

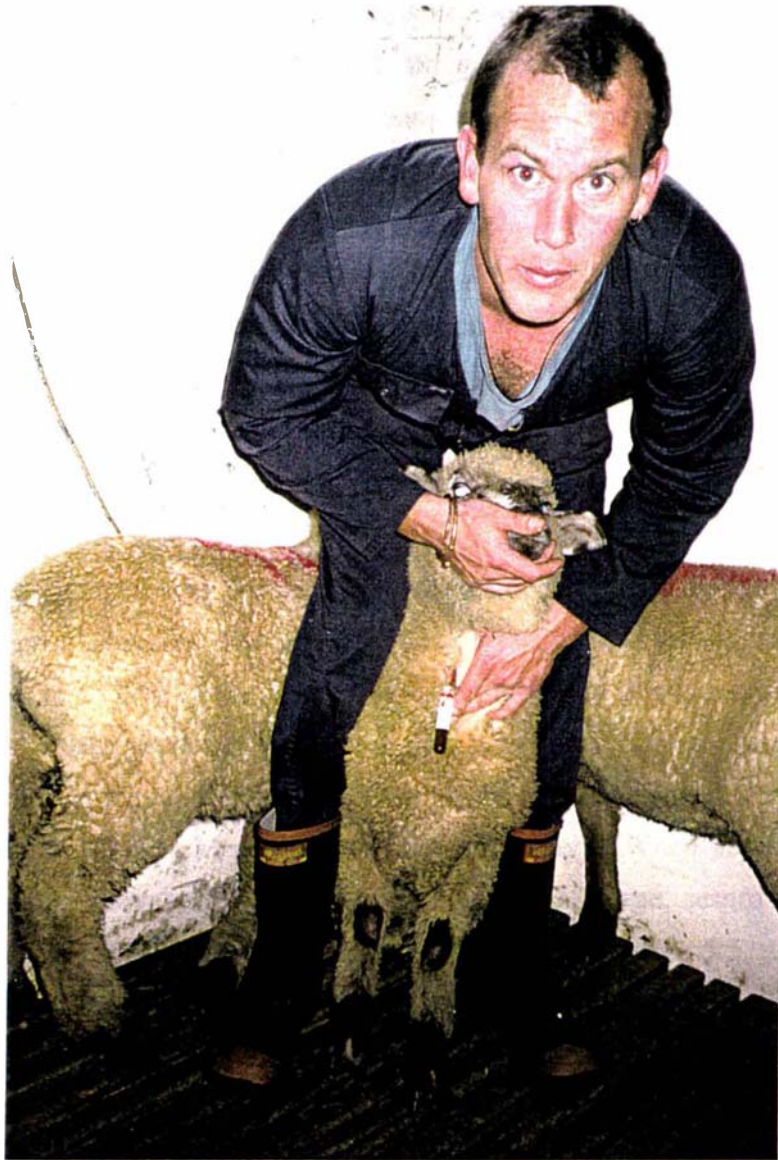
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**ABOMASAL SECRETION  
IN  
PARASITISED SHEEP**

A thesis presented  
in partial fulfilment of the requirements  
for the degree of  
**DOCTOR OF PHILOSOPHY**  
in Physiology  
at Massey University

**DAVID ERIC BENJAMIN LAWTON**

1995



**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 perfusion 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.

A handwritten signature in black ink, appearing to read 'D. Lawton', with a long horizontal flourish extending to the right.

David Eric Benjamin LAWTON

(1995)

## ACKNOWLEDGEMENTS

I am grateful to the Department of Physiology and Anatomy, Massey University, for providing me with the opportunity, facilities and technical support to undertake this study. In particular, I would like to thank Dr Heather Simpson for her humour, assistance, encouragement, patience and tolerance during and prior to the period of my studentship - all of which far exceeded the reasonable expectations of either supervisor, mentor or friend on many occasions. I also wish to express my special gratitude to Jane Candy for her long-suffering help in the laboratory and for the somatostatin assays she performed on my behalf.

I would like to thank Dr Gordon Reynolds for the surgeries he performed and for his assistance in my supervision. My thanks are also due to Professor David Mellor and Associate Professors Peter Davie and Keith Lapwood for their support and to the technical and secretarial staff of the department, in particular that of Debbie Antony and Eddie Hunt. I am grateful to the Department of Pathology and Public Health for the use of the parasitology laboratory and other facilities. I would like to express my gratitude to Associate Professor Tony Charleston and Dr Bill Pomroy for fostering my interest in parasitology and for their helpful advice and assistance in this area, to Barbara Adlington and Shirley Calder for sharing their considerable expertise in this field with me and Dr Alan Murray and Dr Eamon Gormley for encouragement and advice in their specialised areas.

Financially, this work was jointly supported by the C. Alma Baker Trust and the E & C Thoms Bequest. My personal support during this study was generously provided by a Massey University Vice-Chancellor's Doctor of Philosophy Scholarship and a C. Alma Baker Scholarship.

Finally, I wish to thank my companion Luke, my fellow student Elke Haag, my family, Anne and the staff of Wellington Zoo.

## TABLE OF CONTENTS

TITLE.....	i
ABSTRACT.....	ii
STATEMENT.....	iv
ACKNOWLEDGEMENTS.....	v
TABLE OF CONTENTS.....	vi
LIST OF FIGURES.....	xiv
LIST OF TABLES.....	xvii
LIST OF ABBREVIATIONS.....	xx
PREFACE.....	xxii
<b>Chapter 1    <u>LITERATURE REVIEW</u></b> .....	1
1.1 INTRODUCTION.....	1
<b>1.2 ABOMASUM: ANATOMY AND HISTOLOGY</b> .....	2
1.2.1 GROSS ANATOMY.....	2
1.2.2 MICROANATOMY.....	2
1.2.3 BLOOD AND NERVE SUPPLY.....	4
<b>1.3 ABOMASAL SECRETION: PEPSINOGEN, ACID AND GASTRIN.</b>	5
1.3.1 GASTRIC SECRETION IN THE SHEEP.....	5
1.3.2 PEPSINOGEN SECRETION.....	6
1.3.2.1 Cholinergic agents.....	6
1.3.2.2 Gastrin.....	7
1.3.2.3 Cholecystokinin.....	7
1.3.2.4 $\beta$ -Adrenergic agonists.....	7
1.3.2.5 Histamine.....	8
1.3.2.6 Other agents.....	8
1.3.2.7 Somatostatin.....	9
1.3.2.8 Intracellular pathways.....	9
1.3.2.9 Summary.....	9
1.3.3 ACID SECRETION.....	10



1.3.3.1 Biochemical and functional morphology of the parietal cell.....	10
1.3.3.2 Physiology of acid secretion.....	11
1.3.3.2.1 Cephalic Phase.....	11
1.3.3.2.2 Peptone.....	12
1.3.3.2.3 Distension.....	12
1.3.3.2.4 Luminal pH.....	12
1.3.3.2.5 Enterogastrones.....	13
1.3.3.3 Stimulators of acid secretion.....	13
1.3.3.3.1 Histamine.....	13
1.3.3.3.2 Acetylcholine.....	14
1.3.3.3.3 Gastrin.....	15
1.3.3.4 Inhibitors of acid secretion.....	16
1.3.3.4.1 Somatostatin.....	16
1.3.3.4.2 Enterogastrones.....	17
1.3.3.4.3 Epidermal Growth Factor (EGF) and Transforming Growth Factor- $\alpha$ (TGF- $\alpha$ ).....	18
1.3.3.4.4 Prostaglandins.....	19
1.3.3.4.5 Interleukin-1 (IL-1).....	19
1.3.3.4.6 Other Inhibitors.....	20
1.3.3.5 Summary.....	20
1.3.4 GASTRIN SECRETION.....	21
1.3.4.1 Physiology of gastrin secretion.....	21
1.3.4.1.1 Chemical Composition of Ingesta.....	21
1.3.4.1.2 Gastric Acidity.....	22
1.3.4.1.3 Gastric Distension.....	24
1.3.4.2 Regulation of gastrin secretion.....	25
1.3.4.2.1 Cholinergic Agonists.....	25
1.3.4.2.2 Gastrin Releasing Peptide (GRP).....	26
1.3.4.2.3 Vagal or Electrical Field Stimulation.....	27
1.3.4.2.4 Adenosine.....	27
1.3.4.2.5 Somatostatin.....	28
1.3.4.2.6 Catecholamines.....	31
1.3.4.2.7 Peptide Histidine Isoleucine (PHI).....	31
1.3.4.2.8 Prostaglandins.....	32

1.3.4.2.9 Gastric Inhibitory Peptide (GIP).....	32
1.3.4.2.10 Gamma Amino Butyric Acid (GABA).....	32
1.3.4.3 Summary.....	32
1.3.4.4 Trophic and other effects of gastrin.....	34
<b>1.4 GASTRIC MUCOSAL DEFENSE.....</b>	<b>34</b>
1.4.1 GASTRIC MUCOSAL BARRIER.....	35
1.4.1.1 Mucus.....	35
1.4.1.2 Bicarbonate secretion.....	36
1.4.1.3 Mucosal blood flow.....	36
1.4.1.4 Adaptive cytoprotection.....	36
1.4.2 CELL REPLACEMENT.....	37
1.4.3 INFLAMMATION.....	38
1.4.3.1 Cytokines.....	38
1.4.3.2 Eosinophils.....	39
1.4.3.3 Mast cells.....	39
<b>1.5 OSTERTAGIASIS.....</b>	<b>40</b>
1.5.1 LIFE CYCLE OF <i>O. CIRCUMCINCTA</i> .....	41
1.5.2 ABOMASAL MORPHOLOGY.....	42
1.5.3 ABOMASAL FUNCTION.....	45
1.5.3.1 Hyperpepsinogenaemia.....	46
1.5.3.2 Hypochlorhydria.....	49
1.5.3.3 Hypergastrinaemia.....	53
1.5.4 SECRETORY AND EXCRETORY PRODUCTS OF <i>O</i> <i>CIRCUMCINCTA</i> .....	55
1.5.5 IMMUNITY TO <i>O. CIRCUMCINCTA</i> .....	57
<b>1.6 RETROSPECTIVE.....</b>	<b>59</b>
<b><u>Chapter 2</u>    <u>EFFECT OF ADULT OR LARVAL <i>OSTERTAGIA</i></u> <u><i>CIRCUMCINCTA</i> ON ABOMASAL pH AND SERUM</u> <u>GASTRIN AND PEPSINOGEN.....</u></b>	<b>61</b>
<b>2.1 INTRODUCTION.....</b>	<b>61</b>
<b>2.2 MATERIALS AND METHODS.....</b>	<b>63</b>
2.2.1 EXPERIMENTAL DESIGN.....	63

2.2.2 ANIMALS.....	63
2.2.2.1 Surgery.....	64
2.2.3 BLOOD AND ABOMASAL FLUID SAMPLES.....	64
2.2.3.1 Sample collection.....	64
2.2.3.2 Abomasal pH.....	65
2.2.3.3 Serum pepsinogen.....	66
2.2.3.4 Serum gastrin.....	66
2.2.4 ABOMASAL TISSUE SAMPLES .....	66
2.2.4.1 Tissue gastrin.....	67
2.2.5 PARASITOLOGY.....	67
2.2.6 STATISTICS.....	68
2.2.7 INTERPRETATION OF RESULTS.....	68
2.3 RESULTS.....	68
2.3.1 DEFINITION OF NORMAL VALUES.....	68
2.3.2 CONTROL GROUPS.....	69
2.3.3 INFECTION WITH ADULT <i>O. CIRCUMCINCTA</i> .....	70
2.3.4 INFECTION WITH LARVAL <i>O. CIRCUMCINCTA</i> .....	70
2.3.4.1 Serum pepsinogen concentration.....	70
2.3.4.2 Abomasal pH.....	72
2.3.4.3 Serum gastrin concentration.....	72
2.3.4.4 Feeding response.....	74
2.3.5 ABOMASAL WET WEIGHT.....	75
2.3.6 TISSUE GASTRIN.....	75
2.3.7 PARASITOLOGY.....	75
2.3.7.1 Previous infection (Group A).....	75
2.3.7.2 Parasite-naive sheep.....	75
2.3.7.3 Faecal egg floats and egg counts.....	76
2.3.7.4 Examination of abomasal fluid samples.....	76
2.3.7.5 Post-mortem parasitology.....	76
2.4 DISCUSSION.....	77
2.4.1 INTERPRETATION OF RESULTS.....	77
2.4.2 DIRECT TRANSFER OF ADULT WORMS.....	79
2.4.3 PATHOPHYSIOLOGY AND PARASITE DEVELOPMENT.....	82
2.4.4 AETIOLOGY OF THE SECRETORY LESIONS.....	87

2.4.4.1 Disruption of the intrinsic mucosal barrier.....	87
2.4.4.2 Parietal cell inhibition.....	89
2.4.4.3 Gastrin hypersecretion.....	91
2.4.6 PARASITE EXCRETORY/SECRETORY PRODUCTS.....	93
<b>Chapter 3</b> <b><i>INVITRO</i> TECHNIQUES.....</b>	<b>95</b>
<b>3.1 INTRODUCTION.....</b>	<b>95</b>
<b>3.2 GENERAL PROCEDURES.....</b>	<b>97</b>
3.2.1 BASAL MEDIUM (BM).....	97
3.2.2 ABOMASAL TISSUE.....	98
3.2.3 ASSAYS.....	98
<b>3.3 METHOD 1: PERIFUSION OF TISSUE SEGMENTS.....</b>	<b>99</b>
3.3.1 PRINCIPLE OF METHOD.....	99
3.3.2 PREPARATION OF DISPERSED GLANDS OR TISSUE PIECES.....	99
3.3.3 EXPERIMENTAL PROCEDURE.....	99
3.3.4 ASSESSMENT OF RESPONSE.....	100
3.3.5 RESULTS.....	101
<b>3.4 METHOD 2: STATIC INCUBATION OF TISSUE SEGMENTS.....</b>	<b>102</b>
3.4.1 PRINCIPLE OF METHOD.....	102
3.4.2 PREPARATION OF TISSUE SEGMENTS.....	103
3.4.3 EXPERIMENTAL PROCEDURE.....	103
3.4.3.1 Effect of a secretagogue: carbachol .....	104
3.4.3.2 Effect of experimental conditions: osmolarity and pH.....	104
3.4.4 ASSESSMENT OF RESPONSE.....	104
3.4.5 STATISTICS.....	106
3.4.6 RESULTS.....	107
3.4.6.1 Control tissue.....	107
3.4.6.2 Effect of carbachol on gastrin and pepsinogen secretion.....	107
3.4.6.3 Effect of osmolarity and pH on gastrin secretion.....	107
<b>3.5 METHOD 3: DISPERSED CELL PREPARATIONS.....</b>	<b>108</b>
3.5.1 PRINCIPLE OF METHOD.....	108
3.5.2 PREPARATION OF DISPERSED CELL SUSPENSION.....	108
3.5.3 ASSESSMENT OF CELL VIABILITY.....	109

3.5.4 EXPERIMENTAL PROCEDURE.....	109
3.5.5 RESULTS.....	111
3.5.5.1 Homogeneity of antral cell suspensions.....	111
3.5.5.2 Effect of secretagogues on gastrin secretion: carbachol, bethanechol, GRP.....	111
3.5.5.3 Effect of inhibitory substances on gastrin secretion: parasite incubates.....	111
3.5.5.4 Effect of a secretagogue on pepsinogen secretion: Carbachol.....	111
3.6 DISCUSSION.....	112
3.6.1 PERIFUSION METHOD.....	112
3.6.2 STATIC INCUBATION METHOD.....	114
3.6.3 DISPERSED CELLS.....	116
3.6.4 CONCLUSIONS.....	118
<b><u>Chapter 4</u>    <u>PHARMACOLOGICAL STUDIES OF THE OVINE G</u>                   <u>AND D CELL</u>.....</b>	<b>119</b>
4.1 INTRODUCTION.....	119
4.2 MATERIALS AND METHODS.....	122
4.2.1 EXPERIMENTAL DESIGN.....	122
4.2.2 PHARMACOLOGICAL AGENTS.....	122
4.2.3 GASTRIN AND SOMATOSTATIN ASSAYS.....	123
4.2.4 STATISTICS.....	123
4.3 RESULTS.....	124
4.3.1 BOMBESIN-LIKE PEPTIDES.....	124
4.3.1.1 Bombesin.....	124
4.3.1.2 GRP.....	124
4.3.2 CHOLINERGIC AGONISTS AND ANTAGONISTS.....	125
4.3.2.1 Carbachol .....	125
4.3.2.2 Carbachol, atropine and hexamethonium.....	126
4.3.2.3 Bethanechol .....	128
4.3.2.4 Nicotine .....	128
4.3.2.5 Eserine .....	128
4.3.3 VASOACTIVE INTESTINAL PEPTIDE.....	129

4.3.4 GASTRIC INHIBITORY PEPTIDE.....	129
4.3.5 ADRENALINE.....	129
4.3.6 PROSTAGLANDIN -F2 $\alpha$ .....	129
4.4 DISCUSSION.....	129
4.4.1 GASTRIN RELEASING PEPTIDE.....	130
4.4.2 CHOLINERGIC MECHANISMS.....	133
4.4.3 BASAL CHOLINERGIC ACTIVITY.....	135
4.4.4 SOMATOSTATIN.....	136
4.4.5 VIP, GIP AND ADRENALINE.....	137
4.4.6 CONCLUSIONS.....	138
<b><u>Chapter 5</u></b> <b><u>EFFECT OF <i>OSTERTAGIA CIRCUMCINCTA</i></u></b> <b><u>PRODUCTS ON GASTRIN AND SOMATOSTATIN</u></b> <b><u>SECRETION <i>IN VITRO</i></u></b> .....	141
5.1 INTRODUCTION.....	141
5.2 MATERIALS AND METHODS.....	142
5.2.1 EXPERIMENTAL DESIGN.....	142
5.2.2 EXPERIMENTAL PROCEDURE.....	142
5.2.2.1 Separation of worm preparations by molecular weight.....	144
5.2.2.2 Somatostatin antiserum.....	144
5.2.2.3 Atropine.....	144
5.2.2.4 Dispersed cells.....	145
5.2.3 ESTIMATION OF GASTRIN AND SOMATOSTATIN CONCENTRATION.....	145
5.2.4 STATISTICS.....	145
5.3 RESULTS.....	145
5.3.1 PARASITE-NAIVE SHEEP.....	147
5.3.2 SEPARATION OF SOLUTION BY MOLECULAR WEIGHT.....	148
5.3.3 SOMATOSTATIN ANTISERUM.....	148
5.3.4 ATROPINE.....	148
5.3.5 DISPERSED CELLS.....	148
5.4 DISCUSSION.....	149

<b><u>Chapter 6</u></b>	<b><u>EFFECT OF MICROORGANISMS ON GASTRIN AND SOMATOSTATIN SECRETION <i>IN VITRO</i></u></b> .....	156
<b>6.1</b>	<b>INTRODUCTION</b> .....	156
<b>6.2</b>	<b>MATERIALS AND METHODS</b> .....	157
6.2.1	EXPERIMENTAL DESIGN.....	157
6.2.2	TEST SOLUTIONS.....	157
6.2.3	EXPERIMENTAL PROCEDURE.....	157
6.2.4	ESTIMATION OF GASTRIN AND SOMATOSTATIN CONCENTRATION.....	157
6.2.5	STATISTICS.....	158
<b>6.3</b>	<b>RESULTS</b> .....	158
<b>6.4</b>	<b>DISCUSSION</b> .....	158
<b><u>Chapter 7</u></b>	<b><u>GENERAL DISCUSSION</u></b> .....	160
<b><u>REFERENCES</u></b> .....		169
<b><u>APPENDICES</u></b> .....		206

## LIST OF FIGURES

Facing page

- Figure 2.1** Distribution of serum pepsinogen, abomasal pH and serum gastrin values determined from samples collected from parasite-free sheep. 69
- Figure 2.2** Mean serum pepsinogen, abomasal pH and serum gastrin values in Group E sheep prior to and following their infection with 15 000 adult *O. circumcincta* transferred directly into their abomasa. 70
- Figure 2.3** Serum pepsinogen, abomasal pH and serum gastrin values in sheep 23 and 24 prior to and following their infection with 15 000 adult *O. circumcincta* transferred directly into their abomasa. 70
- Figure 2.4** Serum pepsinogen, abomasal pH and serum gastrin values in sheep 25 and 26 prior to and following their infection with 15 000 adult *O. circumcincta* transferred directly into their abomasa. 70
- Figure 2.5** Mean serum pepsinogen concentrations of Group A, B, C and D sheep prior to and following their infection with *O. circumcincta* larvae. 70
- Figure 2.6** Mean abomasal pH of Group A, B C and D sheep prior to and following their infection with *O. circumcincta* larvae. 71
- Figure 2.7** Mean serum gastrin concentration of Group A, B, C and D sheep prior to and following their infection with *O. circumcincta* larvae. 72
- Figure 2.8** Abomasal pH and serum gastrin changes in sheep over the first 10 days following their infection with *O. circumcincta* larvae. 73
- Figure 2.9** Examples of the dissociation between the abomasal pH and serum gastrin response in sheep following their infection with *O. circumcincta* larvae. 74
- Figure 2.10** The effect of feeding on the abomasal pH in a an achlorhydric and hypochlorhydric sheep after their infection with 50 000 *O. circumcincta* larvae. 74
- 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). 75
- Figure 2.12** Mean faecal egg count of sheep in Experiment 3 following their infection with either larval or adult *O. circumcincta*. 75
- Figure 3.1** Schematic drawing of column perfusion apparatus. 100
- Figure 3.2** Gastrin concentration in the effluent from two BioGel/antral chopped tissue columns perfused with BM at a flow rate of 0.5 ml per minute. 101



<b>Figure 3.3</b> Gastrin concentration in the effluent from two BioGel/antropyloric gland columns perfused with BM containing a range of bombesin concentrations.	101
<b>Figure 3.4</b> Gastrin concentration in the effluent from two BioGel/antropyloric gland columns perfused with BM containing a range of carbachol concentrations.	102
<b>Figure 3.5</b> Arrangement of ovine abomasal tissue segments for use in the static incubation method: (top) showing segments mounted in rack of polystyrene; (centre) showing pins conforming with the wells of a Costar 48 well cell culture plate; (bottom) showing tissue suspended in 1 ml of BM.	102
<b>Figure 3.6</b> Concentration of pepsinogen (A) and gastrin (B) in the medium used to incubate control tissue for six successive periods using the static incubation method.	104
<b>Figure 3.7</b> Effect of $10^{-4}$ M carbachol on gastrin secretion by ovine antral tissue segments using the static incubation method.	104
<b>Figure 3.8</b> Effect of osmolarity and pH changes on gastrin secretion by segments of ovine antral mucosa.	108
<b>Figure 3.9</b> Effect of inhibitory solutions on the secretion of gastrin by dispersed ovine antral mucosal cells.	111
<b>Figure 3.10</b> Effect of increasing carbachol concentration on pepsinogen secretion (Rt) by a dispersed ovine fundic mucosal cell preparation.	112
<b>Figure 4.1</b> Effect of GRP on gastrin and somatostatin secretion by segments of antral mucosa (mean $\pm$ SEM).	124
<b>Figure 4.2</b> Effect of carbachol on gastrin and somatostatin secretion by segments of ovine antral mucosa (mean $\pm$ SEM).	126
<b>Figure 4.3</b> Effect of atropine on the gastrin response to carbachol.	126
<b>Figure 4.4</b> Examples of the gastrin and somatostatin response to carbachol from individual experiments (mean $\pm$ SEM).	126
<b>Figure 4.5</b> The gastrin response to $10^{-4}$ M carbachol versus the gastrin response to $10^{-5}$ M atropine within individual experiments.	126
<b>Figure 4.6</b> Effect of nicotine on the secretion of gastrin and somatostatin by ovine antral mucosa (mean $\pm$ SEM).	128
<b>Figure 5.1</b> Effect of increased storage time on the gastrin inhibitory properties of the Batch 1 <i>O. circumcincta</i> incubate.	146

<b>Figure 5.2</b> Effect of worm products on gastrin secretion by dispersed ovine antral mucosal cells and from segments of antral mucosa.	149
<b>Figure 5.3</b> Effect of worm products on pepsinogen secretion by dispersed ovine fundic cells.	149

## LIST OF TABLES

	Facing page
<b>Table 2.1.</b> 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.	69
<b>Table 2.2</b> The mean serum pepsinogen, serum gastrin and abomasal pH change in response to feeding in parasite-naive sheep.	69
<b>Table 2.3</b> Time at which serum gastrin and abomasal pH were elevated after infection of parasite-naive sheep infected with <i>O. circumcincta</i> larvae.	72
<b>Table 2.4</b> Relative wet weight of abomasum to body weight at post-mortem in parasite-naive sheep and sheep infected with <i>O. circumcincta</i> from Experiment 3.	75
<b>Table 2.5</b> Amount of gastrin extracted per gram of antral mucosa collected from parasite-naive sheep and sheep infected with <i>O. circumcincta</i> .	75
<b>Table 3.1</b> Effect of bombesin on gastrin secretion by perfused ovine antropyloric glands.	101
<b>Table 3.2</b> Effect of carbachol on gastrin secretion by perfused ovine antropyloric glands.	102
<b>Table 3.3</b> Effect of carbachol on pepsinogen secretion by segments of ovine fundic mucosa.	107
<b>Table 3.4</b> Effect of the osmolarity of the incubation medium on the secretion of gastrin by segments of ovine antral mucosa.	108
<b>Table 3.5</b> Effect of the pH of the medium on gastrin secretion by segments of ovine antral mucosa.	108
<b>Table 3.6</b> Effect of carbachol, bethanechol and GRP on gastrin secretion by dispersed ovine antral mucosal cells.	111
<b>Table 4.1</b> Effect of bombesin on gastrin and somatostatin secretion by ovine antral mucosa.	124
<b>Table 4.2</b> Effect of GRP on the gastrin and somatostatin secretion by ovine antral mucosa.	124
<b>Table 4.3</b> Effect of somatostatin antiserum on the gastrin response (Rt) to $10^{-7}$ M GRP.	125
<b>Table 4.4</b> Effect of carbachol on gastrin and somatostatin secretion by ovine antral mucosa.	126

<b>Table 4.5</b> Effect of carbachol on gastrin and somatostatin secretion by ovine antral mucosa in individual experiments.	126
<b>Table 4.6</b> Effect of carbachol or atropine on the gastrin response (Rt) by ovine antral mucosa.	126
<b>Table 4.7</b> The gastrin response (Rt) to $10^{-4}$ M carbachol and the effect of atropine on the response to a range of carbachol concentrations by ovine antral mucosa.	127
<b>Table 4.8</b> Effect of atropine on the somatostatin response (Rt) to a range of carbachol concentrations by ovine antral mucosa.	127
<b>Table 4.9</b> Effect of hexamethonium on the gastrin response (Rt) to a range of carbachol concentrations by ovine antral mucosa.	127
<b>Table 4.10</b> Effect of bethanechol on gastrin and somatostatin secretion by ovine antral mucosa.	128
<b>Table 4.11</b> Effect of nicotine on gastrin secretion by ovine antral mucosa.	128
<b>Table 4.12</b> Effect of VIP on gastrin and somatostatin secretion by ovine antral mucosa.	129
<b>Table 4.13</b> Effect of GIP on gastrin and somatostatin secretion by ovine antral mucosa.	129
<b>Table 4.14</b> Effect of adrenaline on gastrin and somatostatin secretion by ovine antral mucosa.	129
<b>Table 4.15</b> Effect of prostaglandin F- $2\alpha$ on gastrin and somatostatin secretion by ovine antral mucosa.	129
<b>Table 5.1</b> Effect of exsheathed <i>O. circumcincta</i> L <sub>3</sub> products on gastrin secretion by ovine antral mucosa.	146
<b>Table 5.2</b> Effect of adult <i>O. circumcincta</i> products (Batch 2) on gastrin and somatostatin secretion by ovine antral mucosa.	146
<b>Table 5.3</b> Effect of adult <i>O. circumcincta</i> products (Batch 3) on gastrin and somatostatin secretion by ovine antral mucosa.	147
<b>Table 5.4</b> Effect of adult <i>O. circumcincta</i> products (Batch 4) on gastrin and somatostatin secretion by ovine antral mucosa.	147
<b>Table 5.5</b> Effect of adult <i>O. circumcincta</i> products (Batch 5) on gastrin secretion by ovine antral mucosa.	147
<b>Table 5.6</b> Effect of adult <i>O. circumcincta</i> products on gastrin secretion by ovine antral mucosal tissue from parasite-naive sheep.	148

<b>Table 5.7</b> Effect of fractions separated on size (3000 daltons) of adult <i>O. circumcincta</i> products on gastrin secretion by ovine antral mucosa.	148
<b>Table 5.8</b> Effect of somatostatin antiserum on the gastrin response to an inhibitory solution (Batch 2) derived from <i>O. circumcincta</i> .	148
<b>Table 5.9</b> Effect of combining $10^{-5}$ M atropine with <i>O. circumcincta</i> products on gastrin secretion by ovine antral mucosa.	148
<b>Table 6.1</b> Two series of solutions (a & b) prepared by the inoculation of Basal Medium with abomasal contents from two sheep.	157
<b>Table 6.2</b> Effect of solutions (Table 6.1) on gastrin and somatostatin secretion by ovine antral mucosa.	158

## 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 <sub>i</sub>	control ratio for individual tissue piece
CV	coefficient of variation
D cell	somatostatin cell
DMPP	1,1-dimethyl-4-phenylpiperazinium
<i>D. viviparus</i>	<i>Dictyocaulus viviparus</i>
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
<i>H. contortus/placoi</i>	<i>Haemonchus</i> spp.
IGF	insulin-like growth factor
IL	interleukin
i.m.	intramuscular
kg	kilogram
L	litre
L <sub>3</sub> /L <sub>4</sub>	third/fourth stage larvae
M	moles per litre
mg	milligram
ml	millilitre
mm	millimeter
mM	millimolar
mOsm	milliosmoles

mU	milli-international enzyme unit
$\mu$	mean
$\mu+1s$	mean plus one standard deviation
$\mu+2s$	upper limit of the normal range (mean plus two standard deviations)
$\mu$ l	microlitre
$\mu$ m	micrometer
$\mu$ g	microgram
<i>N. brasiliensis</i>	<i>Nippostrongylus brasiliensis</i>
<i>O. circumcincta</i> / <i>ostertagi</i>	<i>Ostertagia</i> spp.
<i>Oe. radiatum</i>	<i>Oesophagostomum radiatum</i>
p	probability statistic
pg	picogram
PHI	peptide histidine isoleucine
pM	picomolar
RIA	radioimmunoassay
rpm	revolutions per minute
Rt	response to treatment
$R_t$	response to treatment by individual tissue piece
SD	standard deviation
SEM	standard error of the mean
SMS	somatostatin
<i>T. axei</i> / <i>colubriformis</i>	<i>Trichostrongylus</i> spp.
TGF- $\alpha$	transforming growth factor- $\alpha$
Th	T-helper
TNF- $\alpha$	tumour necrosis factor- $\alpha$
TR	test ratio
$TR_t$	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.