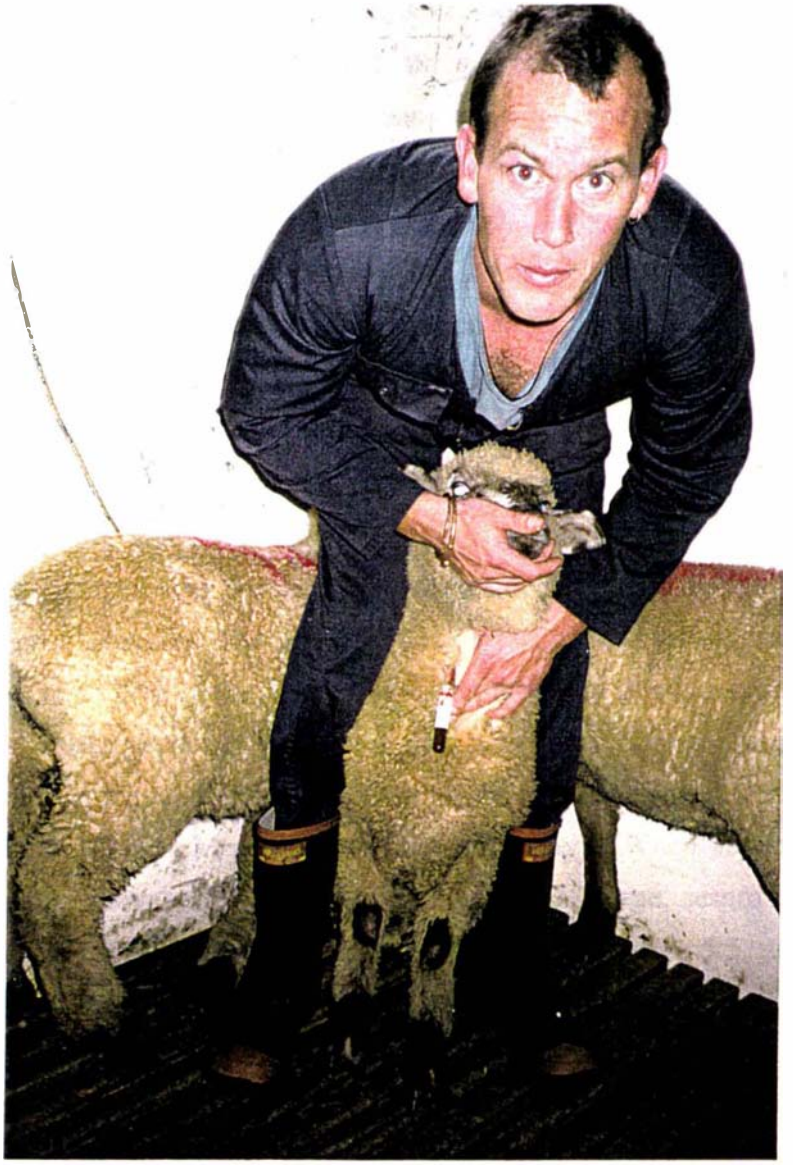
ABOMASAL SECRETION
IN
PARASITISED SHEEP

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 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

(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

LIST OF FIGURES

Facing page

Facing page

LIST OF TABLES

Facing page

Facing page

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.