

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

Spatiotemporal Mapping of the Motility of the *ex vivo* Rabbit Caecum.

A thesis presented in partial fulfilment of the requirements for the degree of Masters of
Physiology in Digestive Biomechanics (Physical Process of Digestion)

at Massey University, Turitea,

New Zealand.

Corrin Hulls

2015

Abstract

This work sought to determine the contractile factors influencing the coordination of inflow and out flow from the caecum, and the mixing and mass transfer within. Specifically, the work was focussed on the ileocaecal junction in the domestic rabbit (*Oryctolagus cuniculus*). The salient questions to answer were;

1. What are the contractile movements in the body of the caecum and associated structures of the rabbit caecum?
2. How are contractile movements coordinated at the body of the rabbit caecum and how does this affect the pattern of motility?

The following two main experimental works of this thesis were all conducted using live gut rabbit caecum preparations maintained *ex vivo*. Spatiotemporal mapping and electromyography was used to visualize and quantify contractile activity and coordination in the caecum.

1. High definition radial, strain rate and intensity spatiotemporal mapping was used to quantify contractile movements of the body and associated structures of the rabbit caecum.
2. Coordination between contractile events at different sites in the basal portion of the rabbit caecum and its associated structures were identified by electrophysiological recordings with simultaneous one dimensional, and a novel two dimensional, spatiotemporal mapping technique.

The following are the main findings and implications of the work.

1. The body of the caecum exhibited two patterns of motility that appeared autonomous, i.e. occurred independently of any contractile activity at the inlet or outlet. Firstly, a pattern termed **ladder activity** consisted of orderly sequential contractions in the spiral turns in the *corpus ceci*. Secondly, less localised, rapidly propagating synchronous contractions that were termed **mass peristalsis**.
2. Movements of the ileum and *sacculus rotundus* occurred at the same frequency and were broadly coordinated. Further, the findings suggest that the action of the *sacculus rotundus* may result from its distension with chyme by ileal peristalsis and

that the subsequent propagation of contraction along the basal wall of the caecum toward the colon may be augmented by this local distension.

3. The caecum and proximal colon/ampulla coli act reflexly to augment colonic outflow. When the caecum is distended and mass peristalsis is instituted, the action of the latter overrides the inherent rhythm and direction of haustral propagation in the adjacent portion of the proximal colon but not in the terminal ileum.

In conclusion, coordination, mixing and mass transfer in the rabbit caecum is a very complex, dynamic and largely autonomous process. Further, spatiotemporal mapping techniques enabled the identification and visualization of previously unknown contractile movements within the rabbit caecum.

Table of Contents

Abstract	ii
Table of Contents	iv
List of Figures	ix
List of Tables	xii
Preface.....	xiii
Acknowledgements	xiv
Copyright and permissions	xvii
Chapter 1- Introduction	1
Chapter 2- Literature Review	4
2.1 Foreword	5
2.2- Ontogeny and Embryonic Development of the Rabbit Gastrointestinal Tract	6
2.2.1 Introduction	6
2.2.2 Embryonic gastrointestinal development in the rabbit	6
2.2.2.1 Genesis of gut cavity: Gastrulation and Neurulation	6
2.2.2.2 Organogenesis and Morphogenesis	9
2.2.3 Postnatal Gastrointestinal Development in the Rabbit	14
2.2.4 Concluding Remarks on the Ontogeny and Embryonic Development of the Rabbit Gastrointestinal Tract	15
2.3- The Digestive Physiology of the Rabbit and the Morphology of its Digestive System .	17
2.3.1 Introduction	17
2.3.2 The Digestive Physiology of the Rabbit	17
2.3.3 The Mouth and Oesophagus	19
2.3.4 Anatomy and Digestion in the Stomach	19
2.3.5 Anatomy and Digestion in the Small Intestine	20
2.3.6 Anatomy and Digestion in the Hindgut	23
2.3.7 Concluding Remarks on the Rabbit Digestive System	26
2.4- Detailed Anatomy of the Rabbit Caecum	28

2.4.1 Introduction	28
2.4.2 The Structure of the Caecum.....	28
2.4.3 Topographical Location of the Caecum.....	30
2.4.4 Mesenteric Attachments	30
2.4.5 Arterial Blood Supply	31
2.4.6 Internal and External Structure of the Caecum.....	32
2.4.6.1 The sacculus rotundus	32
2.4.6.2 The base of caecum	33
2.4.6.3 The corpus ceci	34
2.4.6.4 The appendix.....	36
2.4.6.5 The ampulla coli	37
2.4.6.6 The proximal colon	37
2.4.7 Concluding Remarks on the Structure of the Rabbit Caecum.....	38
2.5- The Ileocaecal Valve of the Rabbit- its Function, Nervous Innervation and Interaction with the Ileum and Proximal Colon.....	41
2.5.1 Introduction	41
2.5.2 The Anatomy of the Ileocaecal Valve	41
2.5.3 Is the ICV a Sphincter?	42
2.5.4 Functional Characteristics of the ICV	43
2.5.5 Nervous Innervation of the Gastrointestinal Tract and ICV	43
2.5.5.1 General.....	43
2.5.6 The Interaction of the ICJ with Ileal and Colonic Contractile Activity.....	46
2.5.7 Concluding Remarks on the Rabbit Ileocaecal valve.....	47
2.6- Comparative Anatomy of the Mammalian Caecum- Strategies of Fermentative Digestion in Mammals.....	49
2.6.1 Introduction	49
2.6.2 The Herbivore Digestive System and Caecum.....	50
2.6.2.1 Foregut fermenters.....	50
2.6.2.2 Hindgut fermenters	54
2.6.3 The Carnivore Digestive System and Caecum	58
2.6.3.1 The Dog Caecum	58

2.6.3.2 The Cat Caecum	60
2.6.4 The Omnivore Caecum	61
2.6.5 The Human Caecum.....	63
2.6.6 Concluding Remarks on the Comparative Anatomy of the Caecum.....	63
2.7- Tonic and Phasic Gastrointestinal Contractile Activity in the Gut.....	66
2.7.1 Introduction	66
2.7.2 The Physiology of Motility in the Gut.....	66
2.7.2.1 Structure	66
2.7.2.2 Smooth muscle cells and contractile filaments	67
2.7.2.3 The signal transduction pathway.....	68
2.7.2.4 Electrical activity	69
2.7.2.5 Slow waves.....	69
2.7.2.6 Action potentials.....	71
2.7.3 Temporal Patterns of Motility of the Intestine	72
2.7.4 Overall Control of Contractile Activity in the Gastrointestinal Tract	73
2.7.4.1 Hierarchical structure of control	73
2.7.4.2 Neuronal and hormonal regulation of gut motility	74
2.7.4.3 Plexus of the rabbit.....	76
2.7.5 Concluding Remarks on Regulation and Control of Gastrointestinal Activity.....	77
2.8- Types of Phasic Contractile Motility in the Rabbit Terminal Ileum, Caecum and Proximal Colon	80
2.8.1 Introduction	80
2.8.2 Motility in the Small Intestine	80
2.8.2.1 The structure of a peristaltic event	81
2.8.2.2 Pendular movement	83
2.8.2.3 Segmentation.....	83
2.8.3 Motility in the Caecum	83
2.8.4 Motility in the Proximal Colon.....	85
2.8.5 Concluding Remarks on the Types of Phasic Contractile Motility in the Rabbit Terminal Ileum, Caecum and Proximal Colon	88
2.9 Conclusion	89

Chapter 3- Spatiotemporal mapping of *ex vivo* motility in the caecum of the rabbit. 90

3.1 Foreword91

3.2 Copy of the Paper- Spatiotemporal mapping of *ex vivo* motility in the caecum of the rabbit.92

Spatiotemporal mapping of *ex vivo* motility in the caecum of the rabbit.....93

3.2.1 Abstract.....93

3.2.2 Introduction95

3.2.3 Method96

3.2.4 Results.....102

3.2.5 Discussion 114

3.2.6 Journal Article References118

3.3 Additional details on the equipment and methods used in the previous chapter ..122

3.3.1 Organ bath design122

3.3.2 Organ bath..... 122

3.3.3 Layout of experimental apparatus123

3.3.4 Additional details on the spatiotemporal mapping technique125

3.3.4.1 ST Mapping Using the Boundaries of Gut Segments: ‘D’ and ‘R’ Maps125

3.3.4.2 Strain Rate Mapping Using Cross-Correlation127

3.3.4.3 Intensity Maps127

Chapter 4- *Ex vivo* motility in the base of the rabbit caecum and its associated structures: an electrophysiological and spatiotemporal analysis. 130

4.1 Foreword131

4.2 Copy of paper- *Ex vivo* motility in the base of the rabbit caecum and its associated structures: an electrophysiological and spatiotemporal analysis.....132

Ex vivo motility in the base of the rabbit caecum and its associated structures: an electrophysiological and spatiotemporal analysis.133

4.2.1 Abstract.....133

4.2.2 Introduction 133

4.2.3 Method 136

4.2.4 Results.....141

4.2.5 Discussion.	153
4.2.6 Journal Article References	155
4.3 Additional details on the measurement and interpretation of electrophysiological recordings and 2D spatiotemporal maps in the previous chapter	159
4.3.1 Electrophysiological recording of Muscle Contractions	159
4.3.2 2D Spatiotemporal Mapping	161
Chapter 5- General Discussion	164
5.1 Contractile motility of the caecum	165
5.2 Possible further work into the motility of the ileocaecal junction.....	166
5.3 Overall context of the work.....	168
References	171

List of Figures

Fig. 2-1(Druckenbrod and Epstein 2005). Summary diagram of colonization of cecum and proximal colon in the mouse with approximate embryonic ages.	8
Fig. 2-2 (Zacchetti et al 2007). Co-expression of seven Hoxd genes in posterior midgut.	10
Fig.2-3 (Evans and Sack 1973). Growth curve and representative developmental stages in the Rabbit (<i>Oryctolagus cuniculus</i>).	13
Fig. 2-4 (Harcourt-Brown 2001). The digestive system of the rabbit.	18
Fig. 2-5 (Harcourt-Brown 2001). The activity of the rabbit digestive system during the excretion of hard and soft faeces.	22
Fig. 2-6 (Leng 2008). Faecal types.	24
Fig. 2-7 (Smith and Norwell 1889). Appendix, caecum and colon of the rabbit.	29
Fig. 2-8 (Harcourt-Brown 2001). Three-dimensional topographical anatomy of the abdominal contents of the rabbit with the caecum removed.	30
Fig. 2-9 (Snipes 1978). Schematic drawing of the arterial supply to the caecum.	31
Fig. 2-10 (Snipes 1978). Scanning electron micrograph from the surface of the sacculus rotundus. ...	32
Fig. 2-11 (Snipes 1978). Schematic drawing of the internal, macroscopic structure of the caecum.	34
Fig. 2-12 An inflated and dried preparation of the corpus ceci of the rabbit caecum.	35
Fig. 2-13 (Abdel-Kaylek et al 2011). Cross-section in caecal wall of rabbit at 6 weeks of age.	36
Fig. 2-14 (Snipes et al 1982). Macroscopic view of the first segment of the proximal colon.	38
Fig. 2-15 (Besoluk 2006). View of the saccorotundocecal orifice from the caecal cavity in the rabbit.	41
Fig. 2-16 (Furness and Costa 1980). Diagrams showing the arrangement of the enteric plexuses.	44
Fig. 2-17 (Stevens et al 1995). Digestive tract of the sheep- a foregut fermenter.	50
Fig. 2-18 (Stevens et al 1995). Digestive tract of the Kangaroo- a foregut fermenter.	52

Fig. 2-19 (Stevens and Hume 1995). Digestive tract of the Horse- a colon fermenter.	54
Fig. 2-20 (Stevens and Hume 1995). The digestive tract of the Rabbit- a caecum fermenter.	56
Fig. 2-21 (Stevens et al 1995). The gastrointestinal tract of the dog.	58
Fig. 2-22 (Stevens et al 1995). The gastrointestinal tract of the cat.....	59
Fig. 2-23 (Stevens et al 1995). Digestive tract of the rat.	61
Fig. 2-24 (Ginsberg and Costoff 2015). Gastrointestinal smooth muscle structure.....	66
Fig. 2-25 (Wood et al 1999). Neural control of the gut is hierarchic with four basic levels of integrative organization.....	73
Fig. 2-26 (Maslennikova 1960). The structure of the nerve plexus in the muscular layer of different sections of the rabbit intestine.....	76
Fig. 2-27 (Lentle et al 2007). Diagram of the suggested mechanism for mixing generated by simultaneous circular and longitudinal contractions during peristalsis.....	81
Fig. 2-28 (Adapted from Ehrlein and Schemann 2006). Peristaltic waves of the caecum produce a shallow constriction resulting in low propulsion associated with backflow.	83
Fig. 2-29 (Adapted from Ehrlein and Schemann 2006). Peristaltic wave at a haustrated colon cause a central flow and mixing of digesta within the haustra.	85
Fig. 3-1. Showing the orientation of the structures at the base of the caecum in the video frames used for spatiotemporal mapping.	96
Fig. 3-2. Showing progression of ladder contractions across the body of the rabbit caecum.	101
Fig. 3-3. Intensity maps of the body of the caecum showing progression of ladder contractions.	102
Fig. 3-4. Profiles of transects through caecal maps showing the relative timing of ladder contractions in three successive turns of the interspiral domain.	104
Fig. 3-5. Mass caecal peristalsis.	106
Fig. 3-6. Spatiotemporal maps of the progression of mass peristalsis across the body of the caecum.	107

Fig. 3-7. SR map of the distal ileum and the sacculus rotundus.....	109
Fig. 3-8. SR maps of motility in the distal ileum, sacculus rotundus and ampulla caecalis during a mass peristaltic event in the body of the caecum.....	111
Fig. 3-9. A Schematic of rabbit caecum organ bath with the associated dimensions.....	121
Fig. 3-10. General experimental setup.	122
Fig. 3-11. Recirculating HBS system.....	122
Fig. 4-1. Showing the morphology of the base of the rabbit caecum and the placement of electrodes.	135
Fig. 4-2. Resting electrophysiological activity in the caecal base.	137
Fig. 4-3. Variation with treatment in mean durations and periods of spike bursts in A) Ileum, B) Sacculus rotundus, and C) Colon.	143
Fig. 4-4. Spatiotemporal maps of longitudinal contractile activity in the distal ileum and sacculus rotundus (A) and colon (B) during and after a mass peristaltic event.....	145
Fig. 4-5. Effect of treatments on the mean frequency of mass peristalses.....	146
Fig. 4-6. Sequences of images of rate of change of area during normal contractile activity in the caecal base and associated structures with perfusion via the ileum (A) or the proximal colon (B).....	147
Fig. 4-7. Sequence of images of rate of change of area in the caecal base and associated structures during repeated episodes of mass peristalses with perfusion via the ileum.....	149
Fig. 4-8. Temporal profiles of transects from ST maps of the ileum, sacculus rotundus and colon (A), and the corresponding integrated spike burst activity (B) during and after a mass peristaltic event.	150

List of Tables

Table 4-1. Effect of treatments on slow wave frequencies in the ileum, sacculus rotundus, and colon.....	141
Table 4-2. Effect of treatments on spike burst duration in the ileum, sacculus rotundus, and colon with site of perfusion.	141
Table 4-3. Effect of treatments on inter spike-burst period in the ileum, sacculus rotundus, and colon with site of perfusion	142

Preface

This thesis is written according to the regulations stipulated in the latest version of the '**Guidelines for the Preparation and Submission of Thesis**', published by Massey University.

All animal works were carried out in strict accordance with the 'New Zealand Code of Practice for the Care and Use of Animals for Scientific Purposes'. The procedures carried in this thesis were also approved by the Massey University Animal Ethics Committee (MUAEC approval no. 08/75 and 12/01).

The thesis format complies with the format of a thesis based on publications, as described on page 63-64 under the section 'Submission of a thesis based on publications'. The journal article has been reproduced in this thesis in its entirety at the relevant chapters. Below, details of the journal article that has been published and the chapter of which it may be found are listed and where it appears in my thesis.

Chapter 3:

Hulls C, Lentle RG, De Loubens C, Janssen PWM, Chambers P, Stafford K (2012)
Spatiotemporal mapping of *ex vivo* motility in the caecum of the rabbit.

Published in- Journal of Comparative Physiology B 2012 Feb; 182(2):287-97. DOI:
10.1007/s00360-011-0610-2.

Acknowledgements

Though only my name appears on the cover of this thesis, a great many people have contributed to its production. I owe my gratitude to all those people who have made this thesis possible- to say it has 'taken a while' is an understatement.

My deepest gratitude is to my Chief Supervisor, Prof. Roger Lentle. I have been amazingly fortunate to have a supervisor who granted me this opportunity, the freedom to explore on my own and at the same time the guidance to recover when my steps faltered. Roger taught me how to question and express ideas. His direction, patience, and support have been unfaltering and his enthusiasm toward the sciences an inspiration.

My Co-Supervisor, Prof. David Mellor, has been always there to listen and give advice. Thank you for being there from Day-1. I am deeply grateful to him for giving me the opportunity to return to Massey University (some 10 long years ago). I am also thankful to him for encouraging and directing my many postgraduate papers and written reports and for carefully reading and commenting on countless revisions of many manuscripts. I am indebted to him for his continuous encouragement, guidance and friendship.

Dr's Patrick Janssen and Gordon Reynolds for their co-supervision, their insightful comments and constructive criticisms at different stages of my research were thought-provoking and they helped me focus my ideas. I am grateful for their immense technological knowledge, advice, competence and skills.

I would like to acknowledge Assoc. Prof. Paul Chambers for all his technical help and veterinary care. Thank you for your flexibility, help and co-operation in all things.

I am grateful to Dr. Clement de Loubens for in whom I found a colleague and a friend. Thank you for all the laughter, encouragement, and practical advice.

Thank you to Ian, Ivanna, Anne G, Bob, Hannah, Tamara and all the other postgraduates on the same journey as myself. Keep on keep'n on! There is a finish line!

Thank you to all the staff (current and former) I share a space with- Anne B, Kim, Fran, Chris, Michelle, Linley, Shampa, Debjit, Gabby, Wei, Fran, Yvonne, Lynne, Marlena, and Miria. The many staff-room 'support group' sessions have been invaluable.

Thank you to my friends, too numerous to name, but you know who you are. Their support and care helped me stay sane. I greatly value their friendship and I deeply appreciate their belief in me.

Thank you to Lois and Alan Wilkinson (My surrogate parents) for proof-reading the manuscript.

Most importantly, none of this would have been possible without the love and patience of my family. To my immediate family and wife to whom this thesis is dedicated, thank you for your constant source of love, concern, support and strength all these years.

I know it seems cliché to say I have the best parents in the world- but it is fact. Mum and Dad, thank you. In my life's brightest and darkest times, you have been there- unflinching, unwavering. The opportunities you have given me in life, the experiences, your generosity and love, I am indebted and will never forget.

In Glenn, I could not find a better brother. Thank you for being there. In what you yourself have achieved and accomplished in life I am extremely proud.

Finally, to my wife Ana- to express what you have done for me and how you have helped me is difficult to put into words and will always be insufficient. You have been my saviour, my rock, my confidante, my friend..... my everything. Together we have built a life. What you have accomplished and achieved, the goals you set for yourself are an inspiration. You have always encouraged me, supported me, and motivated me in every endeavour. Simply put- I am a better person having met you and a greater person by being with you.

Copyright and permissions

Permissions to reproduce the figures from other authors/publishers and material from my published journal articles have either been obtained or are in the process of being approved by the relevant authorities at the time of submission of this thesis.