journal of Agricultural Science*" but "*Asian-Australasian Journal of Animal Sciences*"
206	17	not "Folin Ciocateau" but "Folin-Ciocalteau"
207	7	not "thiomerosal" but thiomersal"
208	6	not "thiomerosal" but thiomersal"

Facing page

73	Fig.2.8	top graph = sheep 15, Group C; middle = sheep 19, Group D; bottom = sheep 17, Group C.
127	Table 4.7	not "Rt (carbachol and atropine) - Rt (carbachol)" but "Rt (carbachol) - Rt (carbachol and atropine).

ADDENDA

1. Somatostatin

The radioimmunoassay used to estimate the somatostatin concentration in incubates from static incubation experiments described in Appendix 1.3 was developed and validated by Prof. C. McIntosh (University of British Columbia) and reported in *Gut* **19**:655-663 (McIntosh C, Arnold R, Bothe E, Becker H, Kobberling J & Creutzfeld W (1978) Gastrointestinal somatostatin: extraction and radioimmunoassay in different species).

The mean somatostatin concentration determined in the pooled control incubates (A, B and C) of both control and test plates (see Chapter 3) was 328 ± 169 pg/ml (mean ± SD). The mean sensitivity of the assay was 98 ± 44 pg/ml. Between 1% and 10% (usually about 5%) of values were close to or below the assay sensitivity. In most cases these samples included both the pooled control and test incubates for individual tissue pieces and as the response of any individual tissue piece (TR_i) was determined with respect to its own basal secretion (see Chapter 3.4.4), the influence of these individual responses on the response of the treatment set was found to be minimal and their responses generally conformed with those of other set members. Consequently, to avoid subjective rejection of samples with marginal somatostatin concentrations, the results obtained from these tissue pieces were retained in the treatment set and contributed to the response (Rt) determined for that experiment. However, where the estimated somatostatin concentration was substantially less than the sensitivity for that assay, the response by the tissue piece concerned was not included in the treatment set. It was not considered that the inclusion of samples with marginal somatostatin concentrations affected the interpretation of the results in any way.

2. Feeding of sheep in *in vivo* experiments (Chapter 2)

All sheep experimentally-infected with *O. circumcincta* in the study reported in Chapter 2 (Groups A, B, C, D and E) were fed at 0900 hours.

3. Parasite status of sheep used to provide abomasal fluid (Chapter 6)

The two sheep that were used to provide abomasal fluid for the inoculation of solutions for the preliminary studies described in Chapter 6 were both raised on pasture and had unknown histories of infection but are likely to have been infected with abomasal and intestinal nematodes, although this was not determined. The identity of the active substance(s), the conditions under which it is generated and the association with parasitism was not examined but is the subject of a subsequent ongoing investigation.