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)

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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.

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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.

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Chapter 1- Introduction

1- Introduction

The mammalian digestive tract is constantly in motion. Segments of gut must break down, mix, propel and absorb dietary nutrients in a highly complex succession of processes. Smooth muscle layers in the wall of the digestive tract exhibit phasic and tonic contractions. The controlled progression of chyme along the gastrointestinal tract is the result of the interplay between spontaneous activity of the intestinal smooth muscle, enteric and extrinsic neural control (Costa and Furness 1982; Huizinga et al. 1998; Hennig et al. 1999). This complex phenomenon of regulation and coordination of motility is best seen at the junctions between various components of the gut, notably the ileocaecal junction.

Gut motility and the process by which food is digested and transported within the digestive tract have long been studied by physiologists. There are numerous techniques available by which the various aspects of motility (intraluminal pressure, tone, compliance, wall motion, myoelectric activity and transit) can be studied (Smout and Mundt 2009). In the rabbit, caecal motility and the flow of digesta within and between adjacent segments has been described myoelectrically (Ruckebusch and Hörnicke 1976; Papasova and Mizhorkova 1976), with florescence radiography (Ehrlein and Ruoff 1982), through the movement of marker particles (Jilge 1982), and in the caecum and proximal colon via strain transducers (Ehrlein et al 1982). Trenelenberg preparations (1917) of various segments of the gut have enabled the physical form of contractions and analysis in living segments of the digestive tract to be directly assessed. Early workers using these techniques have made significant contributions to the understanding of gut motility, particularly in regard to the origin, methods of control, and also the frequency and velocity of propagation of electrical activity that underlies contractile events in this species.

The development of computational image processing in the last decade (Jähne 2004; Ross 2006) has opened new possibilities to quantitatively evaluate the characteristics of gut contractions. Spatiotemporal (ST) mapping techniques can not only quantify tonic and phasic contractions, but the quantified results can form the basis of complex algorithms to analyse the flow and mixing of the lumen contents, contractile processes activated by networks of intestinal cells of cajal (ICCs), and subtle patterns of contraction

and dilation associated with displacement and accommodation of digesta (Janssen and Lentle 2013).

It is the aim of this thesis to use ST mapping techniques to further calculate and quantify the contractile behaviour in the rabbit caecum, and to describe the coordination of various contractions in the caecum. Further, it is hoped the observations presented here may offer some insight into the assessment of motility disorders such as idiopathic ileus, diverticulitis, and stasis in the human caecum.

Chapter 2- Literature Review

2.1 Foreword

The principle objective of this thesis is to describe the motility of the rabbit caecum as a whole, and also automated movements in the adjacent terminal ileum and proximal colon. A close relationship must exist between the motor activity of the caecum and the sections of gut proximal and distal to it so as to regulate and coordinate the orderly flow of digesta. This review is to set the stage of this thesis, summarizing previous relevant work that has a bearing on the objective, most (if not all) of which is from studies conducted on various mammals. The following sections provide an overview of what is currently known about the rabbit caecum and its interrelations with the adjacent segments of gut, each of which will also be considered in the context of their contributions to motility in the rabbit caecum.

2.2- Ontogeny and Embryonic Development of the Rabbit Gastrointestinal Tract

2.2.1 Introduction

The gastrointestinal tract performs the mixing, propulsion, digestion and absorption of nutrients both pre- and postnatally. During gastrulation, tissue layers are formed and the overall body plan is established. In this section the general embryonic development of the gastrointestinal tract will be examined including early gastrulation, neurulation, and nervous innovation of the ileocaecal junction. The important changes that occur due to endogenous (mucosa, absorption, immunity, gut micro flora etc.) and exogenous factors (nutrition, weaning etc.) will also be considered. An understanding of these processes sheds light on the development at this position of the gut and the neural stimulation that controls transit of digesta into and out of the caecum.

2.2.2 Embryonic gastrointestinal development in the rabbit

2.2.2.1 Genesis of gut cavity: Gastrulation and Neurulation

Early embryonic and feto-placental development in the human and rabbit are similar (Fischer et al. 2012). Cells of the embryo are divided into three groups: the ectoderm, which forms skin and central nervous system; mesoderm, which forms blood, bone and muscle; and the endoderm, which forms the lining of the respiratory and digestive tracts. The process by which the cells divide, differentiate, and rearrange themselves into these three distinct germ layers is termed gastrulation.

During the early transformation of the three germ layers into tissue and organs, the notochord is formed along with the neural tube. The development of the neural tube is called neurulation.

The notochord is first visible soon after gastrulation is complete, forming from mesoderm along the dorsal midline of the embryo. It is a flexible rod located along the dorsal midline in the embryos of all chordates, although its function is replaced by the vertebral column which subsequently develops from mesoderm in the vertebrates. After the notochord has been laid down, a layer of ectodermal cells dorsal to the notochord invaginates, forming a

long crease, the neural groove, down the long axis of the embryo. The edges of the neural groove then move toward each other and fuse, creating a long hollow cylinder, the neural tube, which runs beneath the surface of the embryo's back. The neural tube later differentiates into the spinal cord and brain.

Just before the neural groove closes to form the neural tube, its edges pinch off, forming a small strip of cells, the neural crest, which becomes incorporated into the roof of the neural tube. The cells of this neural crest later migrate to the sides of the developing embryo, ultimately laying the ground work for the innervation of all structures within the vertebrate body. At the cranial end of the embryo, the neural crest cells form placodes which develop into the sense organs of the head. Some neural crest cells migrate ventrally toward the notochord and form sensory neurons in the dorsal root ganglia. Others become specialized as Schwann cells, which insulate nerve fibers and permit the rapid conduction of nerve impulses. Still others form the autonomic ganglia and the adrenal medulla.

The neural crest cells that enter the foregut, advance along the entire length of the gastrointestinal tract by a combination of migration and proliferation. Some of these enteric neural crest cells (ENCCs) differentiate into neurons. Coordination of these activities results in the innervation of the gut and establishment of ganglia containing neurons and glia that constitute the enteric nervous system (Druckenbrod and Epstein 2005).

Migration of ENCCs is essential for the distribution of neural cells throughout the gut. In fixed preparations of developing gut, ENCCs are arranged in strands or cords (Epstein et al. 1991; Young et al. 1998; 1999; Conner et al. 2003). The most caudal cells of the strands represent the migratory wavefront, while rostral to the wavefront, the crest cells show a pattern of branches and nodes that preceeds the organization of the adult ENS (Druckenbrod and Epstein 2005). In the ileum and distal colon, strand extension is a continuous process. In the cecum and proximal colon, however, the process shows three distinct differences from the pattern observed in other regions. These differences are the pause in migration at the ileocaecal junction, the variation in extension of strands into the cecum, and the extensive fragmentation of strands resulting in large numbers of advanced isolated cells (ACs). The colonization of the caecum and proximal colon in the mouse are shown in Fig. 2-1.

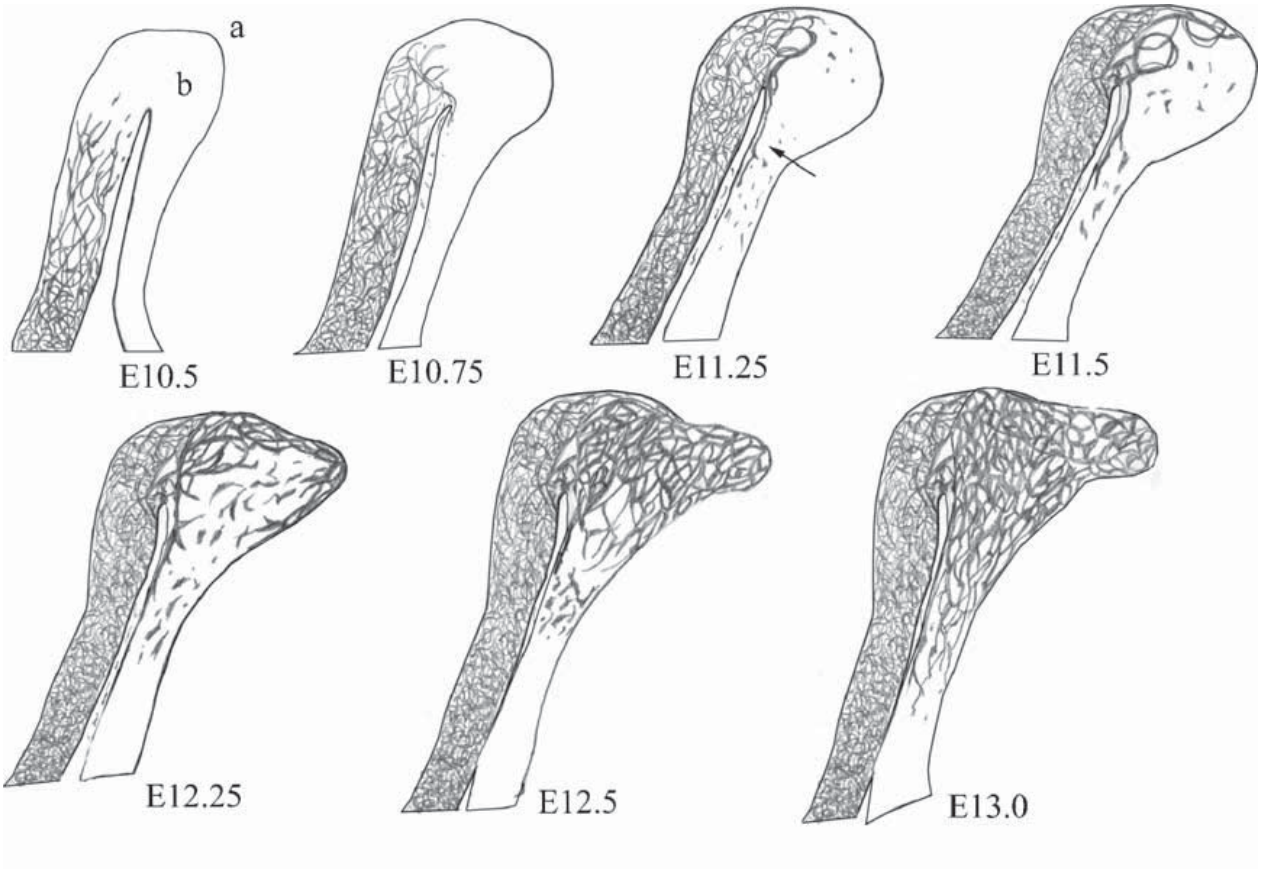

Fig. 2-1. (Druckenbrod and Epstein 2005). Diagram showing the colonization of cecum and proximal colon in the mouse with approximate embryonic ages. Successive diagrams show the point of union between the fore and hindgut in the developing embryo. The ileum is on the left and the colon on the right with the growth comprising the caecal bud. Distal ends of strands form a wavefront that advances through the caudal ileum at E 10.5 and reaches the ileocaecal junction at 10.75. At this stage, a few ENCCs are found in the mesentery. From 10.75 to E11.25, most ENCCs have paused, and have accumulated at the caecal base. A few form a long strand along the mesenteric border of the cecum and colon (arrow at E11.25; mesenteric strand). Close to the termination of the mesenteric strand, isolated ENCCs appear in the caudal cecum. At E11.5, strands of cells are half way to the apex, and at E12.25, they have filled the apex and are located on the anti-mesenteric border of the cecum. At this stage, isolated clusters and solitary cells appear in the body of the cecum and are distinct from those found in the proximal colon. At E13.0, the caecal population has intersected with the clusters in the proximal colon; this merged population forms strands of ENCCs that proceed to colonize the remainder of the colon in a manner similar to that seen in the ileum.

During the colonisation of the ileum and colon, extensions of the strands of ENCC pause only minutes. In contrast, colonisation by ENCC's may pause for hours at the caecal base (Druckenbrod and Epstein 2005). Again, whilst innervation by individual strands of ENCCs advancing through the ileum and caudal colon initially appears random (Young et al., 2004b), strands in the caecum appear more organized and ultimately progress along the mesenteric and anti-mesenteric borders of the caecum before colonizing the caecal body (Druckenbrod and Epstein 2005). The sum of this process results in the ACs of the ileum being sparsely distributed and formed close to the wavefront (Young et al., 2004b) whilst ACs in the cecum and proximal colon are many, and formed far from the wavefront.

ACs in the proximal colon persist for hours, and whilst the cecum is slowly being colonized, the ACs in the proximal colon aggregate, proliferate, and then expand into short strands. As the population of ENCCs subsequently colonize the cecum and proximal colon; it merges with the immature network formed by the ACs. The addition of this advanced population of ENCCs to the distal strands extending from the cecum extends the wavefront caudally. The importance of adding an advanced population to the delayed caecal population is unknown, but it creates an unbroken network of ENCCs that advance through the remaining colon (Druckenbrod and Epstein 2005).

2.2.2.2 Organogenesis and Morphogenesis

At the end of gastrulation the endoderm exists as a layer of cells whose form is characterised by expression of regional determining factors (Noah et al. 2011). The developing intestine expresses combinations of Hox genes along its anterior and posterior axis which are important in patterning the mammalian gut (Zacchetti et al. 2007). After gastrulation, the endoderm is a one cell-layer thick sheet of approximately 500 cells (mouse) that will form the epithelial lining of the oesophagus, lungs, stomach and intestines. Subsequent morphogenetic movements result in the sheet of endoderm being pushed into the inside of the developing embryo, eventually forming a simple tube (Zacchetti et al. 2007). Following induction and molecular patterning, the endoderm undergoes extensive folding to generate the embryonic gut tube. This process is called tubulogenesis and is initiated by the indentation of the cranial and caudal ends of the embryo to form pockets.

As the pockets become deeper, the lateral midgut endoderm folds ventrally to complete tubulogenesis (Noah et al. 2011). The tube will become the gastrointestinal tract, and the buds which project from this tube will grow, branch, and eventually form differentiated functioning organs.

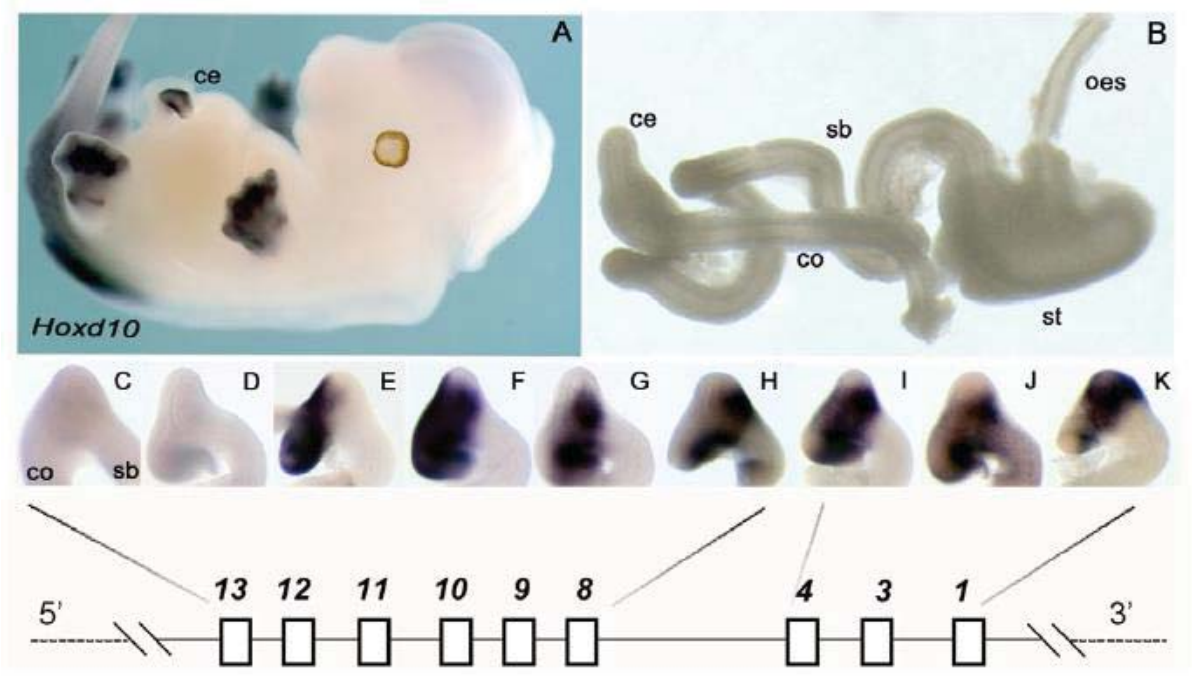

Fig. 2-2. (Zacchetti et al 2007). Co-expression of seven Hox genes in posterior midgut. (A) Whole-mount RNA in situ hybridization detection of Hoxd10 transcripts in E13 mouse embryo, showing some sites of expression, including the intestinal hernia. (B) Anatomical subdivisions of the mid-gestation murine gastrointestinal system at E12. (C-K) Detection of Hoxd13 (C), Hoxd12 (D), Hoxd11 (E), Hoxd10 (F), Hoxd9 (G), Hoxd8 (H), Hoxd4 (I), Hoxd3 (J) and Hoxd1 (K) transcripts in dissected gut of E12.5 mouse embryos. The contiguous loci Hoxd1 to Hoxd10 are all co-expressed in the posterior midgut, in the region that involves the incipient caecum bud (F-K). Hoxd11 is excluded from the anterior (ileal) part, but is expressed in the posterior (colonic) part of the caecum bud (E). Hoxd12 (D) and Hoxd13 (C) expression is not detected in this region. ce, caecum; co, colon; oes, oesophagus; sb, small bowel; st, stomach.

After the gut tube is fully formed, the gut tube lengthens and the circumference increases due to the expansion of the mesenchyme, epithelium and the lumen.

The caecum forms at the limit between the ileum and the colon i.e. at the junction between the foregut and hindgut; in mice, it begins to grow at day 10 of embryonic development (Fig. 2-2). A large number of Hox genes are expressed in this region and are required for the proper formation of the ileum-to-colon transition and the concurrent budding of the caecum (Noah et al. 2011).

After organogenesis a second phase begins in the mid gut with the formation of villi. In the rabbit, the fetal duodenum develops from a simple tube of stratified epithelium to a tube containing villus and intervillus regions which are covered by a simple columnar epithelium. By day 21 of gestation, the first rudimentary villi have appeared and by day 24 the first true villi are visible (Elnasharty et al. 2013). Cells lining the villi differentiate into enterocytes with microvilli, brush-border hydrolases, and nutrient transporters. With this differentiation of cells the intestine continues to grow and mature.

Intestinal organogenesis and the onset of hydrolytic and transport activities around the intestinal lumen begin earlier in mammals with long gestations, but not until about %80 of gestation is complete in rabbits and other species with short gestations. Hence, lactase production develops late in gestation with peak activity occurring at birth (Buddington 1993).

The intestine is not inactive during gestation. Development of the gastrointestinal tract in the fetus is dependent on nutrients provided both by the placenta (Koski and Hill 1990) and by swallowed amniotic fluid (Mulvihill et al. 1985). After the villi are formed and covered with differentiated enterocytes, fetuses begin to swallow and process amniotic fluid whereby nutrients absorbed from the amniotic fluid are incorporated into fetal tissues (Phillips et al 1991). This amniotic fluid contains not only nutrients but biologically active substances such as growth factors and hormones (Buddington 1993) which are required for normal intrauterine growth (Mulvihill et al. 1985).

While the various organs are being formed, the overall shape or morphology of the embryo as a whole undergoes changes. In the case of the vertebrate embryo, the main changes during the period of organogenesis are (Ballinsky 1981):

1. Elongation of the body.
2. Formation of the tail.
3. Subdivisions of the body into head and trunk.
4. Development of appendages.
5. Separation of the embryo proper from the extra-embryonic parts.

Beaudoin et al. (2003) has described embryonic growth in the rabbit fetus (described below). An overall growth curve and a sequence of representative developmental stages of the rabbit are shown below (Fig 2-3).

Two distinct phases are observed; the first a period of rapid growth which continues until day 15; the second a period of relatively slow growth which continues until term (31-32 days). Fetal weight increases rapidly during the last 10 days (d) of pregnancy. At day 20 the foetus weighs less than 5g, thereafter it increases rapidly gaining about 2g per day until day 22, 4g per day until day 24, and 5g per day until day 30 (Lazarus and Volk 1963).

At 8.5d, the rabbit embryo thickens as the first somites appear. At day 9.5 a dorsal curvature appears and the cardiac mass begins to form. From days 10.5 to 13.5 the dorsal curvature increases. After day 13.5 the body thickens in a more cuboid form, and the predominant cephalic region softens and the embryo begins to straighten with the neck visible at 17.5d.

Forelimb development begins at day 9.5d and the hind at 10.5d. At 12.5d the hand plate is present. The foot plate and finger rays are visible at 13.5d. At 14.5d the fore and hind limbs appear parallel with each other. The elbow appears at day 15.5 and the fingers begin to elongate at 16.5d. The knee is visible at 17.5d and the three segments of the limbs can clearly be distinguished at 19.5 days.

At 9.5d the abdominal wall is located under the cardiac mass. It is progressively lifted by the developing liver, and the first intestinal loops appear at 13.5d inside the gut cavity. Rapid

intestinal growth occurs between 14.5 and 17.5d. At 16.5d the caecal bud is visible and the abdominal cavity is enclosed at 18.5.

The head of the embryonic rabbit consists of only one cerebral vesicle at day 9.5. At day 10.5 three arches are seen in the cephalic pole. At 11.5d, three cerebral vesicles can be seen and the optic plate can be differentiated. At 12.5d the face is modelled, developing nasal, maxilla and mandibular buds and five cerebral vesicles exist. The ear is outlined at day 13.5 and becomes more refined at day 14.5. Eyelids appear and cover the eyes at 18.5d.

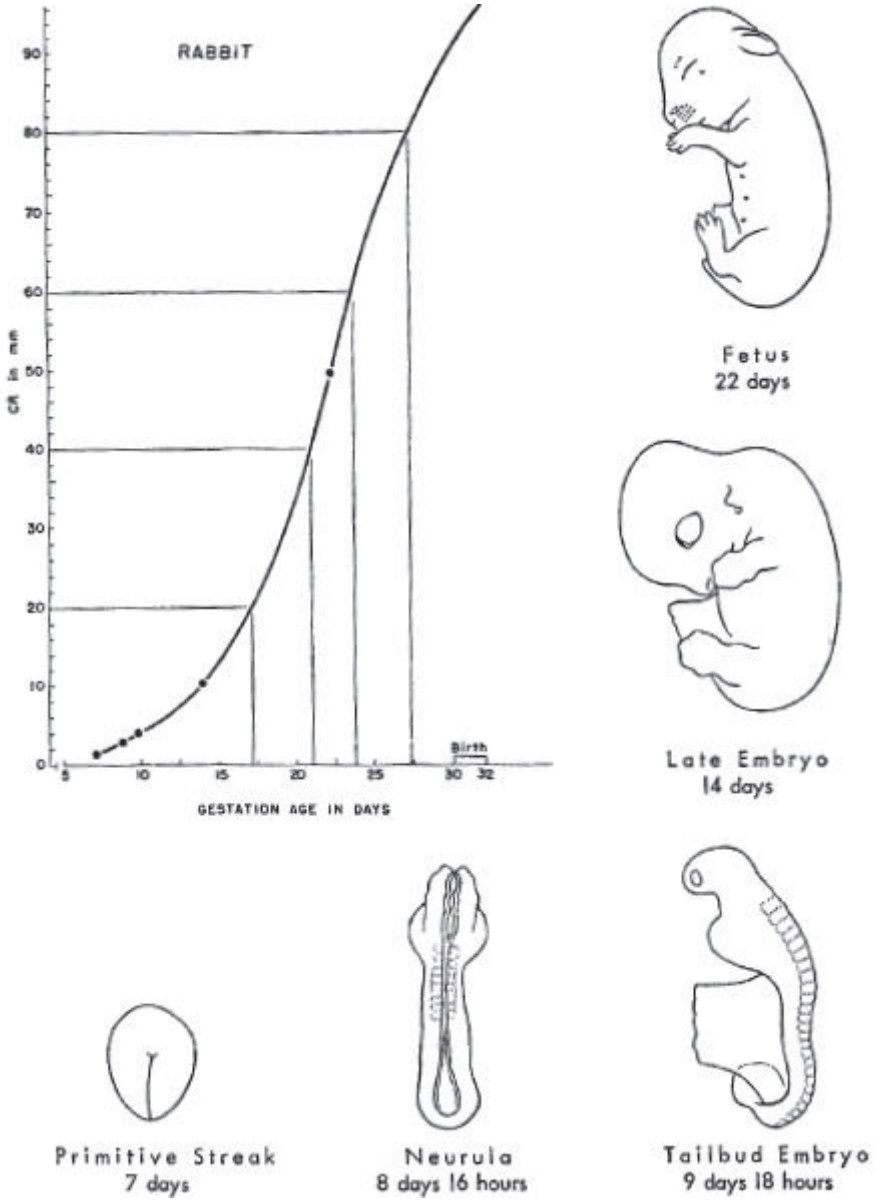

Fig.2-3. (Evans and Sack 1973). Growth curve and representative developmental stages in the Rabbit (*Oryctolagus cuniculus*).

2.2.3 Postnatal Gastrointestinal Development in the Rabbit

Gestation in the rabbit is approximately 26-36 days depending on the species of rabbit (Wilson and Dudley 1952). Birth and the transition to extra uterine life is a critical phase of development, and for the digestive system, is associated with dramatic increases in functional demands.

During birth there is colonization of the gastrointestinal tract by microbiota. The fetus is normally sterile in utero but becomes contaminated at birth with a heterogeneous collection of microorganisms from the maternal birth canal and immediate environment (Berg 1996). In the rabbit, the caecum is colonized by bacterial flora, often strictly anaerobic, that stabilizes a few days after weaning (Padilla et al. 1995). The bacterial community slowly evolves toward a more complex and regular community during the period from birth to 10 weeks of age (Combes et al. 2011).

Once born, rabbit young suckle milk from the mother until about the 11th to 14th day where upon they begin to eat solids. From birth to maturity the gastric and intestinal walls continue to develop. The mucosa thickens and the diameters of villi and mucous crypts increase. The development of the rabbit hindgut follows that of the small intestine which matures at around 8 weeks (Yu and Chou 1997). The caecum increases in weight, length and width from 3 to 12 weeks of age (Abdel-Khaled et al. 2011). The tunicae (Muscular, Submucosa and Mucosa) as well as lamina epithelial all increase in thickness between 3-4 weeks of age. Thereafter, trends of increase in thickness vary in pace, but all reach the maximum at 16 weeks (Abdel-Khaled et al. 2011).

Crypt development by division is the dominant process during the suckling period, but after weaning, development is restricted to mucosal hyperplasia (Cummins et al. 2006).The pre-weaning period of rabbits is a very critical phase because of the change from milk to an exclusively solid feed intake. On weaning, the mode of digestion changes from enzymatic, to a combination of enzymatic digestion followed by bacterial fermentation (Abdel-Khaled et al. 2011). Fermentative activity occurs mainly in the caecum and begins around 18-20 days of age, reaching its maximum development by the 6^{th} week (Pascual 2001).

The development of the caecal tunica mucosa is important for the absorption of microbial products. Dietary fibre may stimulate the division of mucosal cells with differing types of

fibre influencing different mucosal cells (Johnson et al. 1984). Fermentation products i.e. volatile fatty acids generated in the caecum are also suggested to be a simulative factor of mucosal growth (Chou et al. 1994). Thus, the increasing development of the tunica mucosa and lamina epithelial is closely associated with a switch to solid feed at 3 weeks of age (Cheeke 1987). Increasing thickness of tunica mucosa increases its absorptive ability with a corresponding thickening of the tunica submucosa, which contains blood vessels important for absorption (Abdel-Khaled 2000).

2.2.4 Concluding Remarks on the Ontogeny and Embryonic Development of the Rabbit Gastrointestinal Tract

In this section, the origin and development of the rabbit gastrointestinal system was presented, illustrating the complex phenomena involved in the genesis and maturation prior to and following weaning in the small herbivore. Of particular note is the specific neural patterning of the caecum that is synchronous with the differentiation of the gut tube into its various divisions. These processes provide some clues to the modulation of the actions of the caecum. Hence, neural development appears into enter the caecum principally from the hindgut- promotion of potential neural genesis from the small intestine being limited to the proximal most portion of the caecum. Fine tuning of the digestive processes by suitable development of the caecal mucosa and its associated structures only occurs after both structures become colonised by microbiota.

2.3- The Digestive Physiology of the Rabbit and the Morphology of its Digestive System

2.3.1 Introduction

The rabbit digestive system is characterised by the relative importance of the caecum and colon have when compared with that of other species (Portsmouth 1977). Nutrient absorption from hindgut fermentation is also augmented by behavioural means i.e. by caecotrophy, the deliberate of ingestion of soft faeces that are voided directly from the caecum. The digestive system is also evolved to process a high feed intake and to rapidly transport the feed through the gastrointestinal system to meet nutritional requirements (Carabańo et al. 2010).

2.3.2 The Digestive Physiology of the Rabbit

Rabbits are member of the order *Lagomorpha*, which includes hares and pikas. All lagomorphs are terrestrial and herbivorous. Rabbits are hindgut fermenters and rely mainly on microbial fermentation of carbohydrates within the caecum to provide nutrients. Digestion and absorption of nutrients in the stomach and small intestine is similar to that in other monogastric mammals. Small herbivores have evolved several mechanisms that enable them to process cellulose and forage containing a high proportion of fibre (Foley and Cork 1992; Justice and Smith 1992).

The first strategy is that of selective separation of digestible components of fibres from the indigestible fraction of the food in the colon. The proximal colon of the rabbit is specially adapted for the separation of large nutrient poor particles of indigestible fibre from smaller nutrient dense particles that can be degraded and used as substrate for bacterial fermentation in the caecum (Harcourt-Brown 2001). The two components are propelled in opposite directions- indigestible fibre passes down the central lumen of the colon to be rapidly eliminated as hard, dry faecal pellets, and smaller particles and fluids pass along the periphery of the colonic lumen into the caecum for fermentation (Björnhag 1981).

The contents of the caecum are evacuated intermittently. Pellets of soft caecal contents (caecotrophs) (Fig. 2-6.) are periodically expelled from the anus and re-ingested for proximal digestion. The rabbit's habit of consuming caecotrophs directly from the anus is known as

'caecotrophy' (Ebino et al. 1993). Coprophagia is defined as 'the ingestion of dung or faeces' (Blood and Studdert 1999). Faeces are defined as 'body waste discharged from the intestine' and so, strictly speaking, faecal material is not the substance that is ingested by the rabbit but nutritionally rich bacterial protein. This digestive strategy utilizes bacterial fermentation to synthesize nutrients and avoids the need to store large volumes of food in the digestive tract (Harcourt-Brown 2001).

Cellulose is broken down into its component sugars which are absorbed by the walls of the caecum and colon and the bacterial bodies remain to be digested on re-ingestion. A schematic representation of the rabbit's digestive system is illustrated in Figure 2-4.

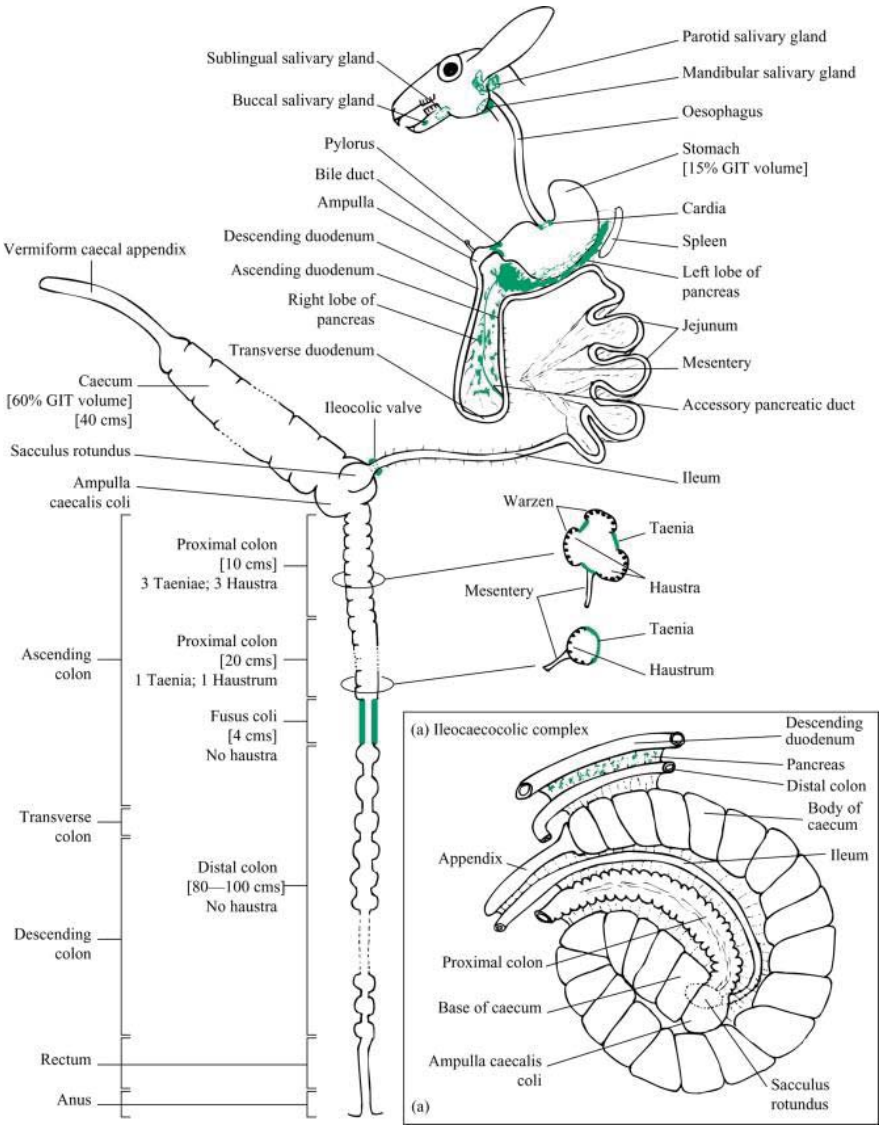

Fig. 2-4. (Harcourt-Brown 2001). The digestive system of the rabbit. The caecum and appendix are shown as a straight tube, but in fact are a helix (See 2- 4a.). 2- 4a shows a ventral view of the ileocaecal region. The area has been slightly unrolled to reveal its component parts.

2.3.3 The Mouth and Oesophagus

The volume of the mouth of the rabbit is relatively small, and the oral cavity and pharynx are long and narrow. The dental formula is i2/1,c0/0,pm3/2,m2-3/3 x 2 = 26 or 28 teeth (Suckow et al. 2002).

Rabbits in the wild selectively eat young succulent shoots. The primary incisors and primary maxillary incisors or “peg” teeth are used to bite, tear, and shear food, the premolars and molars to grind the food into small fragments. Food is located by means of sensitive vibrissae on the lips, as the ocular position in rabbits prevents visualization of objects directly in front of the mouth. The teeth of rabbits grow throughout its life and therefore will continue to grow and lengthen unless there is sufficient wear to the teeth to keep them to an acceptable length. Rabbits normally masticate food with a chewing motion that grinds the food by the movement of the premolars and molars from side to side and front to back.

The rabbit has four pairs of salivary glands, the parotid, sub maxillary, sublingual, and zygomatic. The parotid is the largest and lies laterally just below the base of the ear. Amylase and galactosidase are produced in the saliva, which is produced continuously by the mandibular glands.

The oesophagus serves as a duct from the oral cavity to the stomach. It consists of three layers of striated muscle that extend the length of the oesophagus down to, and including, the cardia of the stomach. This differs from humans and many other species of animals, which have different portions of striated muscle along the length of the oesophagus. There are no mucous glands in the oesophagus of the rabbit.

2.3.4 Anatomy and Digestion in the Stomach

The stomach of the rabbit is a thin-walled, pouch-like organ that comprises about 15% of the volume of the digestive tract (Cruise and Brewer 1994). The cardiac portion of the stomach is thin walled, non-glandular, and intrinsically immobile (Cruise and Brewer 1994). It has a well-developed cardiac sphincter that does not allow regurgitation, and a muscular pyloric area. The stomach is never entirely empty in the healthy rabbit. The gastric contents often include a large amount of hair ingested as a result of normal grooming activity. Together, the caecum and the stomach contain over 80% of the digesta in the digestive tract

(Lang 1981). Water, enzymes and large quantities of hydrochloric acid are secreted into the stomach. The fundus is the major secretory portion of the stomach and has parietal cells (which secrete hydrochloric acid and intrinsic factor) and peptic cells (which secrete pepsinogen, the precursor of pepsin). The postprandial pH can fall to 1-2, which effectively sterilizes the ingesta before it moves into the small intestine. The stomach pH of suckling and juvenile rabbits is higher at approximately 5-6.5, which allows bacteria to pass through the stomach to the hindgut and to colonize the caecum (Harcourt-Brown 2001). During the digestion of caecotrophs the stomach pH rises to 3 (De Blas and Gidenne 1998). The caecotrophs are not chewed before swallowing, but remain intact, protected by their mucinous coat. During the 6-8 hours in the stomach, the caecal material within the caecotrophs is protected from the gastric pH, and microbial fermentation continues. In contrast, food moves through the stomach in approximately 3-6 hours (Carabaõ and Piquer 1998).

2.3.5 Anatomy and Digestion in the Small Intestine

The small intestine of the rabbit is short relative to that of other species and comprises approximately 12% of the total length of the digestive tract (Suckow et al. 2002). The duodenum is slightly enlarged proximally where it receives the bile duct. The rabbit produces around 100-150 ml of bile per kilogram bodyweight daily, independent of secretin stimulation- seven times the rate of production in the dog (Jenkins 2000). Bile acids, such as cholic and chenodeoycholic acids, are synthesized in the liver, and released into the small intestine. Hence the breakdown of fatty or oily material into smaller micelles is likely to be efficient allowing absorption of fats and fat-soluble vitamins in the distal small intestine.

The pancreas is situated in the mesoduodenum of the duodenal loop. It is small and diffuse in shape, often difficult to locate within the mesenteric fat located between the colon, stomach, and duodenum. The main pancreatic duct enters near the end of the duodenum (See Fig. 2-4), well away from the entry of the bile duct (Carabaõ and Piquer 1998). Trypsin, chymotrypsin, and carboxypeptidases are produced in the pancreas and released into the intestinal lumen. These work along with intestinal amino-peptidases to complete protein digestion. Lipases of various forms are also produced. The pancreas is an important source

of bicarbonate ions that neutralize the acidic chyme entering the small intestine from the stomach (Rees Davies and Rees Davies 2003).

Small intestinal digestion and absorption in the rabbit are similar to other species. The acidity of chyme leaving the stomach is neutralised by bicarbonate ions secreted into the duodenum. Most of the digestion of carbohydrates and simple proteins takes place in the duodenum and jejunum and monosaccharides and amino acids are absorbed across the brush border cells. Caecotrophs are digested in this section of the gastrointestinal tract. Having travelled from the stomach whilst being protected by their mucous coating, they are broken down into material such as amino acids, volatile fatty acids, vitamins, and digested microbial organisms (Rees Davies and Rees Davies 2003). Lysis of the microbes within the caecotrophs also releases microbial enzymes which may enhance the rabbits own digestive processes. The ileum regulates and recycles electrolytes secreted by the stomach and proximal small intestine by reabsorbing bicarbonate ions (Rees Davies and Rees Davies 2003).

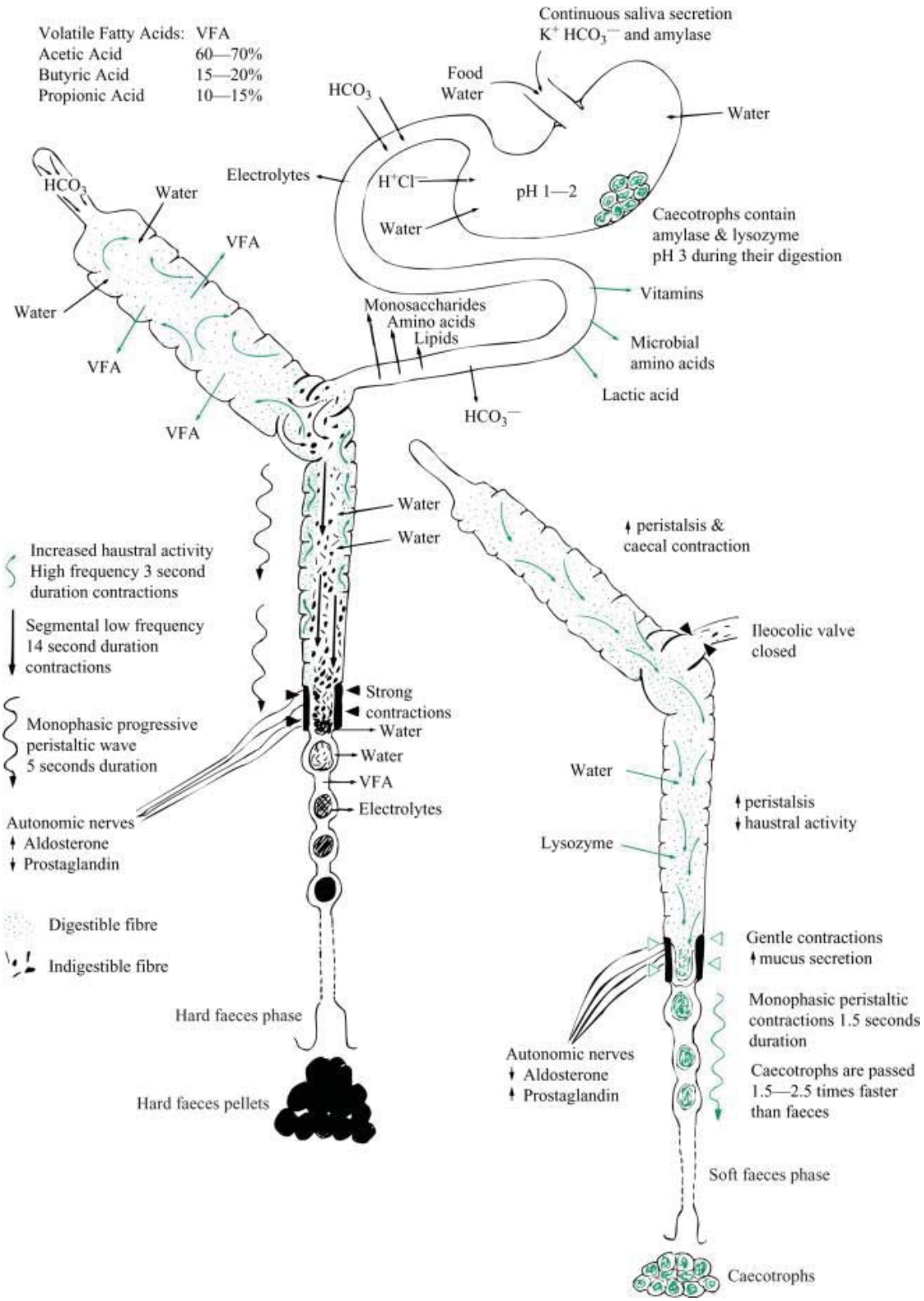

Fig. 2-5. (Harcourt-Brown 2001). The activity of the rabbit digestive system during the excretion of hard and soft faeces. The formation of hard faeces is known as the hard faeces phase (shown in black) and the expulsion of caecotrophs is known as the soft faeces phase (shown in green). The direction of water and electrolyte exchange is indicated by arrows.

2.3.6 Anatomy and Digestion in the Hindgut

The large intestine of the rabbit consists of the caecum and colon. The sacculus rotundus opens from the small intestine into the basal portion of the caecum. Similarly, the colon attaches to the base of the caecum at the ampulla caecalis. Hence, the base of the caecum forms a T-junction between the ileum, caecum and proximal colon. The proximal colon is specifically adapted for mixing, separating and sorting large food particles. Large particles of indigestible fibre are separated from small fermentable particles and fluid. The large particles travel distally along the colon to the anus while small particles and fluid travel proximally into the caecum where bacterial fermentation takes place (Fig. 2-5.).

The rabbit's caecum is proportionally the largest of any eutherian mammal. It is twice the length of the abdominal cavity and contains 40-60% of the total volume of the digesta in the gastrointestinal tract (Jenkins 2000). The flow of chyme into the caecum is regulated by the sacculus rotundus and the ileocaecal valve and prevents back flow into the ileum. Small nutrient rich particles are also propelled from the colon into the caecum, which acts as a large bacterial fermentation chamber to which nutrients and water are continually added.

Studies on the enzymatic activities of the caecal micro flora indicate that ureolysis, proteolysis, and cellulolysis take place in that order (Carabaõ and Piquer 1998). Any simple sugars that are not completely absorbed in the small intestine also undergo bacterial degradation in the caecum. Residual plant proteins are also degraded in the caecum to form ammonia that is metabolized to amino acids by caecal micro flora. Soluble ions are absorbed across the caecal wall as are short chain fatty acids (Harcourt-Brown 2001).

In healthy rabbits, high numbers of large anaerobic metachromatic bacteria are present in the caecum (Lelkes and Chang 1987). Non-pathogenic, gram-negative *Bacteroides* species dominate the flora. Species such as *Bifidobacterium, Endophorus, Clostridium, Streptococcus* and *Acuformis* have been identified (Cheeke 1987; Carabaõ and Piquer 1998). Over 74 strains of anaerobic bacteria have been isolated from the caecal mucosa and many of these species have not been identified (Straw 1988).

A number of these species are susceptible to changes within the caecum. Hence, the population of microorganisms within the caecum changes with the time of day, with caecal pH, and with dietary substrate (Varga 2013). Bacterial fermentation within the caecum and

colon, as previously described, results in the synthesis of amino acids, volatile fatty acids and water soluble vitamins. Most soluble nutrients produced by the caecal and colonic micro flora are absorbed across the caecal and colonic wall. The remaining contents form a soft, dark-coloured paste containing bacteria, and low concentrations of amino acids, vitamins and minerals.

The general structure of the rabbit caecum will be described here and a more detailed description presented later.

The body of the caecum and appendix appear to be a simple, blind ending tube, but the lumen is in the form of a helix (see Fig. 2- 4a) with a larger proximal transverse dimension and small distal transverse dimension. The caecum is thin walled and often translucent. A long spiral fold with 20-30 'turns' (the "spiral valve") extends from the caecocolic ampulla at its junction with the colon, along the first three folds of the caecum. The vermiform appendix forms the final 13cm of the blind end and has thick walls containing lymphoid tissue (Donelly 1997; Barone et al. 1973; Brooks 1997).

The appendix secretes bicarbonate ions into the caecal lumen, which are thought to act as a buffer for the volatile fatty acids produced by caecal fermentation (Cheeke 1987; Williams et al. 1961). Water secreted by the appendix and colon is absorbed across the caecal wall. Because of this, the caecal contents maintain a viscosity varying between a soft paste to a viscous liquid (Harcourt-Brown 2001). The bulk of gut-associated lymphoid tissue (GALT) of the rabbit is situated in the hindgut and represents over 50% of the total lymphoid tissue (Percy and Barthold 1993). GALT and specialized cells (immune, goblet and Paneth cells, responsible for mucus and antimicrobial peptide secretion, respectively) regulate the interaction of the gut mucosa with the microbiota and develop the mechanisms of tolerance and protection against invasion by pathogens (Carabãno et al. 2010).

Fig. 2-6. (Leng 2008). Faecal types. Caecotropes (left of picture) from the fundus compartment of the stomach and hard faeces (right of picture).

The structure of the ascending colon of the rabbit can be described as containing four sections. The proximal end extends from the ampulla caecalis approximately 10cm distally. This section has three longitudinal flat bands of muscular tissue or taeniae spanning longitudinal arrays of haustra. The second section of the colon is approximately 20cm in length and has a single wider taenia, and fewer smaller haustra. The third section of the colon is termed the *fusus coli* and is a muscular tube approximately 4cm long. This section is highly innervated and vascular with its mucosal surface having many longitudinal folds that contain numerous goblet cells. The *fusus coli* is thought to regulate the elimination of hard versus soft (caecotrophs) faecal pellets (Fig. 2-6.). Hard pellets comprise about two thirds of the faecal output (Suckow 2002). The glandular portion of the *fusus coli* lubricates the surface of the colon allowing rapid transport of intestinal contents.

The last section, the distal colon, is 80-100cm long and runs from the *fusus coli* to the rectum. The mucosa of the distal colon is smooth with no surface specialization. The tunica mucosa possesses short crypts with numerous goblet cells which secrete mucus to coat the pellets and prevent the diffusion of electrolytes. This section of the colon is thin walled and usually contains hard faecal pellets (Harcourt-Brown 2001).

During caecotrophy, caecal contents pass rapidly into the proximal colon where there is mechanical separation of solids and liquids and the faecal masses are further divided in the *fusus coli*.

2.3.7 Concluding Remarks on the Rabbit Digestive System

This section summarized the gastrointestinal physiology of the rabbit and provided an overview of the digestive processes. The rabbit has a system that: (1) allows a high food intake compared to that of ruminants; (2) separates out and retains the digestible and easily fermentable components of the diet, whilst; (3) rapidly eliminates the slowly fermentable fibrous waste. The system also eliminates the need for having a large large intestine by completely separating nutrient rich material in the blind ended side pocket that is the caecum. This allows digesta to be retained longer and to be digested more completely. Further, periodic evacuation of the caecum allows for re-ingestion and digestion of bacteria and their by-products in the small intestine.

The review of the overall structure of the rabbit gastrointestinal tract provides no clues as to how the flow of digesta is directed either into the caecum or into the colon. Again there is no detail explaining the means in which flow of digesta into the base of the caecum via the sacculus rotundus is coordinated with nutrient rich fine particles from the proximal colon. Hence, the next section will provide a more detailed investigation into the structure of the caecum.

2.4- Detailed Anatomy of the Rabbit Caecum

2.4.1 Introduction

The rabbit caecum is a complex structure. Specialised musculature in the caecum and colon allow the rabbit to retain fine particulate material in corpus ceci whilst allowing coarse particles in chyme to traverse the sacculus rotundus to the ampulla caecalis and into the colon. The caecum acts as a blind ended storage organ for small nutrient rich particles and the soluble components of digesta which is held in a buffered medium supporting bacterial growth. As well as acting as a fermentative chamber, the caecum actively absorbs soluble nutrients (such as short chain fatty acids) across its wall. It is also prominent in immune surveillance having considerable amounts of lymphatic tissue. Hence, rather than acting as a simple 'T'-junction, the structure and physiology of the rabbit caecum is a highly specialized organ most correlated with the above mentioned processes and the practice of coprophagy.

2.4.2 The Structure of the Caecum

The caecum (Fig. 2-7.) consists of three major components:

1. The bulbous *ampulla coli* which lies proximal to the colon.
2. The base and body of the caecum which consists of the ileocaecal junction and *corpus ceci*; the latter has 18-22 haustra-like bulges and forms the majority of the caecum.
3. The *appendix vermiformis* which is at the terminal end of the caecum. It is rough surfaced, finger like projection with a narrow.

The caecum is connected to the small intestine or distal ileum via the *sacculus rotundus*.

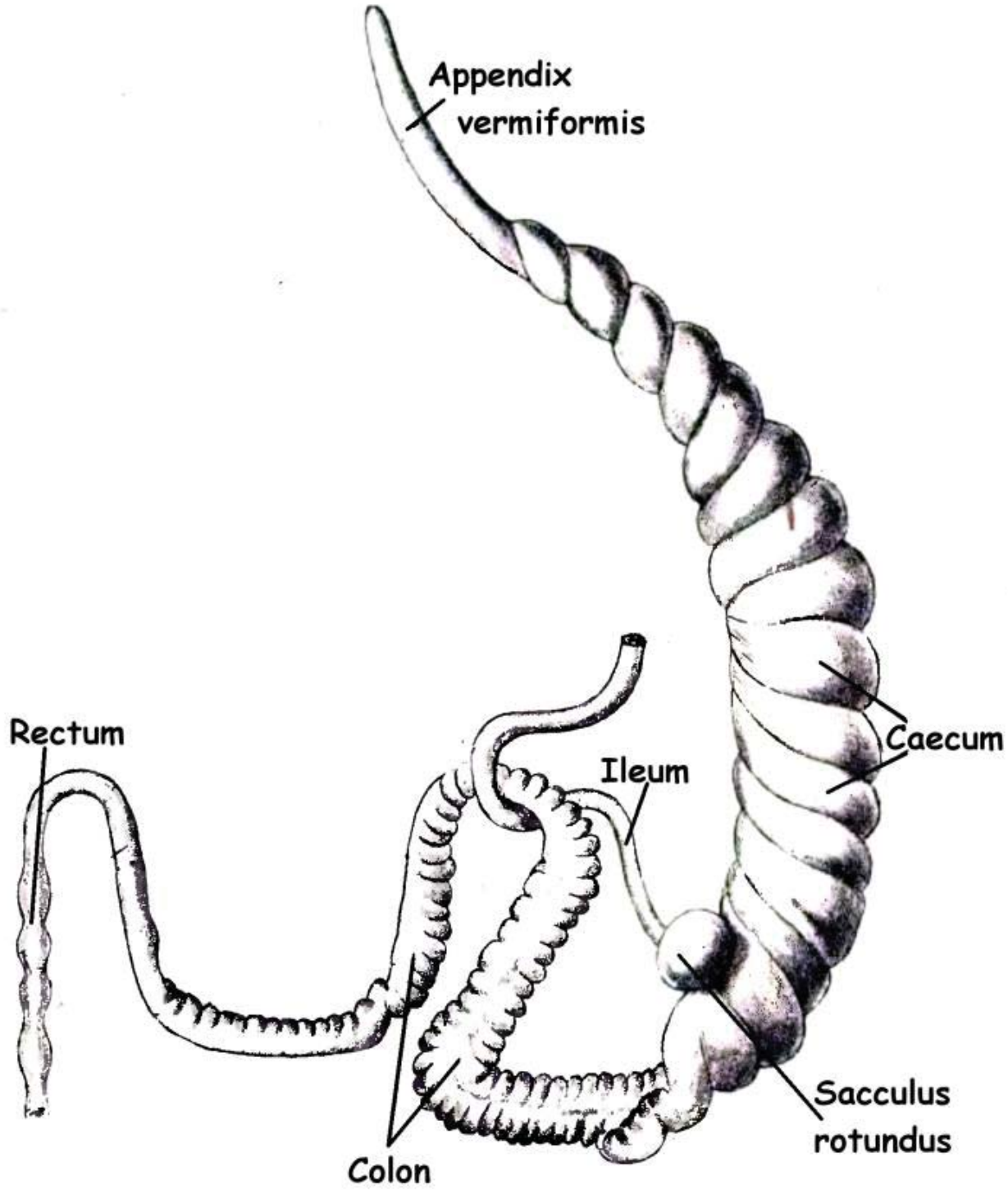

Fig. 2-7. (Smith and Norwell 1889). Appendix, caecum and colon of the rabbit.

2.4.3 Topographical Location of the Caecum

The caecum occupies the major portion of the middle to lower abdomen lying ventrally, and flexed by three major turns (Polesko 1962). The small intestine is displaced laterally and dorsally by the caecum. The stomach lies cranial to the caecum with the ascending duodenum occupying the upper left quadrant of the abdomen and the jejunum and ileum occupying the lower right quadrant (Fig. 2-8.). The proximal colon is located laterally between the third and second turn of the caecum.

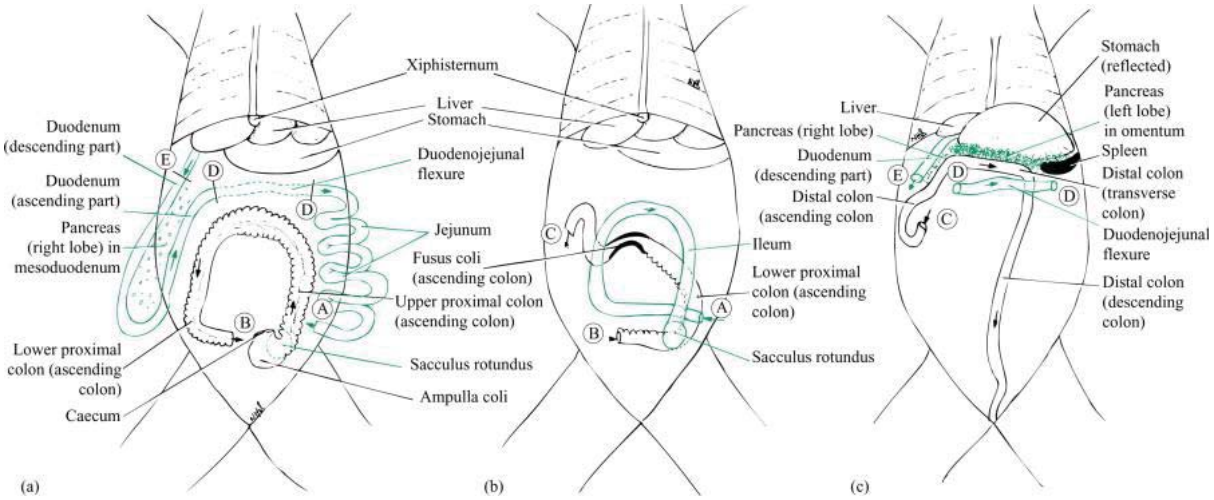

Fig. 2-8. (Harcourt-Brown 2001). Three-dimensional topographical anatomy of the abdominal contents of the rabbit with the caecum removed. Diagram shows the topographical relationship of the liver, spleen, pancreas, small intestine and colon at three levels from the superficial (ventral) to deep (dorsal). Dotted lines show structures that are deeper (more dorsal) than the illustrated layer. The progression through the bowel from stomach to anus is shown by arrows. The small intestine is shown in green, the large intestine in black

2.4.4 Mesenteric Attachments

The small intestine, caecum, and a large portion of the colon are all suspended within the peritoneal cavity by mesenteries which are attached to the dorsal wall under the left portion of the transverse colon (Snipes 1978). The caecum itself is connected to other organs by three major mesenteric plicae. The *plica caecocolic;* which connects with the caecum, proximal colon and ileum, the *plica ileocolica;* which connects with the proximal colon and the distal ileum, and the *plica ileocaecalis;* which connects with the ileum with the distal part of the caecum including the appendix (Barone et al. 1973).

2.4.5 Arterial Blood Supply

The caecum is supplied by the ileocaecocolic artery, which originates caudally from the abdominal aorta and cranial most mesenteric artery respectively. The ileocaecocolic artery branches into at least six sub branches that deliver blood to different sites within the caecum (Fig. 2-9.) The first caecal branch cranially supplies the appendix and a small area of the adjacent ileum. From this branch, the arterial supply to the colon also emerges (*ansa spiralis coli*). At the midpoint of the ileocaecal artery, two braches emerge at approximately right angles from the main arterial stem. One branch supplies the proximal colon and the other the distal third of the corpus ceci and toward the appendix. The main arterial stem of this branch branches further into four sub branches as it approaches the corpus ceci. One branch supplies the ampulla coli, the proximal colon and the sacculus rotundus. The remaining three branches supply the corpus ceci with smaller branches vascularizing the interspiral pouches and caecal walls (Snipes 1978).

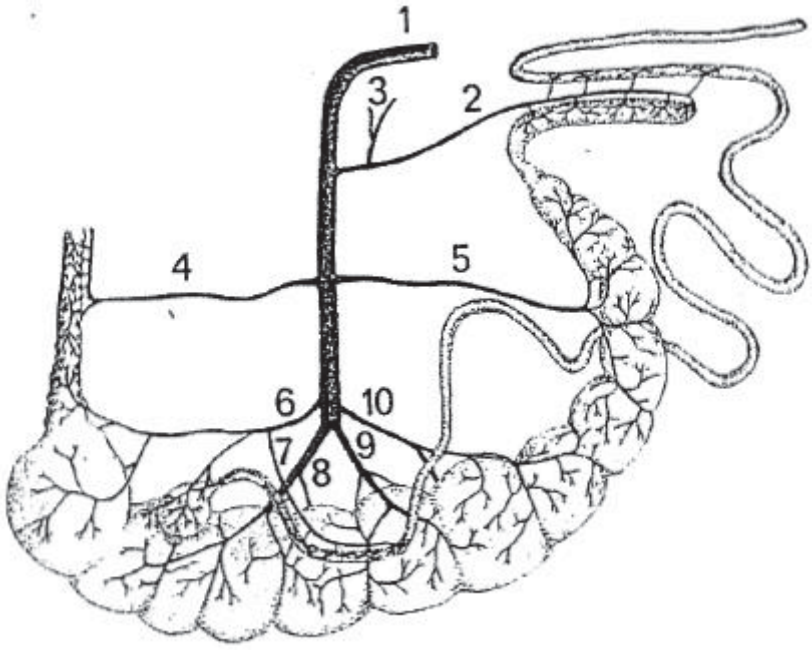

Fig. 2-9. (Snipes 1978). Schematic drawing of the arterial supply to the caecum. 1=ileocaecal artery; 2=branch supplying the appendix and a portion of the ileum; 3=artery to the anas spiralis coli; 4=branch supplying the proximal colon; 5=caecal artery for the distal portion of the corpus ceci; 6=one of the four end branches of the main arterial stem supplying the ampulla coli, the proximal colon and the sacculus rotundus; 7=arterial supply to the distal ileum before it enters the sacculus rotundus; 8,9+10; arteries to the central part of the corpus ceci.

2.4.6 Internal and External Structure of the Caecum

The caecal base is the junction between three physiologically distinct regions; the descending ileum and sacculus rotundus; the corpus ceci and attached appendix; and lastly the ampulla coli and proximal colon. Each region has distinct morphology, structure and function that will be described below.

2.4.6.1 The sacculus rotundus

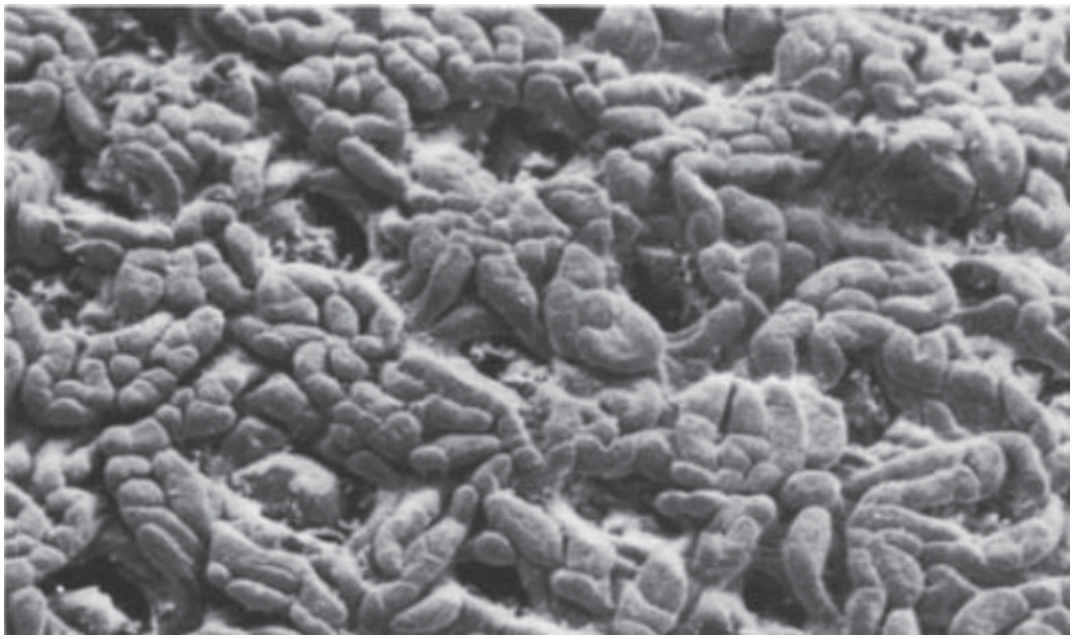

Fig. 2-10. (Snipes 1978). Scanning electron micrograph from the surface of the sacculus rotundus. Depressions are surrounded by small leaf-like structures oriented randomly to each other (x 103.5).

The terminal portion of the ileum of the rabbit is enlarged to form the sacculus rotundus. The sacculus rotundus is similar in structural organization to the appendix, in that it contains a comparable amount of lymphatic tissue (Muthmann 1913). At the junction of the ileum and sacculus rotundus (at the level of the ileal orifice), the ileum protrudes into the lumen of the sacculus rotundus to form an ileal papilla (Fig. 2-14.). It is suggested that the papilla serves to prevent retrograde flow by acting as a valve (Besoluk et al. 2006). The walls of the sacculus are not uniform in thickness: the ventral wall is thin and bears aggregated lymphoid tissue, the dorsal surface very thick with lymphoid structures scattered through it (Alboghobeish and Zabiehy 1996). The lamina muscularis is thickened both at the point of transition between the ileum and sacculus rotundus and between the sacculus rotundus and the base of the caecum suggesting a region of muscular transition similar to that of pylorus

(Besoluk et al. 2006). The increases in muscle thickness at the borders of the sacculus rotundus suggest it has some sphincteric control of flow between the terminal ileum and the base of the caecum.

The mucosa within the sacculus rotundus bears short thick villi and large numbers of crypts along with aggregated lymphoid tissue in the submucosa. There is however a difference in appearance. SEM of the topography (Fig. 2-10.) of the sacculus rotundus shows a series of short ridge-like structures that appear to be randomly orientated to one another and surround a deep oval depression (Snipes 1978).

2.4.6.2 The base of caecum

The caecum base forms a point along the course of the gastrointestinal tract where the small intestine (ileum) ends as it opens into the caecal portion of the large intestine. The interior structure of the caecum base (Fig. 2-10.) contains a complex arrangement of spirals and folds. The point at which the sacculus rotundus enters the caecum is termed the 'ileocaecal orifice'. Directly above the orifice is a rough area of tissue on the inner wall comprised of lymphatic tissue. The ileocaecal orifice is bordered laterally by two folds that lie in a "V" configuration with the orifice of the ileum the middle part of the "V". One fold travels from the ileocaecal orifice toward the corpus ceci approximately one half to two thirds the diameter of the caecum to a point where it thins out and merges with the caecal walls. The other fold extends toward the ampulla coli in a spiral configuration, in effect demarcating the point of division between the ampulla coli and the corpus ceci. Both folds are rigid at the point of attachment to the caecal wall whilst the distal portion projecting into the lumen is thin and flexible. The colon enters the base of the caecum on its ventral side at the ampulla coli.

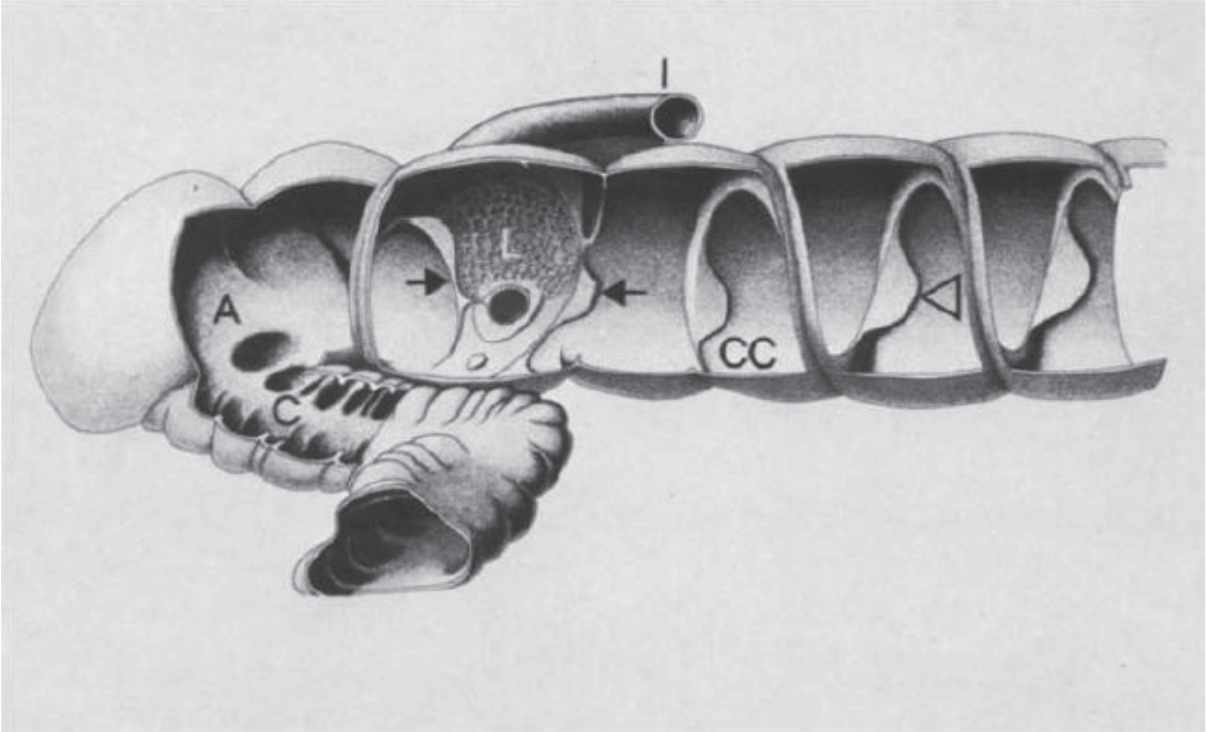

Fig. 2-11. (Snipes 1978). Schematic drawing of the internal, macroscopic structure of the caecum. I = ileum; L = lymphatic plaque; small arrows=fold bordering ileocaecal orifice; A=ampulla coli; C=proximal colon; arrowhead= spiral fold in corpus ceci (CC)

2.4.6.3 The corpus ceci

The corpus ceci appears segmented when viewed externally. This appearance is caused by a spiral fold which starts in the base of the caecum and extends along the length of the corpus ceci to terminate close to the entrance of the vermiform appendix. The fold travels approximately 18 to 22 full turns around the inner circumference of the caecum (Fig. 2-11.). Like the folds of the ileocaecal orifice, the spiral fold is rigid and is set into the caecum wall by muscular tissue with the less rigid flange extending into the lumen. The width of the fold varies. In the distal portion of the cecum, the spiral fold thins out and merges with the caecal wall at the commencement of the appendix lumen (Snipes 1978). Between successive spiral folds, the caecal walls are thin and indented, with an irregularly contoured surface. Lines that demarcate the borders of bundles of smooth muscle are visible through the walls and run from one spiral fold to the other.

The caecal wall of rabbits (Fig. 2-12) is composed of four tunicae- *T. serosa, T. muscularis, T. submucosa* and an inner layer *T. mucosa*. The *T. mucosa* consists of three laminae- an outer muscularis, middle propria and inner epithelial respectively (Abdel-Khaled et al. 2001). The total thickness of the caecal wall is approximately 0.5mm with the thickest layer being the tunica muscularis, followed by the mucosa and submucosa (Snipes 1978). The surface structure of the spiral fold is similar to that of the caecal wall only. The entire intestinal wall including the tunica muscularis extends from the caecal wall into the spiral fold.

The caecal epithelial layer has both absorptive and secretory functions with many multivesicular bodies, autophagic vacuoles and lysosome-like bodies. The luminal surface is lined with characteristically columnar absorptive epithelium and there is a well-developed micro-villous border and glycocalyx (Ross et al. 1988).

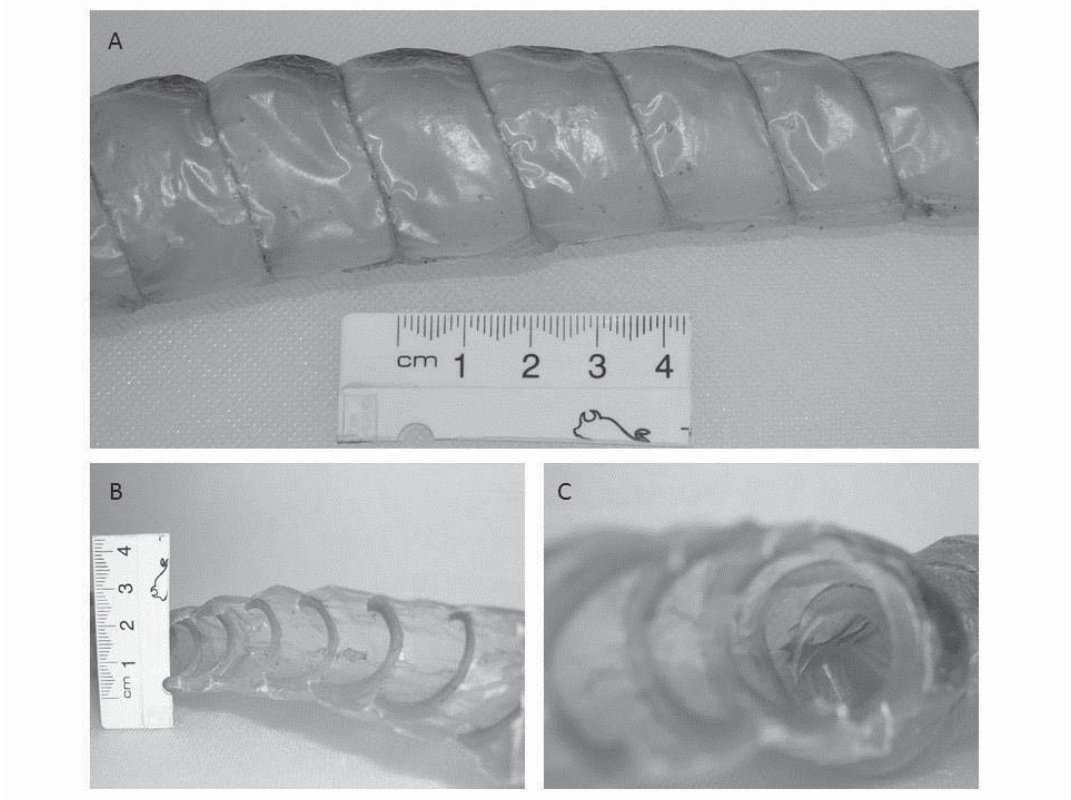

Fig. 2-12. An inflated and dried preparation of the corpus ceci of the rabbit caecum. A) Ventral view of the corpus ceci showing the "segmented" characteristic caused by the spiral fold that runs from the base of the caecum (left) to the beginning of the vermiform appendix (right). B) The ventral surface of the corpus ceci has been removed to show the flange that extends from the wall into the lumen of the ceci. C) Internal view of the corpus ceci showing the helical nature of the spiral fold as it travels toward the vermiform appendix.

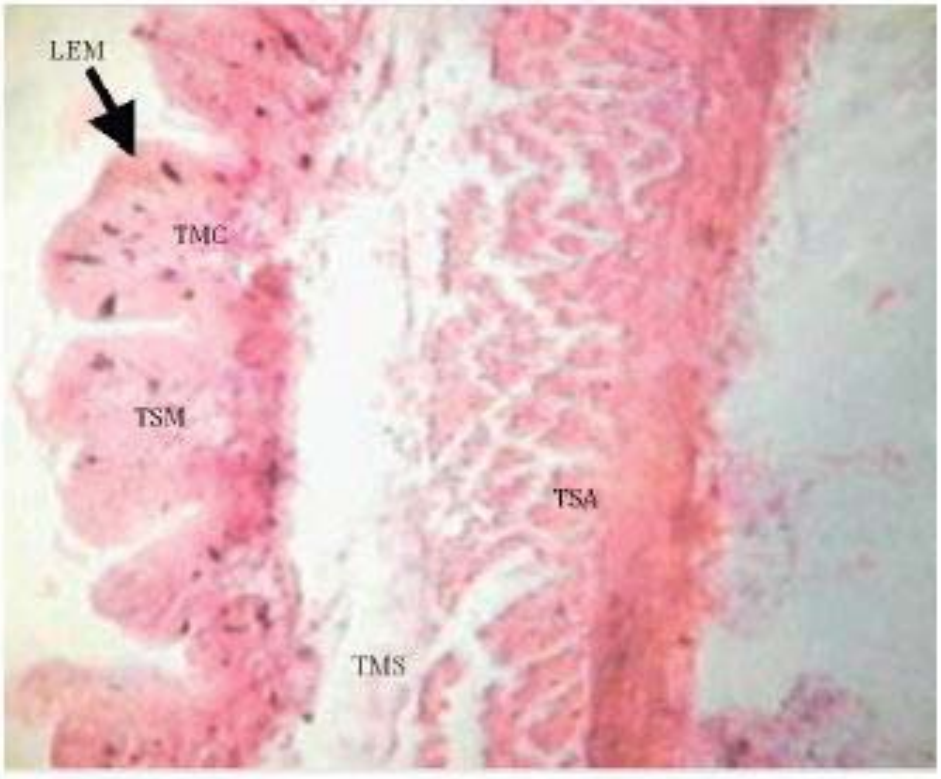

Fig. 2-13. (Abdel-Kaylek et al 2011). Cross-section in caecal wall of rabbit at 6 weeks of age. Slide shows Tunica Serosa (TSA), Muscular (TMS), Submucosa (TSM) and Mucosa (TMC) as well as Lamina Epithelialis Mucosa (LEM). (H and E stains, x200).

2.4.6.4 The appendix

The appendix of the caecum is long and finger like. The walls of the appendix consist of a small outer layer of longitudinal and an inner layer of circular smooth muscle, a thick layer of lymph follicles and lymphoid cells, columnar epithelial cells, goblet cells and reticular connective tissue. The tunica submucosa and muscularis are both relatively thin (Snipes 1978).

Scanning electron microscopy (SEM) of its surface topography reveals slightly elevated, cone-shaped protrusions. These protrusions are covered by columnar epithelium.

2.4.6.5 The ampulla coli

The ampulla coli is situated at the junction of the base of the caecum and the proximal end of the colon. Its location is at the transit point of the 'ileocaecal' and 'caecocolical' orifices. Its bulbous, sac like structure forms the most muscular portion of the caecum, the Tunica muscularis being thicker here than in other portions of the caecum (Snipes 1978). The mucosal surface of the ampulla is thrown into folds that form irregular zig-zag ridges.

2.4.6.6 The proximal colon

The proximal colon lies immediately distal to the ampulla coli. In the area of the taenia the two muscle layers are both very thick. The tunica mucosa as a whole is thicker than that seen in the caecum as is the tunica muscularis (Snipes et al. 1978). The proximal colon has three taeniae with the intervening wall distended to form three rows of sacculations referred to as ''haustrae.'' In the second and third parts of the colon the three taeniae fuse to form a single eccentric taeniae with one row of haustra. In the remainder of the ascending colon the longitudinal muscle is not condensed into taeniae. In the first 3cm of the proximal colon the muscle surface is folded and bears only small protrusions and few crypts. The mucosa of the following 6cm bears prominent wart-like protrusions and many more glands. The cauliflower-shaped protrusions are unique structures that to date have only been described in lagomorphs (Snipes et al. 1982) (Fig. 2-13). These protrusions are termed "Warzen" (Krause 1921) and their irregular surface characteristic results from the presence of widened furrows that lead to crypt openings (Snipes et al. 1982). They may serve to increase the surface area of the colon to increase absorption. They may also assist mechanical processes leading to retention of the large solid components of digesta flowing from large intestine (Björnhag 1972; 1981).

Fig. 2-14. (Snipes et al 1982). Macroscopic view of the first segment of the proximal colon. Area of transition between the portion neighbouring the caecum processing a number of ridges (*arrowheads*) and the subsequent distal portion displaying prominent cauliflower-like protrusions. X 7.5.

2.4.7 Concluding Remarks on the Structure of the Rabbit Caecum

In this section a detailed description of the rabbit caecum has been presented illustrating in detail its complexity. Its position in the rabbit gastrointestinal tract means as well as having a sorting, fermentative, immunological and absorptive importance the region must also coordinate the passage of digesta. The detailed anatomy of the caecum suggests that inward flow of digesta from both the small and large intestine may be forcible and hence accounts for the ability of the caecum to become inflated by sifted digesta. The spiral fold in the corpus ceci may act to increase the area from which nutrients can be absorbed. The flange like structure associated with and around the sacculus rotundus and ampulla caecalis may serve to direct flow of incoming chyme across the base of the caecum. Were this so,

there must be an ability to alter the function when material is entering from ileum or the colon. Hence, it is important to investigate in more detail the physiology and morphology of the ileocaecal valve.

2.5- The Ileocaecal Valve of the Rabbit- its Function, Nervous Innervation and Interaction with the Ileum and Proximal Colon

2.5.1 Introduction

Located between the small and large intestine, the ileocaecal junction (ICJ) and ampulla caecalis (AC) determine how intestinal contents move between the two physiologically and anatomically distinct segments of the gut (Phillips et al. 1988). Chyme must be held in the small intestine until enzymatic digestion is complete; from where it is passed into the caecum and large bowel for fermentative digestion. The ICJ must not only synchronise the passage of digesta between the terminal ileum, caecum and colon, but must also prevent large scale reflux of colonic contents into the small intestine. The region possesses anatomical, structural and physiological characteristics that may help to coordinate this activity. Normal gut motility is maintained firstly, by a mechanical "valve", a physiological sphincter at the ICJ and, secondly, specialized propulsive forces at the distal ileum and proximal colon.

2.5.2 The Anatomy of the Ileocaecal Valve

The ''ileocaecal valve'' (ICV) is thought to prevent reverse flow of digesta from the caecum into the ileum, and to direct the flow of ileal chyme into the base of the caecum (Jenkins 2000) via the muscular action of the sacculus rotundus. The ICV begins at the saccorotundocecal orifice where the walls thicken to form the globular structure of the sacculus rotundus. Within the sacculus the lamina muscularis thickens to form an annular structure that is called the saccorotundal papilla that protrudes into the lumen of the caecal base (Besoluk et al 2006). Around the distal end of the saccorotundocecal orifice are two lateral folds or frenula whose function is unknown but which may constrain the incoming flow of chyme so as to prevent it entering the corpus ceci (Fig. 2-14.). These structural elements form the rabbit ICV.

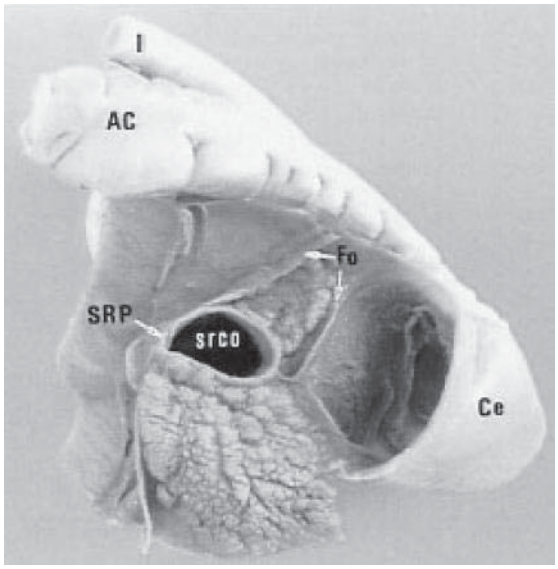

Fig. 2-15. (Besoluk 2006). View of the saccorotundocecal orifice from the caecal cavity in the rabbit. AC=ascending colon; Ce=caecum; Fo=fold; I=ileum; SRCO=saccorotunocaecal orifice; SRP=saccorotundal papilla.

2.5.3 Is the ICV a Sphincter?

Sphincters possess particular functional properties (Papasova 1989)-

1. The intraluminal pressure in a sphincter region is always characteristically higher at rest than in the non-sphincter tract adjacent to it.
2. Suitable stimulation of the area proximal to the sphincter causes the sphincter to relax and intraluminal pressure to decrease.
3. Suitable stimulation distal to the sphincter causes the sphincter to contract and intraluminal pressure to increase.

Hence, gastrointestinal sphincters are generally characterised morphologically by a relative accumulation of the circular muscle cells within a defined region, with the thickness of the longitudinal muscle layer varying to different degrees.

The ICV in the human, cat, dog, monkey, (Gazet and Jarrett 1964) guinea pig (Kubota 1982) and rabbit (Stremmel et al. 1977) possess these characteristics. The sphincters demonstrate a basal tone with a contractile response to distal distension and an inhibitory response to proximal distension (Ouyang 1992).

2.5.4 Functional Characteristics of the ICV

In the rabbit the ileocaecal valve (actually sited between the ileum and the sacculus rotundus) retards reverse flow of fluid into the ileum, and directs chyme via the sacculus rotundus to the caecum (Jenkins 2000). The presence of a sphincter, however, does not negate a role for a mechanical barrier that retards the subsequent flow of digesta in certain directions. Frenula, narrow membranous ridges are thought to contribute to this barrier. The saccorotundocecal orifice in the rabbit is bordered by two such folds (Besoluk et al. 2006) and similarly situated distinct labia incorporating muscular elements have been described in the dog (Quigley et al. 1985), pig, cat, and monkey (Wesson 1937). Evidence suggests an increase in intracaecal pressure causes the frenula to be stretched, causing the sphincter to be restricted to slit, the orifice of which is progressively reduced by increasing intracaecal pressures (Phillips et al. 1998). In humans, enlargement of the venous bed in the ileocaecal papillae may also contribute to this function. Here the increased density and size of submucosal veins creates an enlarged venous bed which may act as a 'compressible venous cushion' that facilitates narrowing or closing of the ileal outlet (Ferraz de Carvalho et al. 1972). As previously described, the rabbit ileal papilla protrudes into the sacculus rotundus an action that is thought to prevent retrograde flow of its content into the ileum (Besoluk et al. 2006). If this is so then the role of the frenulae may be redundant, unless their role is to direct flow rather than to occlude.

2.5.5 Nervous Innervation of the Gastrointestinal Tract and ICV

2.5.5.1 General

The nervous system is the part of an animal's body that coordinates its voluntary and involuntary actions and transmits signals between different parts of its body. In most animal species it consists of two main parts, the central nervous system (CNS) and the peripheral nervous system (PNS). The CNS contains the brain and spinal cord. The PNS consists mainly of nerves, which are enclosed bundles of nerve fibres or axons that connect the CNS to every other part of the body. The PNS includes motor neurons, mediating voluntary movement; the autonomic nervous system, comprising the sympathetic nervous system and

the parasympathetic nervous system, which regulate involuntary functions, and the enteric nervous system, which functions to control the gastrointestinal system.

In mammals as a broad class, sympathetic innervation of the gastrointestinal tract is provided by neurons located in the paravertebral chains of sympathetic trunk ganglia and in larger prevertebral ganglia, the celiac and cranial mesenteric, and caudal mesenteric ganglia. The sympathetic trunk ganglia have neurons involved in controlling blood flow, whereas prevertebral ganglia control motility, secretion, and blood flow as well (Furness 2006a). Fibres from the sympathetic nervous system (SNS) innervate tissues in almost every organ system, providing at least some regulatory function. The SNS (Adrenergic; uses Nor-epinephrine (NE) as a neurotransmitter, or epinephrine (aka adrenaline, hence the name adrenergic)) and the Parasympathetic Nervous System (Cholingeric; uses Acetylcholine (ACh) as a neurotransmitter) directly oppose one another and are collectively called the Autonomic Nervous System (ANS). Efferent nerves from the sympathetic and parasympathetic nervous system synapse with elements of the enteric nervous system and mediate their effects on digestive function through the enteric nervous system (Furness and Costa 1980). Elements of the ENS also connect with afferent neurons.

The enteric nervous system (ENS) consists of a mesh-like system of neurons that ultimately governs the function of the gastrointestinal system (Furness 2008). Hence elements of the enteric nervous system are embedded in the walls of all components of the gastrointestinal system (Fig. 2-15.), from the oesophagus to the anus (Hall 2011). In vertebrates the enteric nervous system includes efferent neurons, afferent neurons, and interneurons, all of which make the enteric nervous system capable of carrying reflexes and acting as an integrating centre in the absence of CNS input. The network of cell bodies and fine processes in the region between the longitudinal and circular muscle layers are termed plexuses. Cells within the plexus are termed interstitial cells of Cajal (ICC) and there are different types with different functions. Myenteric Interstitial cells of Cajal [ICC-MY] serve as pacemakers which create the bioelectrical slow wave potential that leads to contraction of the smooth muscle. Intramuscular Interstitial cells of Cajal [ICC-IM] are involved in the stimulation of smooth muscle cells. Through intestinal muscles, the motor neurons control peristalsis and churning of intestinal contents

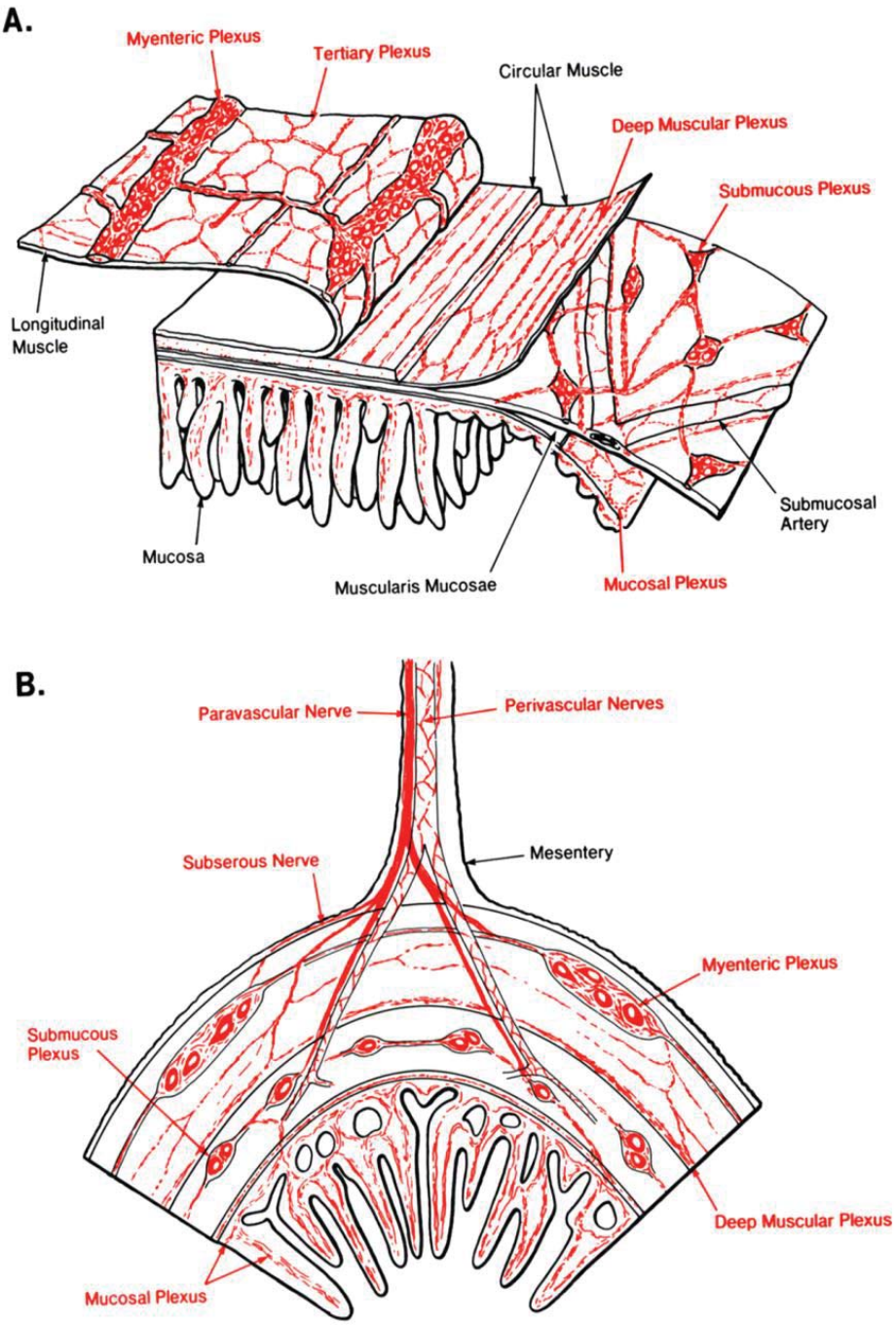

Fig. 2-16. (Furness and Costa 1980) Diagrams showing the arrangement of the enteric plexuses. In (A), a segment of intestine which has been partly separated into layers is shown and in (B) the arrangement of the nerves in a section through the intestinal wall is illustrated. Nerves are shown in red

2.5.6 The Interaction of the ICJ with Ileal and Colonic Contractile Activity

Studies in man have shown that tone in the ICV may not be the only means by which the passage of digesta into the caecum is controlled. Hence, specialized contractile activity in the distal ileum and proximal colon may also contribute to this (Quigley 1988).

Ileal smooth muscle innervation and physiology have unique motor properties. In comparison to the proximal small intestine, the ileum contracts rather than relaxes when alpha-blockers are administered to tissue (Munro 1953). Functionally, the distal ileum shows unique propulsive properties with isolated distal ileal segments being capable of moving fluid in an aborad direction (Weems and Seygal 1981). These propulsive properties of the ileum are dependent on its neural connections, rather than being caused by the presence of specially adapted longitudinal or circular muscle (Phillips 1988).

Recordings of myoelectric activity in the ICJ in conscious animals also suggest functional specialisation within the ileum. The progressive reduction in slow wave frequency and velocity of aboral migrating motor complexes might be expected to slow the movement of chyme, increase contact time with intestinal mucosa, and increase absorption (Quigley et al. 1983).

Evidence for coordinated functioning of the distal ileum, ICJ and proximal colon has been defined in the canine ICJ (Quigley et al. 1984). While sustained tone is confined to the ICJ, unique phasic patterns are observed over a longer, functionally distinct area, which not only included the ICJ but also the first 30cm of the distal ileum and the first 5cm of the proximal colon. Propagating contractions are observed to move rapidly through the ileum and can progress into the proximal colon.

As described above, ileal motor patterns sometimes cross the ICJ and into the proximal colon. The colon is capable of generating high pressure waves which propel chyme distally (Spiller et al. 1986). It also has the ability to relax, a phenomenon which could influence the capacity of the caecum and colon to accommodate flow from the ileum (Phillips 1988).

The ICJ has properties that are determined not only by its anatomy but also of the motility of the adjacent distal ileum and proximal colon. Transit across the ICJ depends on not only

the coordinated motor patterns between these regions but also the localised properties of tone within the junction itself.

2.5.7 Concluding Remarks on the Rabbit Ileocaecal valve

In this section the function of the ileocaecal valve and its relative contributions to the transit of digesta from the ileum to the colon was discussed. It is evident that the function of the ICV may be associated with other actions in associated structures at least in species other than the rabbit.

The work on this action in man highlights the problem as to whether the propagation of contractions occurs directly from ileum to colon (which is physiologically unlikely) or whether the arrival of ileal digesta distends the ampulla caecalis which then initiates colonic contraction.

2.6- Comparative Anatomy of the Mammalian Caecum- Strategies of Fermentative Digestion in Mammals

2.6.1 Introduction

Given the action of associated structures other than the ICV in species such as man, it is useful to consider the comparative anatomy of other mammalian species as it may shed additional light on the mechanisms that allows transit of digesta across the caecal base.

The word "caecum" means "blind" in Latin, reflecting the fact that the caecum is a blind pouch (a dead-end or cul-de-sac). In most vertebrates- particularly herbivores, the caecum is a large and complex being specialized for digestion of oligosaccharides (Kardong 2002). The size and complexity of the caecum varies between species, but in general the size of the caecum is proportional to the amount of plant matter in a given animals diet. Hence, it is largest in obligate herbivores, animals whose diets consist entirely of plant matter (Theobald 2007).

The structure of the caecum is specialized to increase the efficiency of cellulose digestion by fermentation. It houses a large, dense, colony of specialized bacteria. Being a dead-end sac at the beginning of the large intestine, it allows food to remain in the gut for longer and so that it is fermented more completely. In comparison, the digestive systems of carnivores are more dependent on the small intestine, which can be related to the general digestibility of their food, so the caecum is reduced or absent. Omnivores have more complex gastrointestinal tracts, with a hindgut caecum in which some microbial fermentation takes place. Thus variation in digestive strategy results in a wide variety of caecum forms and functions adapted to the wide range of nutritional niches found among mammals. Again, different diets may result in differences in the physiological characteristics of digesta. Hence there may be an accompanying difference in the structures associated with ileo-colonic transit.

2.6.2 The Herbivore Digestive System and Caecum

Mammalian herbivores have been grouped on the basis of the main site in the gut at which microbial fermentation of vegetable matter takes place. The two primary groups of herbivore are foregut fermenters and hindgut fermenters.

Hindgut fermentation is a digestive process seen in monogastric herbivores, animals with a simple, single-chambered stomach. The hindgut fermenter diet includes large quantities of insoluble plant carbohydrates, such as cellulose. Cellulose is digested with the aid of symbiotic bacteria. The microbial fermentation occurs in the segments of the gut distal to the small intestine i.e. the large intestine and caecum. The advantage to a hindgut fermenter is that soluble carbohydrates, such as glycogen, are available to the animal before they are available to the microbes. In contrast, foregut fermentation is the form of cellulose digestion seen in ruminants such as cattle which have a four-chambered stomach which digests cellulose. Microbes in the foregut can convert non-proteinaceous sources of nitrogen, like ammonia and urea to all of the amino acids. Microbial protein is available to the ruminant when the microbes die and pass down into the abomasum and small intestine. Hence ruminants can survive on a poor quality source of nitrogen. Microbial protein is not available to hindgut fermenters because when the microbes in the large intestine die, they are excreted with the faeces and there is no further opportunity for their digestion. Microbes in the foregut synthesise vitamins, which are also available to the animal further on in the digestive tract. Again, they are not available to the hindgut fermenter.

2.6.2.1 Foregut fermenters

In foregut fermenters the primary site for microbial fermentation is in the expanded forestomach (Fig. 2-17.). Many foregut fermenters have a secondary site of microbial fermentation in the hindgut. However, the hindgut makes a relatively minor contribution to the gross energy economy of the animal (Hume et al. 1980).

Foregut fermenters can be subdivided on the basis of the gross morphology of the forestomach. In large herbivores like ruminants, camelids, peccaries and hippos the forestomach consists of one or more sac-like diverticula which maximises the retention of digesta for fermentation and results in high digestibility of plant material (Freudenberger et

al. 1989). These large herbivores eat and process bulk plant material that is high in cell wall content.

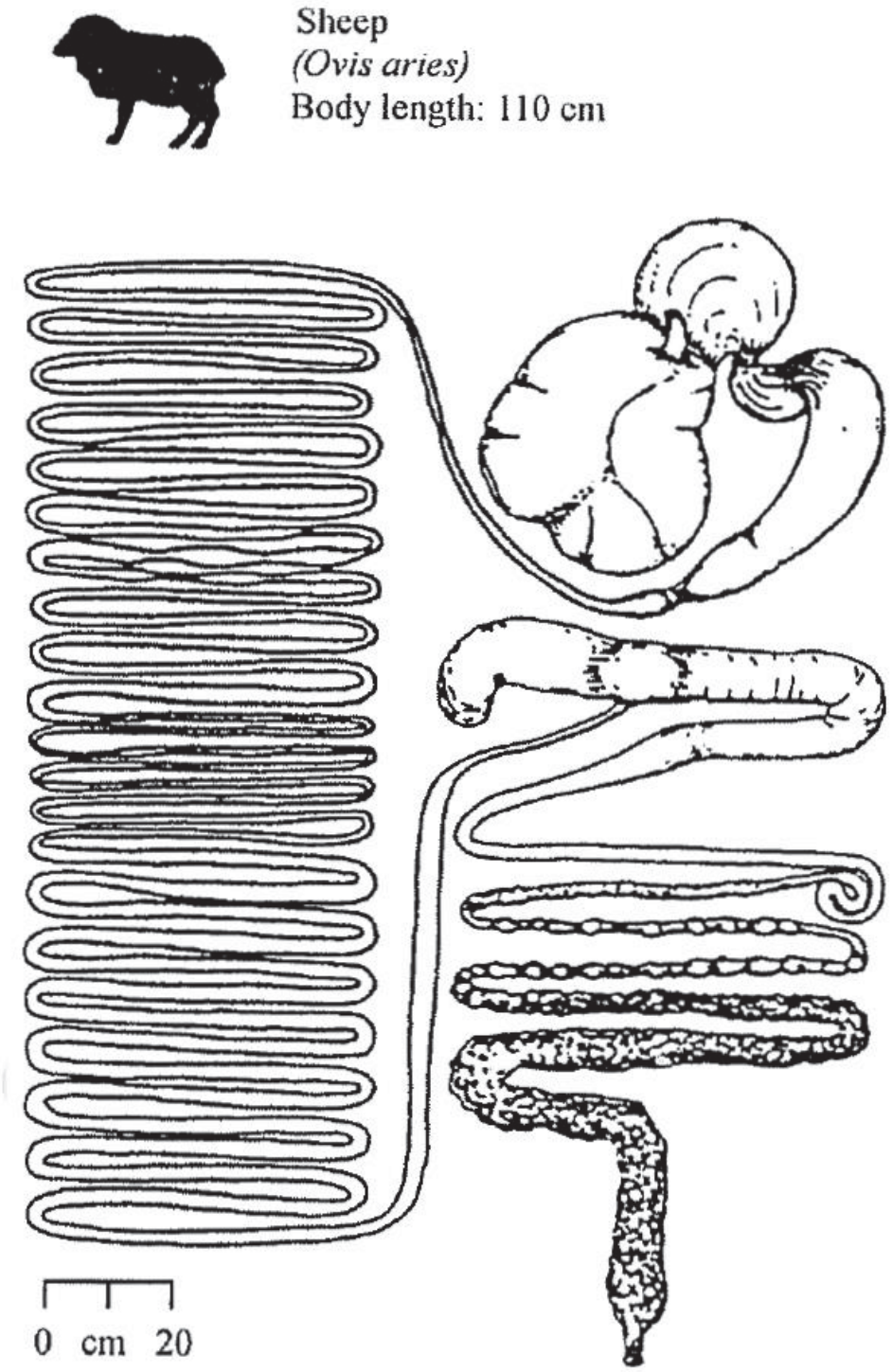

Fig. 2-17. (Stevens et al 1995) Digestive tract of the sheep- a foregut fermenter.

A large fermentation chamber is consistent with the need for prolonged retention of slowly fermenting plant material that consists of mainly plant walls.

Large foregut fermenters use one of two alternative strategies for utilizing plant material with high cell wall content.

The first strategy is that of the ruminant. Ruminants are mammals that are able to acquire nutrients from plant-based food by fermenting it in a specialized stomach prior to digestion, principally through bacterial actions. In the rumen, vegetative particles are held for a prolonged period of time until they are broken down to a certain size by rumination. The rumen, the first of the forestomach chambers, stores and processes plant material. It is also the site of bacterial fermentation. The rumen holds plant material until it is broken down sufficiently to be passed onto the other chambers of the stomach. Volatile fatty acids are released, and the fermentation of protein and carbohydrates is begun here. Once food has been broken down enough, it passes from the reticulorumen through the reticulo-omasal orifice to the omasum. The omasum wall is highly folded, giving a large surface area which allows for the efficient absorption of water and salts released from the partially digested food. This strategy of maximising extent rather than rate of cell wall digestion would seem to be suited to ecosystems in which food availability is sometimes limited or of poor quality (Hume 2002). However, few present day ruminants live in the sort of environments for which their special ruminant adaptions evolved.

The second strategy of foregut fermenters is seen in the large kangaroos, which are primarily grazers (Hume 1999). These herbivores the forestomach is mainly tubiform rather than sacciform (Fig. 2-18.) so they are unable to ruminate. The kangaroo stomach lacks the special features of the ruminant forestomach designed to maximise the retention of large particles e.g. rumen and omasum. Rather, the kangaroo strategy appears to be designed for maximising rate of fermentation at the cost of complete plant cell wall digestion, and also rapid throughput of poor quality feed. Digestive strategies that emphasise the passage of food rapidly rather than maximising its extent of digestion are best suited to environments in which forage is often of low quality but only rarely is limited in quantity (Hume 2002).

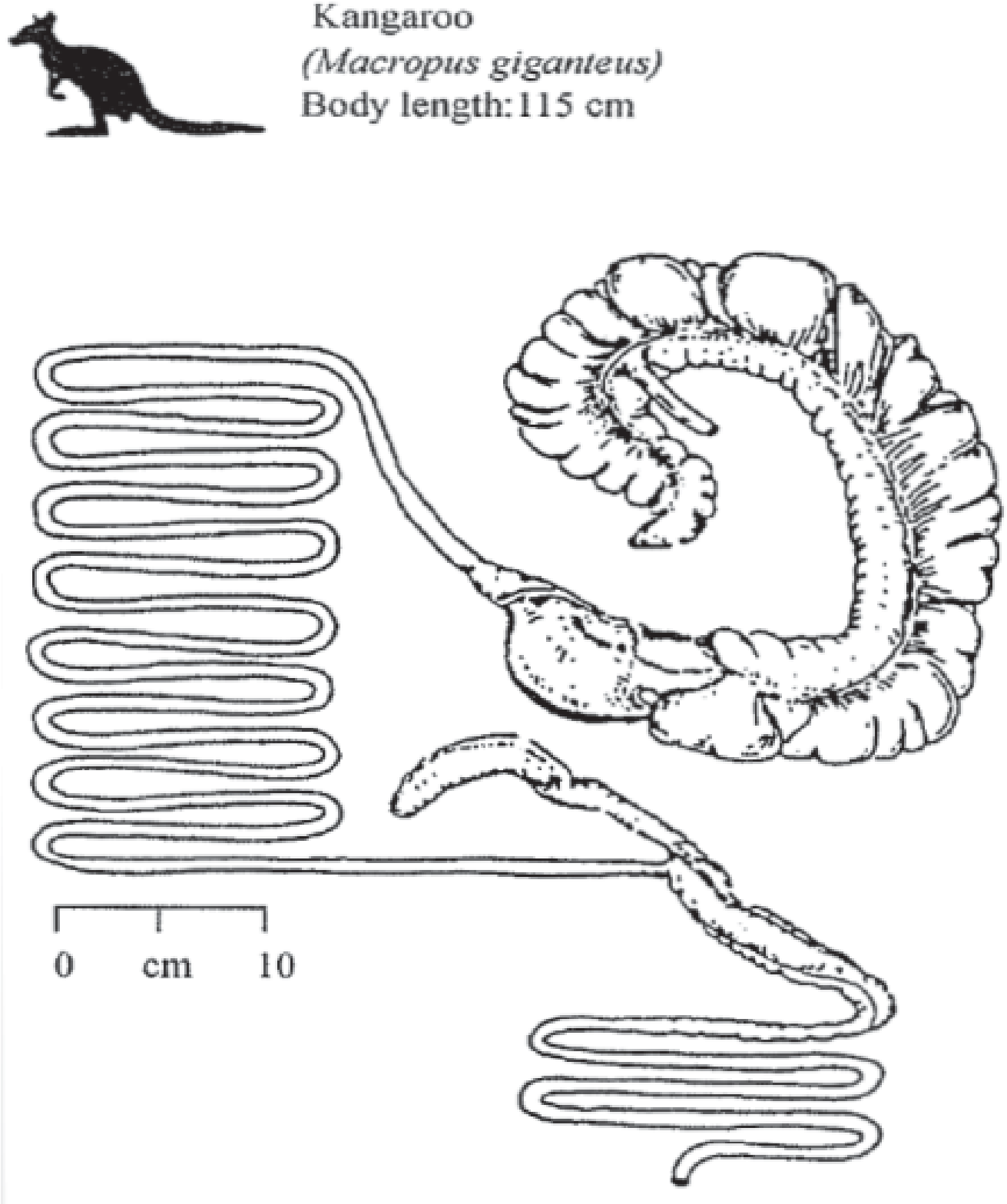

Fig. 2-18. (Stevens et al 1995) Digestive tract of the Kangaroo- a foregut fermenter.

2.6.2.2 Hindgut fermenters

In hindgut fermenters, enzymatic digestion takes place in the stomach and small intestine. Bacterial digestion is largely confined to the large intestine- the caecum or colon, or combinations of the two. Broadly speaking, hindgut fermenters can be classified as either colonic fermenters or caecum fermenters.

2.6.2.2.1 Colon fermenters

In colonic fermenters (>10kg adult body mass) e.g. the horse (Fig. 2-19.), the main site of bacterial digestion is in the proximal colon. A caecum may or may not be present (Hume 1989). When present it functions as a simple extension of the proximal colon and serves to mix digesta between the two regions. The digestive strategy in the colon fermenters follows a similar pattern to that of large kangaroos where a haustrated tubiform organ is the principle fermentation chamber. This tubiform organ is the forestomach in kangaroos, and the colon of the large hindgut fermenters (Hume 1989). The digestive strategy of colon fermenters emphasises the maintenance of food intake at the expense of digestion (Van Soest 1965).

An important morphological characteristic in colon and caecal fermenters is the haustration of the proximal colon and caecum. The action of the muscles within the haustrae may be responsible for the active retention of small nutrient rich particles (Hume 2002). Segmental contractions separate the digesta into faecal pellets and force them slowly aborad, whereas the movements of the haustra carry the liquid and nutrient rich smaller particle contents back orad towards the caecum so that they are retained longer for digestion (Ehrlein and Schwinger 1983). Note that the bulk of fermentation takes place in the colon with little if any caecal development or retention.

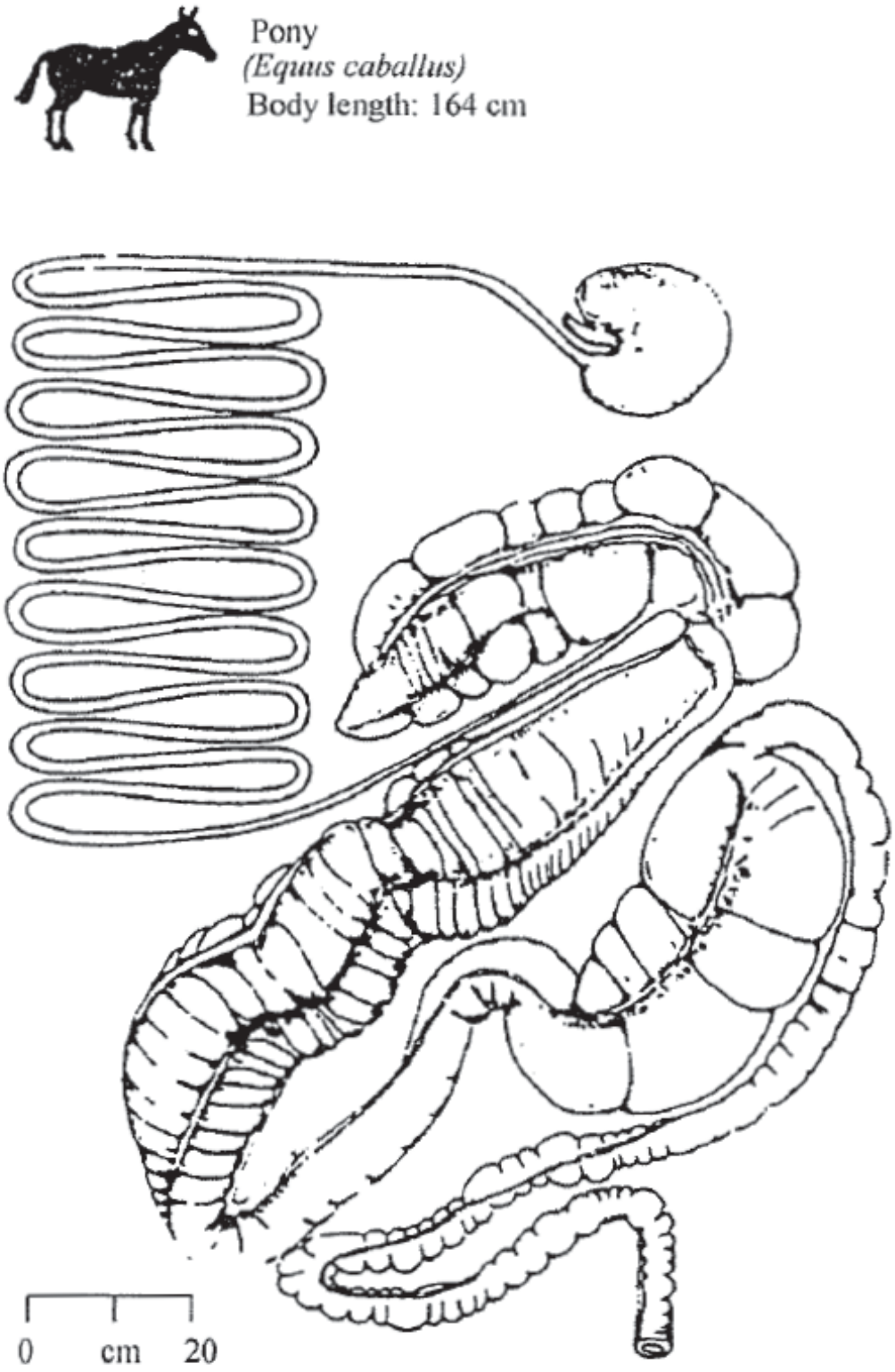

Fig. 2-19. (Stevens and Hume 1995) Digestive tract of the Horse- a colon fermenter.

2.6.2.2.2 Caecal fermenters

Caecum fermenters are generally small bodied, less than 10kg of adult body mass (Hume 2002) (although the capybara at 45 kg is an exception) (Stevens and Hume 1995). Microbial fermentation is mostly confined to the large and complex caecum. In the proximal colon digesta is sorted into fine particles and solutes which are returned to the caecum for fermentation and larger particles are forwarded into the colon. Not all caecum fermenters have a proximal colon sorting mechanism or a well-developed colon. Two types of colonic sorting mechanism (CSM) have been identified, the “wash-back” and the “mucus trap” systems (Hume 2002).

In the wash-back system, there is a net secretion of fluid from the blood into the proximal colon. Muscular activity of the haustrae creates a rolling action that washes out fine particles from larger (Björnhag 1994) which are then carried back into the colon. Fluid, fine food particles and bacteria accumulate in the caecum. The larger particles that are separated travel distally within the lumen of the colon. In the colon of the rabbit, mechanical separation of liquid and solid digesta results in a concentration of coarse particulate matter more than twelve times as high in the distal colon than in the caecum (Björnhag 1972); continuous weak retrograde contractions squeeze out fluid and fine material from the colonic content as it is transported from haustrum to haustrum, from the fusus coli towards the initial part of the proximal colon and then to the caecum by antiperistalsis (Ruckebusch and Hörnicke 1977).

Many wash-back fermenters are coprophagic e.g. the rabbit (Fig. 2-20.). The CSM is variable in duration and often times ceases. In caecotrophic species like the rabbit, while the CSM is operating, usually during the active phase of the animal, the larger particles passing into the distal colon form the hard faecal pellets. During the rest phase, the CSM is switched off, the caecum partially empties and caecotrophes or soft faecal pellets are produced during one or a few periods per day and are eaten directly from the anus (Hume 2002).

In caecum fermenters with a “mucus-trap” system the proximal colon is divided into a main channel and a narrow channel by mucosal folds (Sperber et al. 1983). Mucus secreted by the proximal colon traps bacteria and other fine particles which are transported into the caecum via the narrow channel by antiperistaltic contractions. The bacteria and mucus mix

with the caecal contents. Nearly all myomorph rodents (rats, mice, dormice, jerboas) have mucus trap systems.

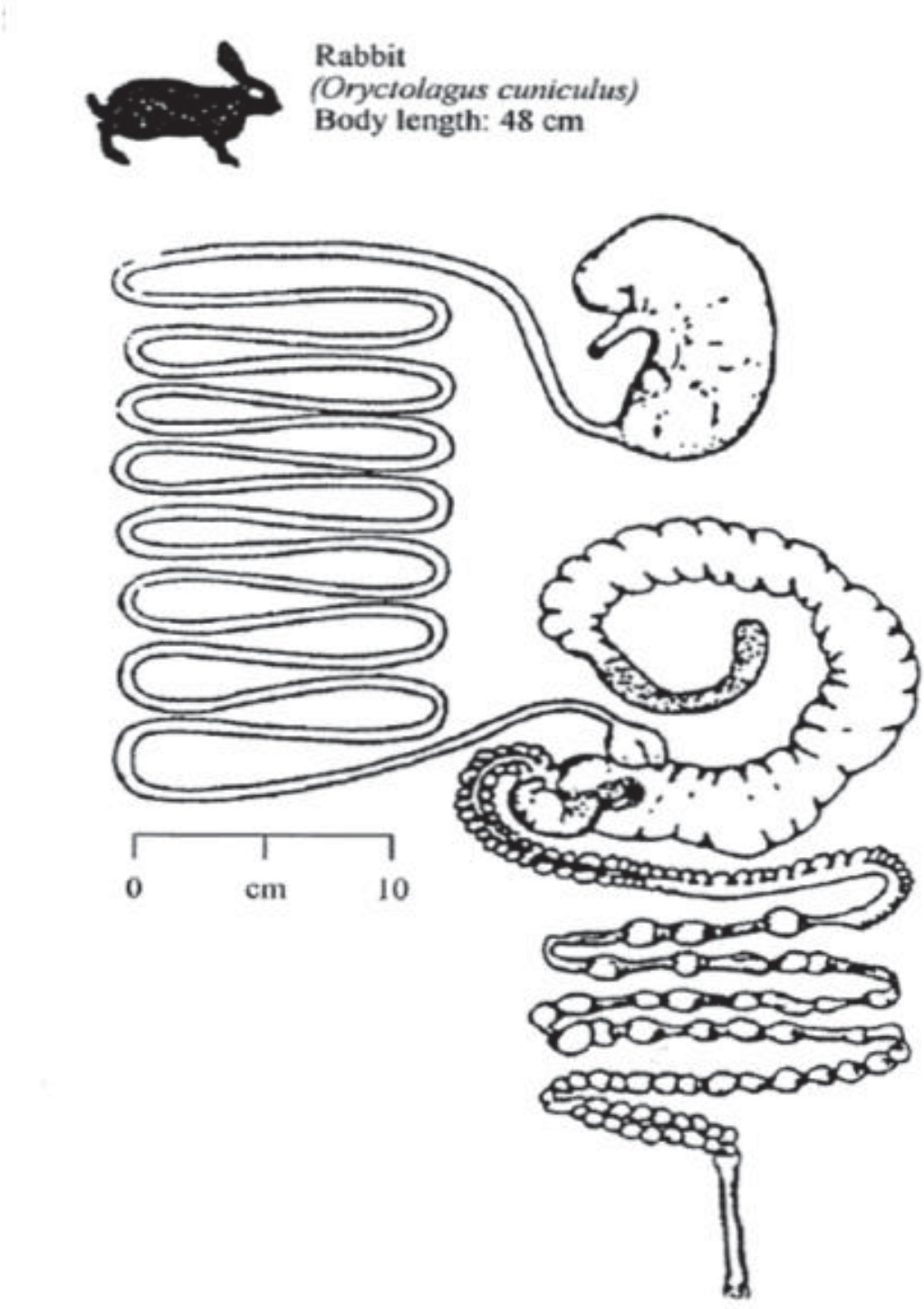

Fig. 2-20. (Stevens and Hume 1995) The digestive tract of the Rabbit- a caecum fermenter.

The common features of both CSM systems is an enlarged caecum with commensal bacterial colonies, and also a highly mobile colon which can be cleared relatively quickly. The caecum is also a highly efficient fermenter which allows for high forage diets.

2.6.3 The Carnivore Digestive System and Caecum

The carnivore stomach is simple, without diverticula, but can often be expanded to accommodate large items of prey (Hume 2002). The small intestine is short, but nevertheless is the dominant feature of the carnivore gut in most species (Stevens and Hume 1995). The large intestine or hind gut is also short, with a small caecum and short, non-sacculated but often wide colon. In all carnivores the main substrates for the gut microbes that comprise the normal gut flora are endogenous secretions (mucus, sloughed mucosal cells, spent digestive enzymes) (Hume 2002). The relative simple morphology of the digestive tract of carnivores correlates with the generally high digestibility of their food (Hume 2002). Because meat is easily digested, the gastric system of carnivores is typically short and simple. Food digestion begins in the monogastric stomach from where it moves into the small intestine. The small intestine is a long and narrow 'tube' with a structure and epithelium that maximises surface area for absorption. This is important because the small intestine is the primary site of digestion by enzymes. In comparison, the colon is short with its primary role the reabsorption of water, vitamins and electrolytes.

2.6.3.1 The Dog Caecum

The caecum of the dog is extremely variable in size and form. It is about 5cm long and 2cm in diameter at its colic end. It irregularly tapers to a blunt apex, which is less than 1cm in diameter with a total length of between 8- 30cm (Abd-El-Hady et al. 2013).

The apex, body and base, are located to the right of the median plane and within the duodenal loop of the abdomen. The caecum (Fig. 2-21) is a short, and forms an irregularly twisted tube which is attached to the ileum and ascending colon by short peritoneal folds (Nickel et al. 1979).

The terminal part of the small intestine joins only with the colon, and the caecum exists as a diverticulum of the proximal portion of the colon (Miller et al. 1993; Kumar 2004; Budras et al. 2007). The caecum joins the ascending colon via the caecocolic orifice, this opening lies

approximately 1cm from the ileocolic orifice. The caecocolic sphincter is a specialisation of the inner circular smooth muscle coat which guards the caecocolic orifice. Like other species, the dog has an ileocaecal valve which has two distinct functions, namely the alternate retarding and forward propulsion of chyme (Quigley et al. 1983). The caecum is attached to the terminal portion of the ileum by fascia and peritoneum throughout most of its length (Miller et al. 1993).

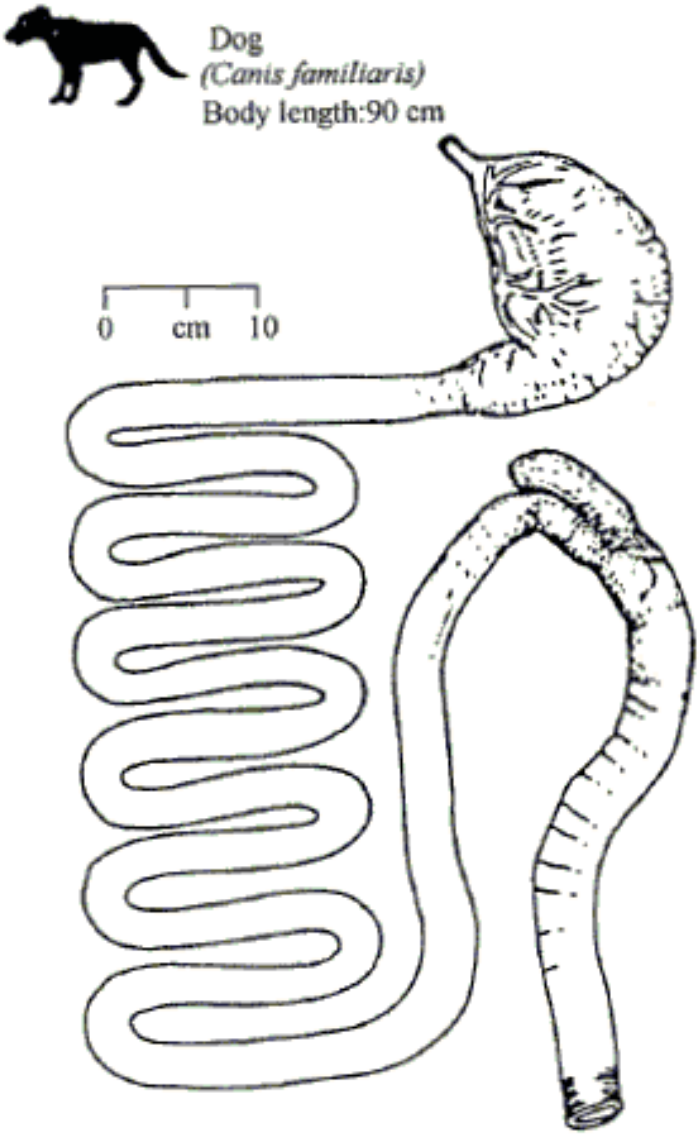

Fig. 2-21. (Stevens and Hume 1995) The gastrointestinal tract of the dog.

2.6.3.2 The Cat Caecum

The caecum of the cat is small and relatively undifferentiated in comparison to that of herbivores. It is bulbous in form showing a subtle concave ventral surface and a convex dorsal surface (Fig. 2-22.). Distal to the entry of the ileum is a slight constriction. Teniae are not present. A vermiform appendix is missing (Snipes 1984).

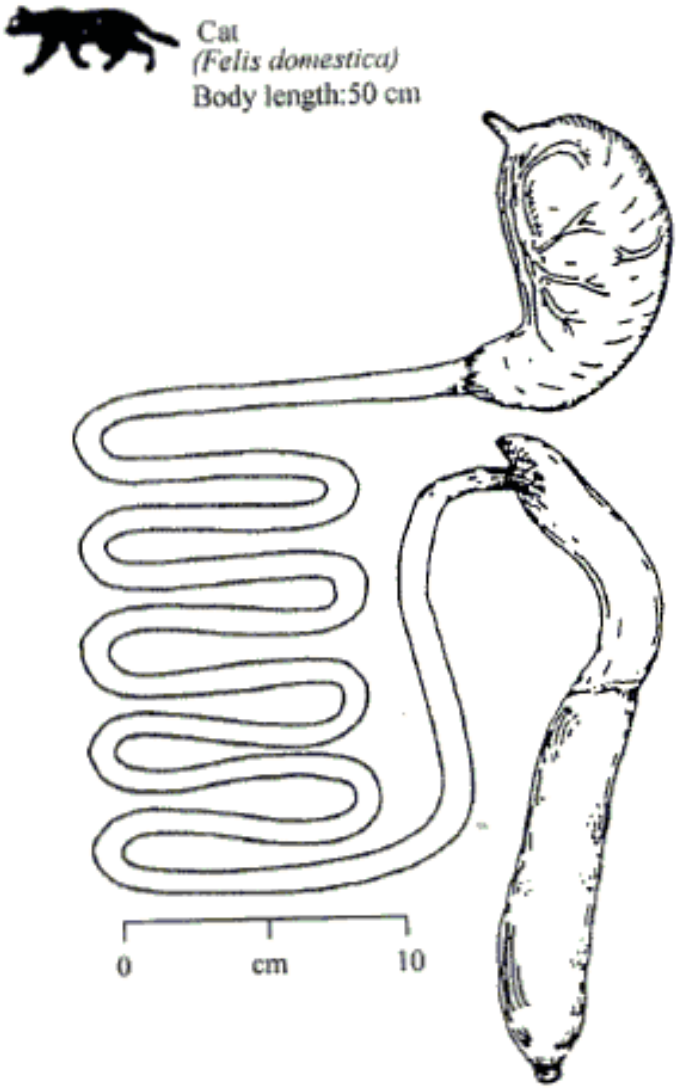

Fig. 2-22. (Stevens and Hume 1995) the gastrointestinal tract of the cat.

The ileum enters between the colon and caecum creating a junction and marks the limitation between these two structures (Kostanecki 1926) and also the location of the ileocaecal valve (Wesson 1937).

Like other animals the cat's caecum provides space for relative retention of partially digested foodstuffs and an increase of mucosal surface for absorption (Snipes 1984). This is associated with the development of localized areas of lymphoid tissue. However, caecal secretory function may be the more important in the cat (Snipes 1984). The secretions are rich in mucus, the product of goblet cells lining the epithelial mucosa (Snipes 1984). The mucus covers the gastrointestinal mucosal surface functioning as a source of nutrients for endogenous micro flora (Sakata and von Engelhardt 1981), antibacterial and antiviral agent, a binding source for ions, and a barrier to prevent contact of mucosa with pathogens present in the lumen (Allen 1983).

2.6.4 The Omnivore Caecum

It is useful to consider the caecum of the omnivore as it could be seen to represent a baseline in terms of caecal specialization.

The caecum and large intestine of the rat and of man lack specialization (Snipes 1981) (Fig. 2-23.). The rat is a versatile omnivore that is capable of filling the most diverse dietary niches (Perrin and Curtis 1980) and thus could be considered a true generalist and thus to represent the baseline condition. The length of the rat's alimentary canal is similar to that of an herbivore as compared to a carnivore. However, omnivores lack the fermenting specialisation found in herbivores. This middling position is reflected in the caecal form of the species. The caecum of the rat is small and relatively undifferentiated when compared to the complex caecal form of some herbivores e.g. the rabbit, but has a relatively larger caecum than that of carnivores.

Though small, the rat caecum and colon enable significant quantities of plant fibre and cellulose to undergo fermentative digestion (Rérat 1978) when their diet is primarily of this feed type. They can also process simple carbohydrates, when in nature these food types are available during certain limited time periods (Snipes 1981).

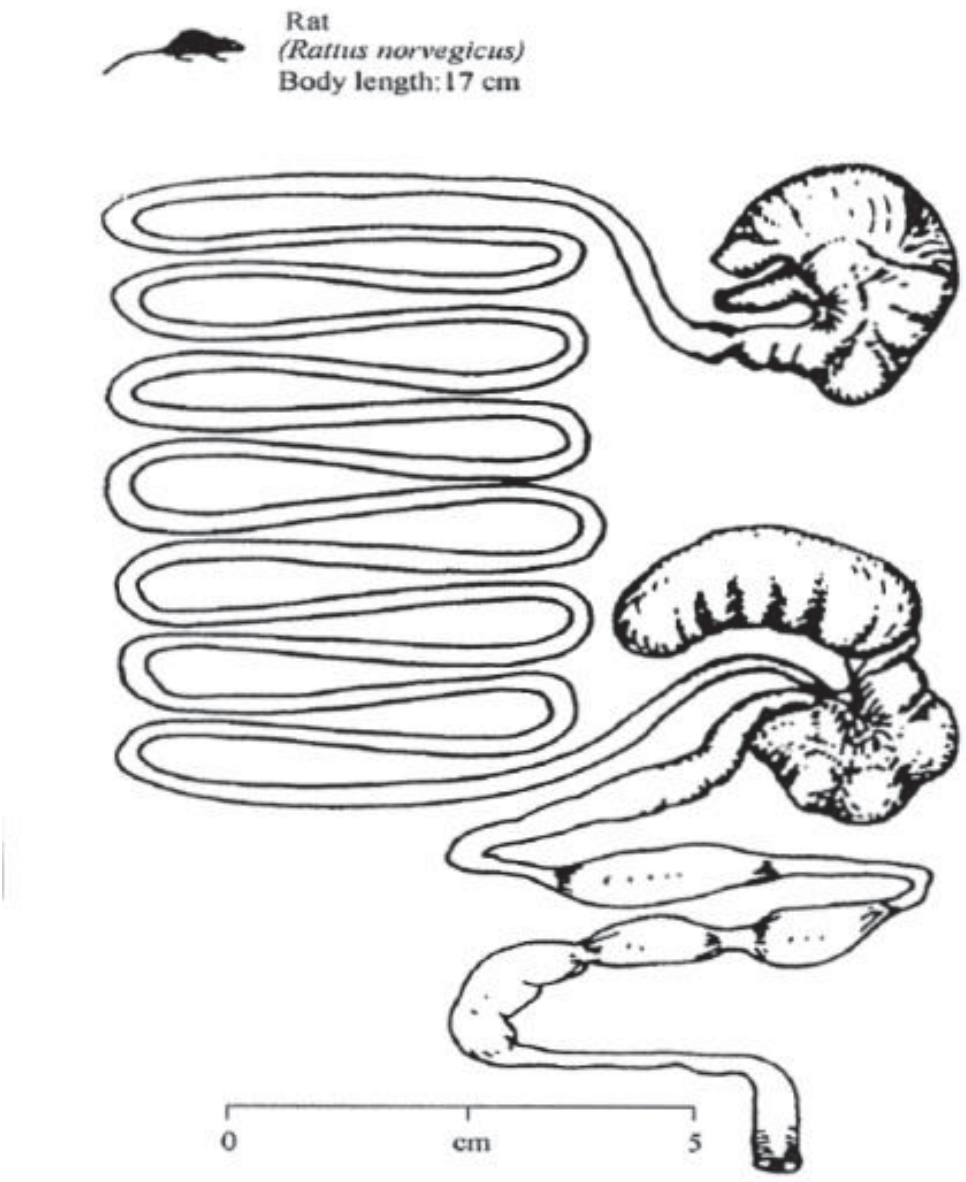

Fig. 2-23. (Stevens and Hume 1995) Digestive tract of the rat

The rat appears to be able to retain unabsorbed residues in the caecum and colon, allowing for microbial growth and fermentation. Hence, around 10-20% of carbohydrate and protein that escapes digestion in the small intestine can be further broken down and utilized (Rérat 1978). Rats are also coprophagic. It has been suggested that this strategy overcomes the disadvantage of having a smaller caecum where fermentation is somewhat limited in comparison to species with a large caecum (Snipes 1981).

2.6.5 The Human Caecum

In the human, the caecum is the first part of the large intestine and begins caudally from the ileocaecal valve. A sphincter at the ileocaecal junction separates the caecum from the ascending colon. The sphincter comprises a semilunar or circular fold approximately 5-8cm from the caecal pole (Pellegrini et al. 1995). The sphincter is thought to be poorly developed anatomically and functionally (Phillips 1988).

A similarly small caecum is also found to varying degrees in several species of New World and Old World monkeys with appropriate dietary niches (Fisher 2000; Hill 1974; Scott 1980). Hence, in species of primates, including humans, the size of the caecum is broadly proportional to the volume of cellulose-bearing plant material that is consumed in their diets. The plant matter that humans and other apes eat tends to be high-energy, starchy tubers, legumes, nuts, grains, and fleshy fruit and is minimal in cellulose fibrous parts so there is no longer a need for a large caecum.

The human appendix is approximately 10cm by 7-8mm with an internal diameter of 1-3mm (Smith et al. 2009). The appendix has been considered an evolutionary vestige. Charles Darwin (1871) suggested that because it had no obvious function that it must be an evolutionary remnant from a primate ancestor that ate leaves. However, it has recently been shown that the human appendix contributes to the GALT immune-mediated maintenance of commensal bacterial flora of the gut (Bollinger et al. 2003).

2.6.6 Concluding Remarks on the Comparative Anatomy of the Caecum

This review of the comparative anatomy of the caecum seen in mammals indicates that the rabbit is a caecal fermenter with a wash back particle sorting mechanism that practices coprophagy. The size of the caecum is large and even in omnivorous species the caecal base is preserved as are the internal flanges which are most likely to function in directing digesta from the small to the large intestine. Carnivorous and omnivorous species have a simplistic or vestigial caecum. The herbivore caecum (in particular the hindgut fermenters i.e. rabbit), in comparison, is much specialised. Similarly, specialisation of structure and function would

require similar specialisation of regulation, control and propulsive motility types. This will be discussed in the succeeding sections.

2.7- Tonic and Phasic Gastrointestinal Contractile Activity in the Gut

2.7.1 Introduction

Gastrointestinal motility is an integrated process including myoelectrical activity, contractile activity, tone (compliance) and transit (Hansen 2003). The instigation of phasic i.e. short lived propulsive contractions of gastrointestinal smooth muscle depends on three principle factors: the intrinsic properties of the musculature, which undergoes rhythmic changes in excitability that differ along the length of the intestine; the influence of circulating hormones; and the influence of nerves (Costa and Furness 1982). These complex functions of gastrointestinal motility are required to transport chyme through the digestive tract and to ensure their adequate mixing and exposure to absorptive surfaces.

2.7.2 The Physiology of Motility in the Gut

2.7.2.1 Structure

The smooth muscle coat of the intestine is organised in two layers; an outer layer- composed of thin longitudinally orientated smooth muscle; and an inner layer of circular orientated smooth muscle which is thicker and densely innervated (Hansen 2003). A further thin layer of smooth muscle, the muscularis mucosa, separates the submucosa from the mucosa. The thickness of the inner radial and outer longitudinal layers varies both between species and regions (Olsson and Holmgren 2001). The circular muscle layer is thicker in the ileocaecal region where it forms the ileocaecal sphincter (Costa and Furness 1982). In each layer the component myocytes are separated by laminar septae into bundles which act as contractile units. The muscle cells are embedded in connective tissue consisting mainly of elastic and collagen fibrils. These layers include glial cells, fibroblasts and interstitial cells of Cajal (Hansen 2003).

Two ganglionated ICC plexuses are found in the wall of the intestine. The first, the myenteric plexus, is located between the longitudinal and circular muscle layers. The second, the submucous plexus, is located in the submucosa. Nerves arising from these plexuses form non-ganglionated nerve plexuses in the longitudinal muscle and throughout the circular muscle.

2.7.2.2 Smooth muscle cells and contractile filaments

Intestinal myocytes are uninucleate and spindle shaped. The surface of the myocyte is smooth and regular except for cell processes when at rest. However, when the muscle is fully stretched the cell surface develops shallow longitudinal grooves (Gabella 1989). The cell membrane is studded with patches of electron dense material that are called *dense bands*. Three types of filaments extend from the dense bands on the body of the cell. These are (Fig 2-24):

1. Thin actin filaments
2. Thick myosin filaments
3. Intermediate desmin filaments

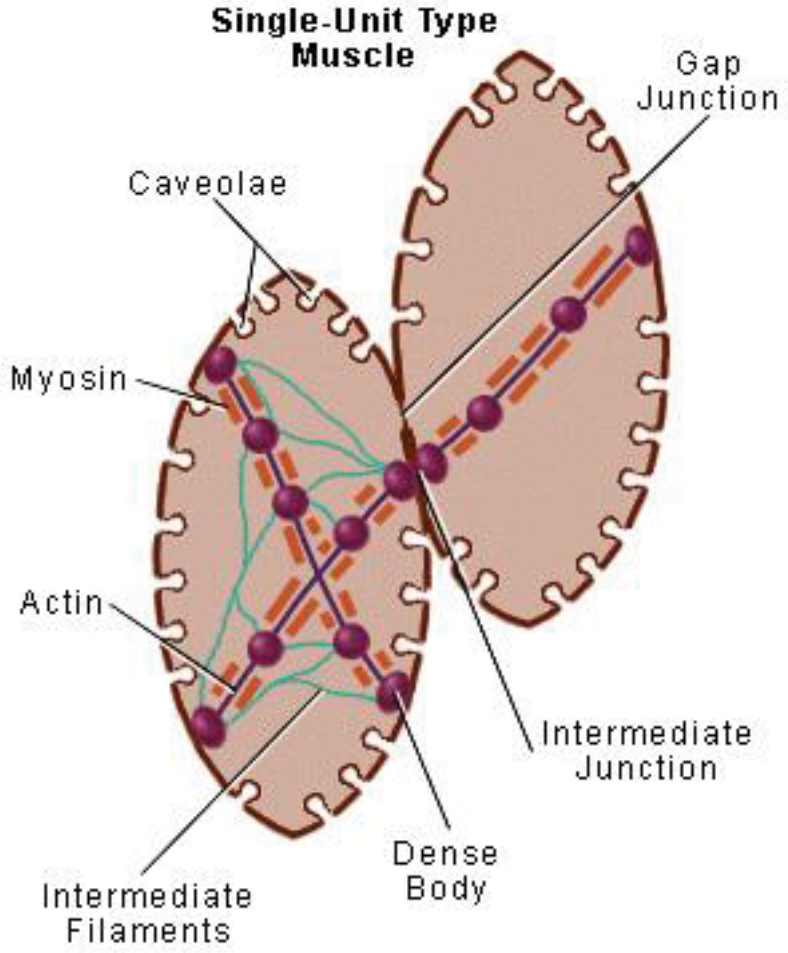

Fig. 2-24. (Ginsberg and Costoff 2015). Gastrointestinal smooth muscle structure

These filaments interlock with one another. Following electric or mechanical stimulation, smooth muscle contraction is generated. The sliding filament theory describes the process used by muscles to contract. It is a cycle of repetitive events that cause a thin actin filament to slide over a thick myosin filament and generate tension in the muscle (Saladin 2012). Desmin is important for the maintenance of structural and mechanical integrity of the contractile apparatus in muscle tissue during deformation by contraction (Paulin and Li 2004).

Contraction of smooth muscle involves phosphorylation by myosin light chain kinase. Contractions in vertebrate smooth muscle are initiated by agents that increase intracellular calcium. The intracellular calcium binds with calmodulin, which then binds and activates myosin light-chain kinase. The calcium-calmodulin-myosin light-chain kinase complex phosphorylates myosin. The rate of phosphorylation of the myosin light chains correlates well with the shortening velocity of smooth muscle. During this period, there is a rapid burst of ATP utilization as measured by oxygen consumption due to the formation of actin-myosin cross bridges. Within a few minutes of initiation, the calcium level markedly decreases, the myosin light chains' phosphorylation decreases, and energy utilization decreases; however, force in tonic smooth muscle is maintained. Crossbridge cycling causes contraction of myosin and actin complexes, in turn causing increased tension along the entire chains of tensile structures, ultimately resulting in contraction of the entire smooth muscle tissue.

Relaxation is governed by actin/myosin phosphatase. ATP hydrolyses to ADP + P, the myosin heads move back to their high energy position. In phasic contraction this happens quickly and in tonic contraction it happens slowly.

There are differences in the myosin heavy and light chains that correlate with these differences in contractile patterns and kinetics of contraction between tonic and phasic smooth muscle, but the primary regulator of the spread of relaxation is nitric oxide.

2.7.2.3 The signal transduction pathway

Regulation of smooth muscle contraction and gut motility takes place at several hierarchical levels. Hormones and neurotransmitters are the dominating components, which act directly and indirectly on muscle cells and will be discussed latter.

In smooth muscle, contraction or relaxation can be initiated or inhibited by agonists that act mainly by the means of intracellular messengers to induce the release or stimulate the reuptake of calcium (Ca^{2+}). Signal transduction occurs when a signalling molecule activates a specific receptor located on the cell surface or inside the cell. In turn, this receptor triggers a biochemical chain of events inside the cell, creating a response e.g. contraction in the smooth muscle cell. In the smooth muscle, the signal transduction pathway of an external neurohumoral signal into an internal signal is a process that involves sequential activation of a least three membrane proteins: a receptor, a guanosine triphosphate (GTP)-binding protein, and phospholipase C (PLC), which is capable of mobilizing intracellular Ca^{2+}(Hansen 2003).

2.7.2.4 Electrical activity

The resting membrane potential of myocytes in the gut wall is in the range of -40 to -80mV and is largely determined by the activity of the Na^{+}-K^{+} pump and K^{+} channels. High conductance channels with mixed selectivity for K^{+} and Na^{+} carry an inward depolarizing current and are activated at membrane potentials negative to -70mV (Hansen 2003). Ca^{2+} channels and Ca^{2+} activated K^{+} channels maintain the rhythmicity in the smooth muscle. The frequency, amplitude and duration depends on the relative proportions of active Ca^{2+} and K^{+} channels, controlled by neurohumoral agents, other membrane channels, the coupling of muscle cells to each other and to pacemaker cells (Murphy 1998; Makhlouf 1995).

Intestinal smooth muscle shows two types of electrical phenomena; *slow waves*, which are cyclical variations of the membrane potential not accompanied by mechanical activity, and *action potentials*, which initiate contractions.

2.7.2.5 Slow waves

A slow wave potential is a rhythmic electrophysiological activity that propagates along the gastrointestinal tract and is one of the key regulators of gastrointestinal motility (Huizinga and Lammers 2008). Slow waves are generated and propagated by a class of pacemaker cells called the interstitial cells of Cajal (ICC), which also act as intermediaries between nerves and smooth muscle cells (Hanani et al. 2004). The synchronous discharge of slow waves across the whole thickness of the muscle is achieved by electrical coupling of ICC to nearby smooth muscle. Cell membrane potentials within an ICC depolarize by an increase in

Na^+ and/or Ca^{2+}. This depolarization depolarises adjacent myocyte cells by local circuit current (Bolton 1989). The near instantaneous spread of slow waves across the longitudinal and circular muscle layers is results in their simultaneous depolarization

There are three types of ICC-

1. Myenteric Interstitial cells of Cajal [ICC-MY] - serve as a pacemaker which creates the bioelectrical slow wave potential that leads to contraction of the smooth muscle (Hennig et al. 2010; Sanders et al. 2006).
2. Intramuscular Interstitial cells of Cajal [ICC-IM] - are involved in the stimulation of smooth muscle cells, neurotransmitters act through them (Kito 2011).
3. Submucosa Interstitial cells of Cajal [ICC-SM] - are the cell types found in the submucosa and submucosa plexus at the interface between the sub mucosal connective tissue and the innermost circular layer (Al-Shboul 2013).

There is a fast propagation of slow waves around the circumference of the intestine through the circular muscle. The almost simultaneous spread of the slow waves around the circumference of the intestine, involves a large number of ICC's that acts to synchronise contractions of the muscle fibres to generate a mechanically effective constriction of the lumen (Costa and Furness 1982). ICC-MY are located at the border of the myenteric plexus and circular muscle while ICC-IM are within the muscle layers. The ICC-MY generate slow waves that propagate into the muscle to reinforce slow waves and muscle contractions (Galligan 2010).

Propagation of slow waves along the long axis of the intestine is much slower (Lammers et al. 2002). In the rabbit, slow waves propagate anally along the longitudinal axis of the intestine. The speed of propagation decreases along the intestine, being 3cm/s in the duodenum of the rabbit (Lammers et al. 1985) and 1.14 cm/s in the ileum (Mathias et al. 1976).

The frequency of the slow waves varies between species and in different parts of the intestine (Prosser and Bortoff 1968). The frequency of slow waves generally decreases from the duodenum to the ileocaecal region in a step wise fashion so that consecutive lengths of intestine have the same slow wave frequency. Hence, in the rabbit, the frequency decreases

from 14cpm in the duodenum to 11.8cpm in the jejunum to 10.4cpm in the ileum (Grasa et al. 2004) to 9.2cpm (Lentle et al. 2008) in the proximal colon. The stepwise decrease in slow wave frequency results in each segment acting as an individual oscillator that is incompletely coupled with adjacent oscillators. The oscillator with the highest frequency drives the slower caudal oscillators. At a certain longitudinal distance it appears that the frequency differences are too great for the next oscillator to follow and a new plateau at a lower frequency is formed (Costa and Furness 1982). From this new plateau a slow wave of a different frequency will be generated.

2.7.2.6 Action potentials

Action potentials or spike bursts initiate contraction of intestinal myocytes (Daniel 1968; Bass 1968). Action potentials are only generated when the sum of the changes in potential from the slow wave and other stimulus causes the potential of a muscle to exceed a threshold level of depolarisation (Bass et al. 1961). Hence, action potentials generally occur during the limited period of slow wave depolarisation, usually at the peak or just after it (Blass 1968). Action potentials, unlike slow waves do not propagate for more than 5mm from an ICC in the intestine as the voltage change is dissipated between adjacent myocytes (Bass 1968). This means that any propagated contraction can only result from a sequential firing of action potentials along the intestine. During the interdigestive period in rabbits, the contraction of the caecal wall is characterised by strong bursts of spike potentials lasting 4-5 seconds. The frequency of contractions varies from 0.5 to 1.2/min and they rapidly propagate from the either base to the apex (ascending contractions) or in the reverse direction (descending) (Ruckebusch and Hörnicke 1977).

The intestine undergoes both phasic and tonic changes of length and tension. Phasic contractions, which depend on action potentials for their generation, can join to produce a tonic contraction. However, some tonic contractions can occur without action potentials or even without changes in muscle membrane potentials (Costa and Furness 1982). Tonic contractions which started as an action potential can continue as an ongoing tension from maintenance of actin/myosin bridges.

2.7.3 Temporal Patterns of Motility of the Intestine

The migrating motor complex (MMC) is a cyclic, temporal pattern of motility that occurs in the stomach and small bowel during fasting. These motor complexes help trigger peristaltic waves which move distally along the gut from the stomach to the small intestine, past the ileocaecal valve, and into the colon. The MMC is a distinct pattern of electromechanical activity observed in gastrointestinal smooth muscle during the periods between meals. It is thought to serve a "housekeeping" role and to sweep residual undigested material from segments of the digestive tube. Hence, MMC's help trigger peristaltic waves that transport digesta from the stomach, through the small intestine, past the ileocaecal junction and into the colon. The temporal pattern of incidence of MMC's varies cyclically, often occurring during the post-prandial or interdigestive period, and is thought to consist of four phases (Carlson et al. 1972):

1. Phase I represents a quiescent phase with few or no contractions and therefore no mechanical activity occurs. Occurs immediately postprandially.
2. Phase II consists of irregular non-propagated contractions of the circular muscle which results in movements to and fro of the intestinal contents with some net aboral transport of chyme. Often occurs toward the end of the postprandial period.
3. Phase III is characterized by phasic contractions occurring at a maximum frequency. During this phase of MMC each slow wave is accompanied by action potentials and the resulting contractions are propagated rapidly in the caudal direction along the whole length of the active segment.
4. Phase IV, which may not always be present, has intermittent contraction of short duration.

In herbivores, such as the rabbit, MMC's migrate from the stomach to the ileocaecal valve (Grivel and Ruckebusch 1972). The duration of the active MMC cycle in the rabbit is 136-153min (Romański 2009) and a new cycle starts when the other arrives at the ileocaecal junction (Costa and Furness 1982). In sheep jejunum, phase I comprises of 33% of the cycle; phase II of irregular electrical spiking activity, 60%; and phase III of regular electrical spiking activity, 7% (Ruckebusch and Bueno 1975). During phase II in sheep, propagating contractions travel for up to 150cm at the speed of 5-12cm/s. These propagating

contractions propel bolus at regular intervals of about 1min (Ruckebusch and Bueno 1977b). A similar pattern of activity occurs in the rabbit (Grivel and Ruckebusch 1972).

At the ileocaecal junction of the rabbit, the interdigestive frequency of slow waves at the terminal ileum is 15.3/min, and 16.3/min on the oral part of the proximal colon (Ruckebusch and Hörnicke 1977). Hence, there is a difference in the frequency as well as a change in the plexus through which it is conducted i.e. from the myenteric plexus in the small intestine to the sub mucosal plexus in the colon.

2.7.4 Overall Control of Contractile Activity in the Gastrointestinal Tract

2.7.4.1 Hierarchical structure of control

Afferent sensory nerves transmit information on the physical state and also the chemical state of the contents of the gut to the brain for processing. Information is also sent to local components of the ENS. These various impulses contribute to a hierarchical system of control, with four basic levels of integrative organization that each effect the myocytes via their associate systems of ICC's (Fig. 2-25)-

- Level 1 is the enteric nervous system (ENS), an integrative nervous system within the walls of the gut. The neural networks at level 1 within the walls of the gut integrate contraction of the muscle coats, transport across the mucosal lining and intramural blood flow into organized patterns of behaviour. These networks form the ENS, which is considered to be one of the three subdivisions of the autonomic nervous system together with sympathetic and parasympathetic divisions.
- Level 2 consists of the prevertebral ganglia of the sympathetic nervous system. Efferent and afferent sympathetic innervations to and from the gut are connected to the CNS via the thoracic and lumbar regions of the spinal cord. Cell bodies in the prevertebral ganglia project efferent fibers into the digestive tract, where they synapse with neurons of the ENS in addition to innervating the blood vessels, mucosa, and specialized regions of the musculature.
- Levels 3 and 4 lie within the central nervous system (CNS). Extrinsic innervation is both cranial and spinal. In general, the anterior gut is innervated by the vagus nerve while the posterior parts are innervated by the splanchnic nerves. The third level of hierarchy comprises sympathetic and parasympathetic nerves. The fourth level of

hierarchy includes contribution from higher brain centers that integrate with control at level 3.

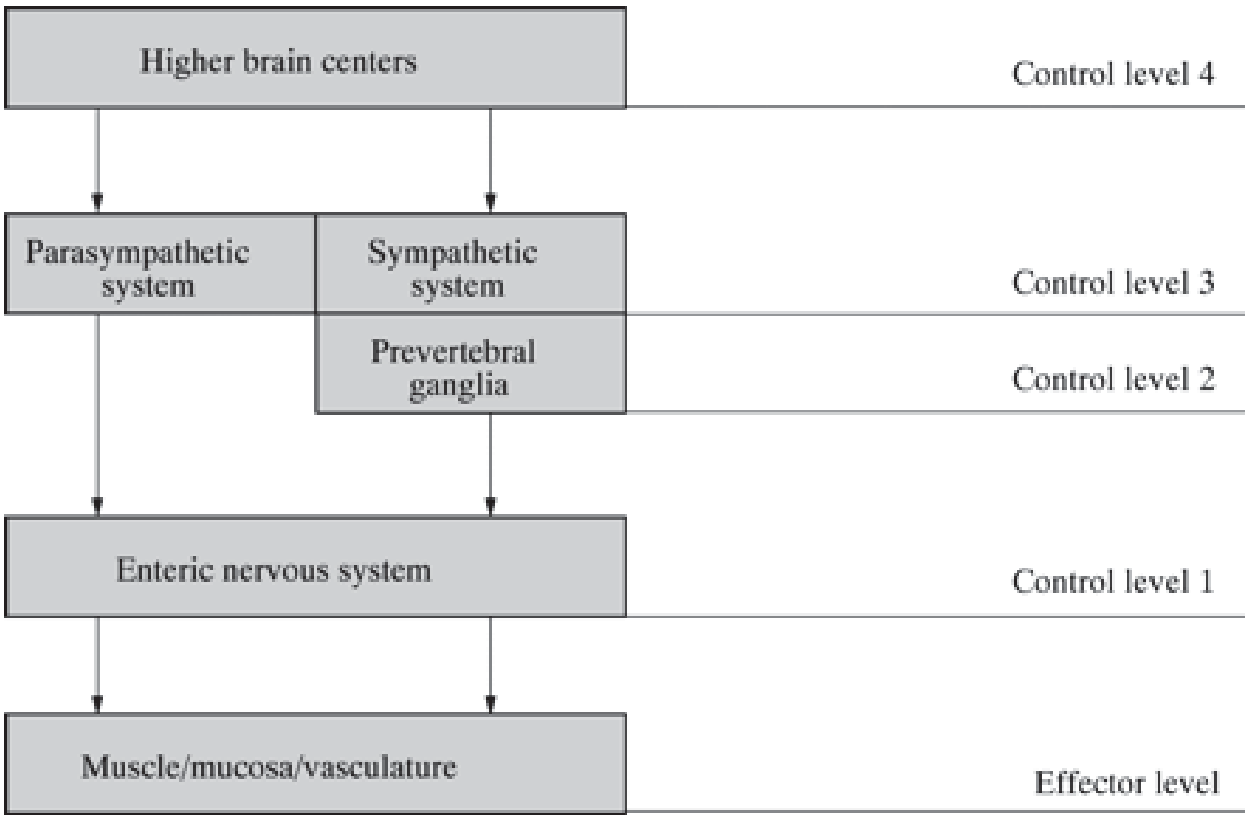

Fig. 2-25. (Wood et al 1999). Neural control of the gut is hierarchic with four basic levels of integrative organization.

2.7.4.2 Neuronal and hormonal regulation of gut motility

Hormones and neurotransmitters act directly and indirectly on myocytes to regulate both phasic and tonic contractile activity.

The neuronal regulation of the gastrointestinal motility involves intrinsic as well as extrinsic nerves. The intrinsic innervation involves the ENS which consists of ganglionated and non-ganglionated plexi. The extrinsic innervation involves the vagus nerve and splanchnic nerves to the stomach and upper intestine, while pelvic nerves supply the distal intestines. Extrinsic neurons of the sympathetic and parasympathetic systems influence smooth muscle indirectly by acting on neurons of the myenteric plexus. Intestinal myocytes are innervated by excitatory and inhibitory neurons as are ICC's in the IM. Via neurotransmitters, control of

motility is similar between species (Hansen 2003). Muscular contractions are inhibited (relaxing motorneurons) by vasoactive intestinal polypeptide (VIP), pituitary adenylate cyclase activating polypeptide (PACAP) and nitric oxide (NO), and stimulated (contracting motorneurons) by tachykinins, acetylcholine (Ach) and serotonin (5-HT).

Elements of the ENS can be influenced by hormones accompanied by the consumption of a meal. The postprandial endocrine response includes the release of insulin, neurotensin, cholecystokinin, gastrin, glucagon-like-peptides (GLP-1 and GLP-2), glucose-dependent insulinotropic polypeptide (Hansen MB 2003).

Elements of the ENS and ICC may act as mechano-, chemo-, and thermoreceptors. Cell bodies of these neurons are in ganglia. Mechanoreceptors sense mechanical events in the mucosa, musculature, serosal surface, and mesentery. They supply both the enteric minibrain and the CNS with information on stretch-related tension and muscle length in the wall and on the movement of luminal contents as they brush the mucosal surface.

Two types of mechanosensory neurons exist. Vagal afferents mediate physiological messages in low threshold Aδ and C fibers and spinal afferents sense nociceptive messages in a wide range of high threshold Aδ and C fibers. Hormones are released locally from the endocrine cells in the mucosal lining (e.g. 5-HT) and modulate activity by activating receptors on sensory fibers, the extrinsic and intrinsic primary afferent neurons, and again back on the endocrine cells in an auto regulatory fashion. They supply both the ENS and the CNS with information on stretch-related tension and muscle length in the wall and on the movement of luminal contents as they brush the mucosal surface. Mesenteric receptors code for gross movements of the organ. Chemoreceptors generate information on the concentration of nutrients, kind of nutrient (e.g. lipids), osmolality, and pH in the luminal contents. Thermoreceptors supply the brain with deep-body temperature data used in regulation and perhaps sensations of temperature change in the lumen. Secretion, motility, blood flow and absorption throughout the length of the gastrointestinal tract are modified according to the needs of digestion, as determined by the volume and nature of the luminal contents.

2.7.4.3 Plexus of the rabbit

Maslennikova (1960) observed 3 main enteric nerve plexuses in the rabbit gastrointestinal tract: a subserous, a myenteric and a submucous plexus. His observations on the structure and density of these plexus are summarized below-

In the myenteric plexus in the muscular layer of the ileum small meshes of elongated oval shape predominate (Fig. 2-16A.). Additional ganglia can be frequently seen outside the meshes. In the region of the free edge of the ileum 2000 nerve cells, and along the mesenteric edge 2500 nerve cells can be found per cm^2.

The myenteric plexus in the central part of the caecum is of somewhat different structure. In the spiral valve it consists of a wide meshed network (Fig. 2-16B.). The meshes have the shape of elongated triangles or irregular rectangles. The strands of the plexus are thicker than in higher sections of the intestine and the ganglia have numerous processes. On average, 1760 nerve cells can be found per cm^2.

In the colon the meshes of the myenteric plexus show an uneven distribution. In the region of the haustra (Fig. 2- 16C) they are mainly of rhomboid shape, the longitudinal axis of the rhomboids stands almost perpendicular to the main axis of the intestine. In the region of the taeniae all plexuses consist of narrow dense meshes. The total number of nerve cells per cm^2 reaches 3375.

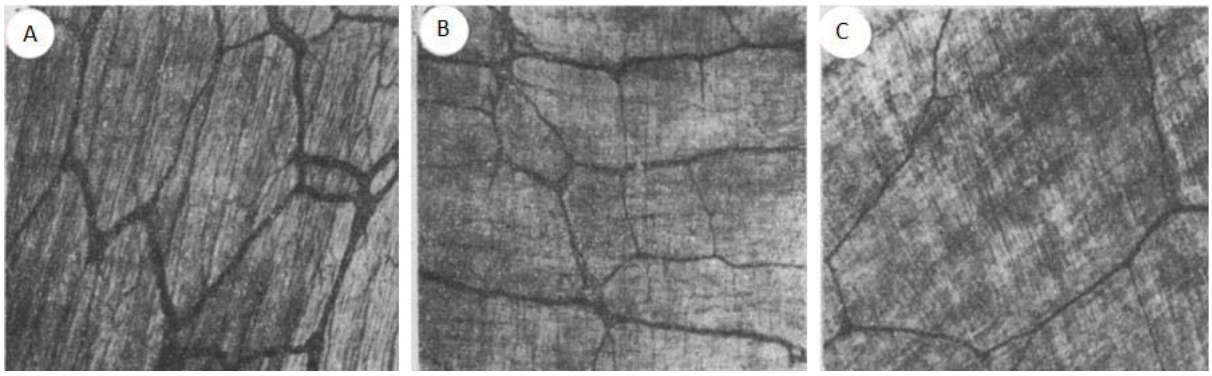

Fig. 2-16. (Maslennikova 1960). The structure of the nerve plexus in the muscular layer of different sections of the rabbit intestine. Staining with methylene blue according to Dogiel-Vorob'ev. Microphotograph. Eye piece x7 objective x24 Microsummar; The photographs are arranged corresponding to the longitudinal axis of the intestine, A) ileum; B) cecum; C) colon.

To summarize, in the rabbit, the cells (ICC's) of the plexus are relatively sparse in the caecum, increase in density to reach a maximum in the middle part of the colon, and become less dense progressively toward the rectum (Christensen et al. 1992).

Neural tissue and plexus in the ICJ of the rabbit caecum is rich in adrenergic type innervation, or tissues most of which are innervated by adrenergic fibers of the SNS (Hollands and Vanov 1965). Sympathetic nerves cause inhibition of the gastrointestinal muscle coats (Lister 1858). Neurons of the SNS slow transit along the gut by inhibiting non-sphincteric muscle through inhibition within the enteric ganglia (Russo et al. 2010). The effects are mediated primarily by α-receptors in the sphincter and ganglia and β-inhibitory adrenoceptors on non-sphincter muscle (Furness 2006a; Lomax et al. 2010).

Sympathetic stimulation also causes contraction of the ICJ (Malbert 2005). There are however species variations in the response of this region to sympathetic stimulation. In the dog and guinea pig, sympathetic stimulation causes not only contraction in the ICJ but also in the distal ileum (Munro 1951; Balsiger and Sarr 2003). In the cat, only a 1cm broad band either side of the sphincter shows a response to stimulation.

In summary, a dense muscle innervation at junctional regions, as seen in the rabbit, is associated with excitatory effects of the sympathetic nerves, which reduces the passage of contents (Furness 2006a). This suggests that the sympathetic innervation contributes to sphincter function, and along with the venous engorgement of venous bed in the ileocaecal papillae to close the ICV.

2.7.5 Concluding Remarks on Regulation and Control of Gastrointestinal Activity

In this section the complex methods of gastrointestinal regulation and control were outlined. Like any other physiologic process, proper function of the digestive system requires robust mechanisms for control and communication. Maintaining adequate control requires intimate participation from both the nervous and endocrine systems, and the gastrointestinal tract has built-in versions of both. This section was important in highlighting why it is necessary to understand the underlying mechanisms of control before phasic forms

of motility are identified, characterised, examined and measured. The various phasic contraction types of the ileocaecal junction will be described in the concluding section.

2.8- Types of Phasic Contractile Motility in the Rabbit Terminal Ileum, Caecum and Proximal Colon

2.8.1 Introduction

Four distinct types of contractile activity can be identified in the hindgut of the rabbit (Lentle et al. 2008): mass peristalsis, fast phasic contractions, haustral progression and interhaustral ripples. These regionally dependent patterns of motility promote the slow aboral progression of digesta, mixing of contents, absorption of water, electrolytes and salts, during which faecal pellets are formed prior to excretion by defecation (Dinning 2012 et al). This is accompanied by the retrograde propulsion of fine, nutrient rich material from the colon. A pacemaker area can be identified in all vertebrate species from which spontaneous contractions originate and travel both proximally towards the caecum and distally toward the anus. In the rabbit, this pacemaker area is located in the proximal flexure which separates the proximal part from the distal part of the colon and termed the *fusus coli.* In the proximal colon of the rabbit orad to the *fusus coli* there is a marked reduction in both slow-wave frequency and differentiation of the two kinds of faeces produced. This indicates the existence of a pacemaker area from which contractions may propagate in an orad or aborad direction (Ruckebusch and Fiorimonti 1976).

2.8.2 Motility in the Small Intestine

The principle function of the intestine musculature is to elicit aboral movement of chyme and to mix its contents. The shortening of the circular or longitudinal elements of the intestinal wall can alter lumen volume, and hence displace the contents (Lentle and Janssen 2011). The viscous nature of digesta in terminal ileum restricts mixing to vorticial flow and folding of the digesta (Melville et al. 1975) - where a vortex is a fluid motion having a closed or spiralling streamline. Because the gastrointestinal tract is variable, the wall exhibits a complex pattern of active and passive longitudinal and radial movements resulting in the pulsatile flow. All these factors can generate vortices and sequences of stretching and folding that serve to mix the digesta contents (Lentle and Janssen 2011).

In the terminal ileum, peristaltic contractions propel and mix digesta within the lumen of the gut whilst pendular and segmentative contraction mix digesta but do not propel it over

distance. The term ‘peristalsis’ has been used to describe prolonged propagating contractile events that propel digesta along the lumen by totally or partially occluding it (Huizinga and Lammers 2009). Distension of the lumen of the gut by a bolus of digesta generates ascending, excitatory electrical activity. This triggers smooth muscle contraction proximal to the point of distension while at the same time generating descending inhibitory electrical activity distal to the point of distension (Costa et al. 2000).

2.8.2.1 The structure of a peristaltic event

A peristaltic contraction results from the coordinated contraction of radial and longitudinal musculature comprising of a zone of lumen distension that travels in advance of a zone of constriction. This action can propagate in an orad or aborad direction (Lentle et al. 2007).

The zone of constriction has an upstream and downstream shoulder with a zone of occlusion between them which is of variable length and of varying degree of occlusion (Lentle and Janssen 2011). Longitudinal and circular contractions in the zone of constriction cause a peristaltic event to move forward in a series of rhythmic movements. The form of the leading edge of the peristaltic contraction is ultimately determined by the patterns of progression of circular and longitudinal muscle contractions within the gut wall and their effects on the lumen around the peristaltic event. This pulsatile rather than smoothly progressing longitudinal constriction may bring about mixing at the periphery (Lentle et al. 2007) (Fig. 2-26.).

In the ileocaecal region, there is a corresponding change from rapid transit of watery digesta along a relatively narrow tube to a more tardy transit of comparatively viscous digesta along a tube of larger diameter (Stevens and Hume 1995). In the rabbit this point of transition is marked by the morphological specialisation called the sacculus rotundus (Snipes 1979). As previous described, the ICJ has a sphincter which generates higher intraluminal pressure that results from localised intrinsic tone and phasic activity. Elevation of contractile tone has been shown to limit the on flow of digesta causing it to be retained in the terminal ileum, from where episodic phasic activity in the wall of the ileum moves the accumulated digesta into the caecum (Lentle and Janssen 2011). Thus, the terminal ileum is in effect acting as an ‘intestinal stomach’ (Hurst 1931).

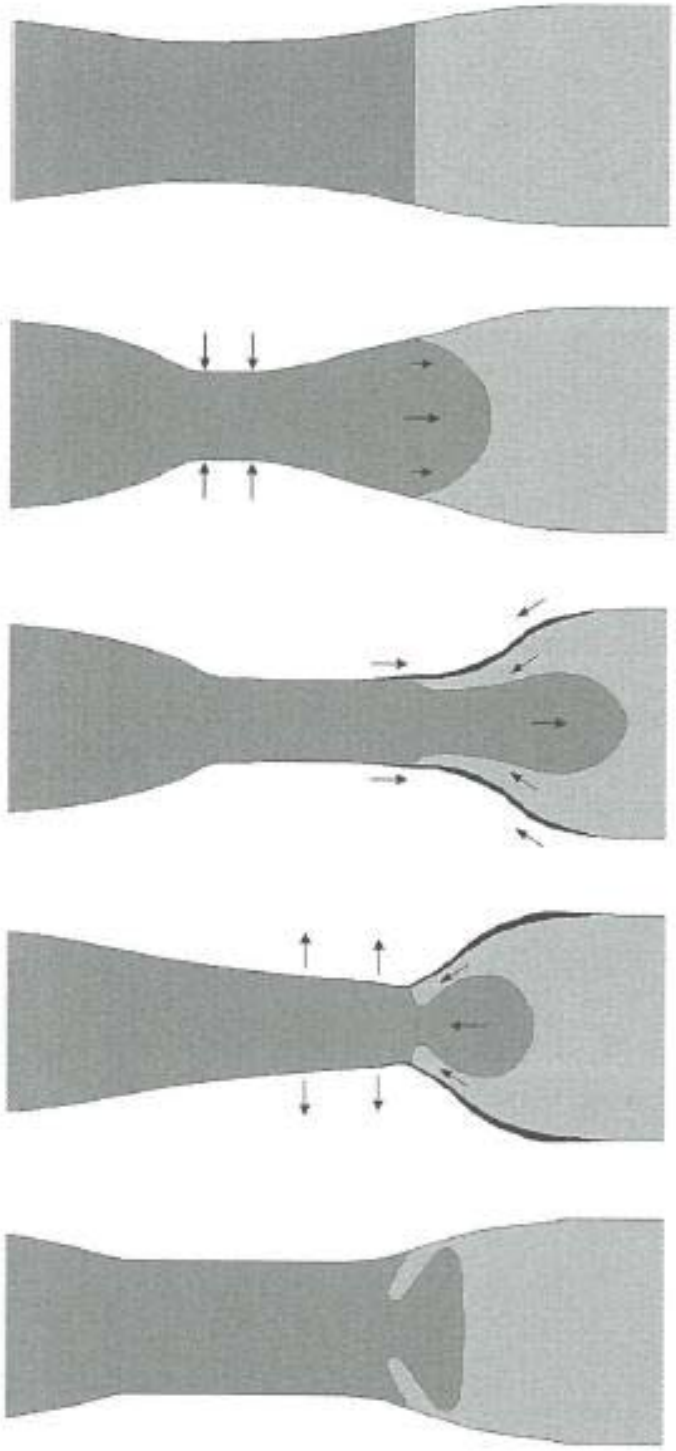

Fig. 2-27. (Lentle et al 2007). Diagram of the suggested mechanism for mixing generated by simultaneous circular and longitudinal contractions during peristalsis.

2.8.2.2 Pendular movement

Pendular movement is a to-and-fro movement of the intestine, without any propelling or peristaltic action due to asymmetric contraction of the longitudinal musculature. Pendular movement is thought to be mediated largely by slow waves (Lammers 2005). Pendular movement has been shown to increase centriluminal mixing as well peripheral mixing. The latter results from cyclic folding of the intestinal mucosa into transient microfolds (Lentle et al. 2013). These microfolds generate short-lived areas of crowding and separation of the tips of the overlying villi and have been shown to augment mixing and absorption of nutrients at the peripheral lumen (de Loubens et al. 2014).

2.8.2.3 Segmentation

Segmentative contractions result from localised contractions of circular and longitudinal smooth muscles in alternating arrays (Lentle and Janssen 2011). Segmentation contractions occur in the large intestine and small intestine, while predominating in the latter. While peristalsis involves one-way motion in the aborad direction, segmentation contractions move chyme locally in either direction. As a result of segmentation; a process by which some physical digestion occurs in the small intestine, the chyme sloshes back and forth between segments of the small intestine that form when bands of circular muscle briefly contract.

2.8.3 Motility in the Caecum

At the distal end of the ileum in rabbits, as previously described, is a muscular ampulla referred to as the sacculus rotundus. It is one of the most common sites for foreign body obstruction of the rabbit intestine (Jenkins 2000). Segmental contractions of the terminal ileum separate digesta into small boli which are transported into the caecum usually during an anti-peristaltic wave at the ileocaecal junction (Ehrlein and Ruoff 1982). The ileocaecal valve (sited between the ileum and the sacculus rotundus) retards reverse flow of fluid into the ileum, and directs chyme via the sacculus rotundus to the caecum (Jenkins 2000).

The contractile patterns of the caecum are incompletely described in the existing literature. The characteristic motor pattern of the caecum and proximal colon has been shown to be

peristaltic in form (Ehrlein and Schemann 2006). It is said that peristaltic and anti-peristaltic contractions of circular muscles are shallow and do not completely occlude the caecal lumen. Antiperistaltic waves move ileal digesta into the caecum where it mixes with caecal contents (Ehrlein and Ruoff 1982). The peristaltic waves are said to move digesta toward the caecal apex and vermiform appendix (Ehrlein and Schemann 2006). During the progression of waves, digesta are stated to be simultaneously retropelled through the central orifice of the wave (Ehrlein and Schemann 2006) (Fig. 2-27.). In this way, digesta of the caecum are moved from base to tip, and from tip to base, and are intensively mixed.

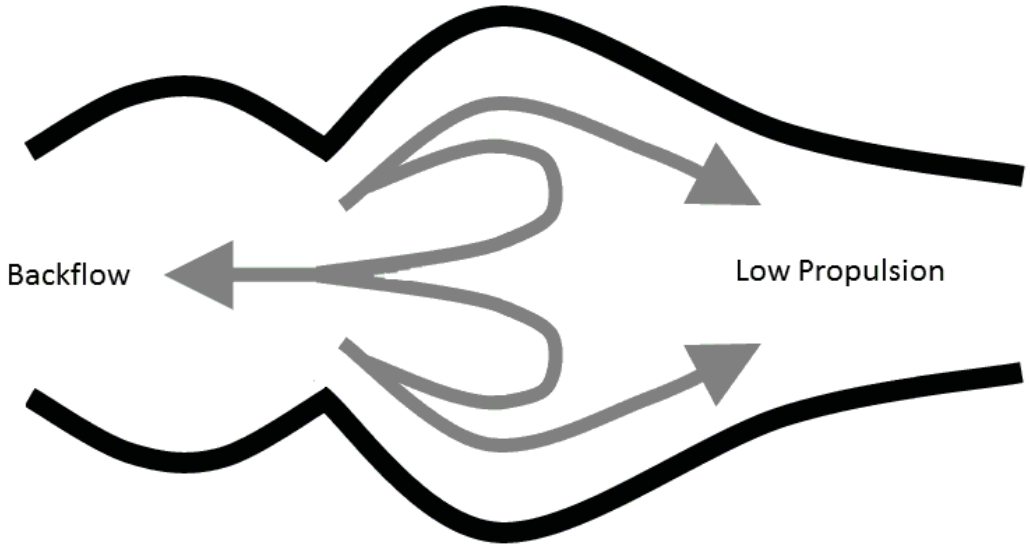

Fig. 2-27. (Adapted from Ehrlein and Schemann 2006). Peristaltic waves of the caecum produce a shallow constriction resulting in low propulsion associated with backflow.

Periodically, small amounts of digesta are passed from caecum to colon and often back again from colon to caecum. Caecocolic flux and reflux is thought to aid in the microbial digestion of material retained in the caecum and proximal colon while selective retention of fluid in the caecum preserves the microorganisms and protects the caecum from possible impaction (Pickard and Stevens 1972).

2.8.4 Motility in the Proximal Colon

The proximal colon of the rabbit is haustrated, as is that of man. Haustra are segments of the colon that are demarcated by annular contractions of circular muscles. Haustra contain small sacculated pouches brought about by contraction and relaxation of the radial smooth muscles and are not permanent structures. The condensation of colonic longitudinal muscle into taenial bands reduces the thickness and increases the compliance of the wall in the domains between taeniae (Lentle and Jannsen 2011). This gives the colon its segmented appearance. Movements of the haustra of the colon are characterised either by alternating contractions and relaxations of circular musculature muscle resulting in mixing of the digesta or by an aboral rolling of movement causing transport of liquids in a definite direction (Ehrlein and Schemann 2006).

Four types of contractile activity have been identified in the proximal colon of the rabbit (Lentle et al. 2008);

1. Haustral progression - the persistent radial constrictions that demarcate haustrae move aborally at a steady rate. Their positions and propagation speeds are coordinated across adjacent intertaenial domains. In the rabbit the rate of progression and frequency is 0.13±0.02mm/s and 0.53 cycles/min respectively (Lentle et al. 2008).

2. Mass peristalsis - resembles small intestinal peristalsis in that there is coordinated contraction of circular and longitudinal muscle. Mass peristaltic events are of relatively long duration (2.8-9.7 s in the rabbit) and propagate rapidly (8-21 mm/s in the rabbit) (Lentle et al. 2008).

3. Fast phasic contractions - are of shorter duration than mass peristaltic events. They result from alternate circular contraction and dilation and propagate predominantly in an aborad direction. They occur at intervals of around 2.3, 3.5, 5.0 and 7.5 s in the rabbit (Lentle et al. 2008). The activity may aid in reducing the apparent viscosity of the contents, particularly at the periphery of the digesta plug, which may promote the plug flow of viscous digesta (Lentle et al. 2007).

4. Ripple contractions - are a fine short-lived motility which occurs within the confines of the haustra in the rabbit (Lentle et al. 2008). Ripples are more pronounced when digesta is of a watery nature and propagation is mainly in an orad direction. Thus, it has been suggested that the motility may serve to propel the liquid phase of digesta.

The various types of motility types observed in the proximal colon of the rabbit suggest that the haustrated colon is adapted to deal with a variety of digesta types of differing rheological properties. Ripple contractions may transport the expressed liquid phase from a digesta plug in an orad direction and when haustra are not present, the same ripples may mix digesta by coordinated 'rolling' contractions of circular muscles within a haustrum. When haustration is present, haustral progression may serve to transport digesta in an aboral direction and when combined with fast phasic contractions of longitudinal musculature, displace digesta to adjacent haustra (Lentle et al. 2008). Haustra also sweep liquid toward the caecum while the indigestible solid particles are simultaneously pushed distally by the migrating segmenting contractions (Ehrlein and Schemann 2006). Mass peristalses (Fig. 2-28) compress the contents of the colon and may express digesta from the proximal into the distal colon. Any watery liquid and contained fine particle material that has been expressed from digesta during mass peristalsis will flow more readily into the relaxed proximal segment of the haustrated colon than will the compacted mass of digesta from which it has been expressed. Hence the fluid and fine particulate phase may be selectively refluxed following mass peristalsis (Lentle and Janssen 2011).

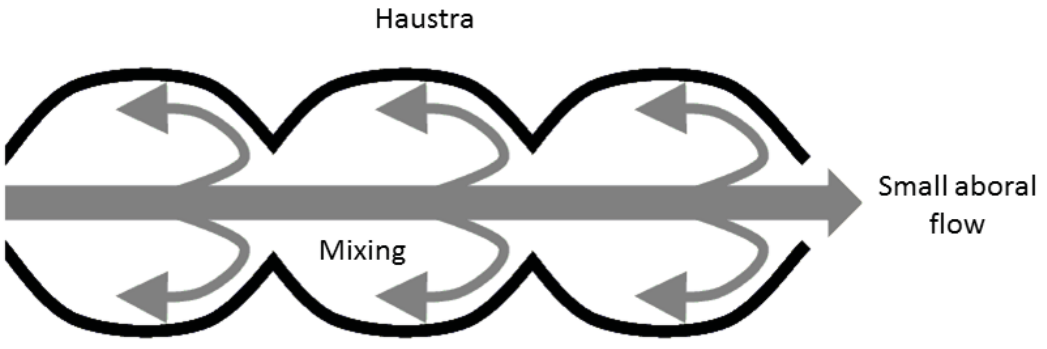

Fig. 2-28. (Adapted from Ehrlein and Schemann 2006). Peristaltic wave at a haustrated colon cause a central flow and mixing of digesta within the haustra

2.8.5 Concluding Remarks on the Types of Phasic Contractile Motility in the Rabbit Terminal Ileum, Caecum and Proximal Colon

Each part of the gastrointestinal tract performs a unique function of digestion. As a result, each part has a distinct type of motility. The motility of the small intestine is chiefly designed to mix and propel digesta. Hence, chyme from the distal ileum is transported into the caecum with no fractionation of particulate or liquid phases. In order for some fractionation to take place, digesta must transit directly to the colon. The sacculus rotundus of the rabbit may have evolved to provide this propulsion across the base of the caecum. This must be coordinated with sphincteric action at the site of the caecal base which serves to periodically ration digesta from the ileum and prevent backflow from the caecum.

The proximal colon motility is designed to separate fluid and fine particulate material from the indigestible and un-fermentable coarser particles and propel them back into the caecum where slow mixing and fermentative digestion can take place. Optimally, digesta should travel from the ampulla caecalis to the body of the caecum. How this movement is achieved in the face of ongoing distal on flow of digesta from the ileum to the colon has yet to be determined.

2.9 Conclusion

The ontogeny, digestive physiology, morphology and contractile behaviours of the components associated with the caecum ultimately influence the ordered transfer of digesta from the ileum to the colon, and of the liquid and fine particulate material from the colon to the base of the caecum. More information is required before we can understand how this is achieved. In conclusion, from the survey of literature, two principle questions arise:

1. How are contractile movements coordinated at the base of the rabbit caecum so as to facilitate the swift transfer of whole digesta from the ileum to the colon and the transfer of fine particulate material from the rabbit colon to the caecum?

2. What are the contractile movements in the body of the caecum and how do they arrest the formulation and accumulation of fine nutrient particles salvaged from the rabbit colon?

Experimental and spatiotemporal mapping work on identifying contractile movements and coordination within the rabbit caecum are developed and discussed in the following two chapters.

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

3.1 Foreword

This chapter details the study of the spatiotemporal mapping of the motility of the rabbit caecum. The detailing of the work and the results presented are in the form of a published peer-reviewed paper. This will be followed by a presentation of additional information on the development, design and construction of the apparatus used in this work plus additional information on the interpretation of spatiotemporal maps.

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

The following pages contain a copy of the published journal article with the following bibliography;

Hulls C, Lentle RG, De Loubens C, Janssen PWM, Chambers P, Stafford KJ (2012) Spatiotemporal mapping of ex vivo motility in the caecum of the rabbit. Comparative Physiology B 2012 Feb; 182(2):287-97. DOI: 10.1007/s00360-011-0610-2

The main format of the published peer-reviewed article is reproduced in this chapter section with its format and content maintained. All references cited in section 3.2 are listed in a separate sub-section after the main body of the article. These references would be reproduced in the main bibliography of this thesis (i.e. after the thesis appendix) only should they be used elsewhere beyond this section (i.e. of this published article). All further work beyond this section will be citing the work presented in this section where appropriate.

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

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3.2.1 Abstract

We used high definition radial, strain rate and intensity spatiotemporal mapping to quantify contractile movements of the body and associated structures of the rabbit caecum when the terminal ileum was being perfused with saline at a constant rate. This perfusion caused gradual distension of the caecum as a result of relative restriction of outflow from the ampulla caecalis.

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, the pattern that we termed *ladder activity* consisted of an orderly sequential contraction of bundles of axially-oriented circular muscle between the spiral turns of longitudinal muscle and proceeded either from base to tip or from tip to base at a similar frequency and velocity. Secondly, less-localised, rapidly-propagating synchronous contractions of both circular and

longitudinal muscle, which were more common when the caecum was distended, that were termed *mass peristalsis*.

Movements of the ileum and sacculus rotundus occurred at the same frequency and were broadly coordinated. Distension of the distal sacculus occurred synchronously with contraction of the ileum and did not propagate in an orderly manner across the structure, i.e. was instantaneous. This pattern was consistent with hydrostatic distension. Contractions propagated through the ampulla caecalis in either an orad or an aborad direction at a similar frequency to, and broadly correlated with, those in the ileum. The frequencies of distension of the sacculus and of contraction in the ileum and ampulla were momentarily augmented during mass peristalsis.

The authors conclude that there was coordination between the contractile activity of the terminal ileum and the caecal ampulla during periods of ongoing inflow from the ileum and that the frequency of their contraction was augmented during distension-induced mass peristalsis.

Key words

Spatiotemporal map, Caecum, Sacculus rotundus, Ampulla caecalis, Motility

3.2.2 Introduction

The physical processes involved in the fermentative digestion of plant material in the hindgut of vertebrates are complex and not fully understood. The rate at which nutrient substrates are broken down by fermentative digestion is generally more tardy in comparison to the rate at which they may undergo enzymatic digestion (Van Soest 1994). This may necessitate prolongation in the time that nutrient rich elements of digesta reside within a segment in which fermentative digestion is conducted (Stevens and Hume 1995). Hence, an animal that combines the strategies of enzymatic and fermentative digestion in successive segments must integrate the flow of digesta between them.

A relative increase in the diameter of a segment in which fermentative digestion is conducted, such as is seen in the hindgut (Stevens and Hume 1995), may be considered to be sufficient adaptation to prolong residence time as this will increase its capacity (Warner 1981). However, in situations where fermentative digestion follows enzymatic digestion in successive tubular components, a common contractile physiology and velocity of propulsion would cause a plug of digesta to travel at a similar rate through the two components. Given that the mean distance through which nutrients must diffuse to the absorbing mucosa will be greater in the wider bored component, then either the rate at which digesta transits must be slowed or the extent of mixing increased so as to maintain efficiency in nutrient absorption. The application of either strategy poses additional problems. Hence, the augmentation of mixing will require higher energy investment as the solid volume fraction and apparent viscosity of hindgut digesta are generally increased following the absorption of water in more proximal segments (Lentle et al. 2009). Again a reduction in the rate at which digesta transits may cause congestion at the junction of the two components and necessitate the development of an ancillary structure that can temporarily accommodate any excess in inflow from the enzymatic to the fermentative compartment. Further complexity may result from morphological adaptations that facilitate the relative retention of fine with respect to coarse fractions of particulate matter and hinder bulk on flow (Janssen et al. 2009).

The rabbit possesses a large and structurally complex caecum (Snipes, 1978) which can accommodate on flow from the small intestine as well as any backflow of fine nutrient rich particles from the proximal colon so as to allow their residence time to be extended for

ongoing microbial fermentation (Björnhag 1981, 1987). Whilst the morphology of the rabbit caecum has been described in detail (Besoluk et al. 2006; Snipes 1978), few studies have described its electrophysiology (Ehrlein and Ruoff 1982; Fioramonti and Ruckebusch 1974; Ruckebusch and Hörnicke 1977) and only one has examined motility *in vivo* (Ehrlein and Ruoff 1982).

The purpose of the current study is to describe and quantify the patterns and directions of motility in various sections of a rabbit caecum and associated structures maintained '*ex vivo*' in an organ bath when there is steady inflow from the ileum. This was undertaken to gain greater understanding of the manner in which these activities regulate on flow into the colon and the accommodation, mixing and fermentation of material in the body of the caecum.

3.2.3 Method

It is important to note that the anatomical nomenclature used in this study differs in some respects from that used by previous workers for species with haustrated caeca such as the guinea pig. Specifically, the structure of the rabbit caecum differs from that of the guinea pig (Schulze-Delrieu et al. 1996) in that the longitudinal musculature, rather than being condensed into taeniae, forms a spirally configured gyrus (Snipes 1978) or spiral fold (Ehrlein and Ruoff 1982) that permanently defines the boundaries over which axially-oriented bundles or 'slats' (Schulze-Delrieu et al. 1996) of circular smooth muscle operate. To avoid confusion, we used the terms *axial* and *transverse* to refer to direction with respect to a particular segment of gut undergoing spatiotemporal analysis, and the terms *longitudinal* and *circular* to refer to the layers of smooth muscle within its walls. We also use the term *interspiral domain* rather than *haustrum* to denote the contiguous convoluted region that lies between the spiral turns of longitudinal muscle.

Preparation of caecal segments

All procedures were approved by Massey University Animal Ethics Committee (MUAEC approval no 08/75) and complied with the New Zealand Code of Practice for the Care and Use of Animals for Scientific Purposes. Eight domesticated rabbits of either gender, of between 2 and 3 kg body weight, were anaesthetised in an induction chamber with 5%

halothane in 33% oxygen and 66% nitrous oxide. They were subsequently maintained on 1.5% halothane in oxygen and nitrous oxide via a face mask attached to a Bain's circuit during the surgery. Once surgical anaesthesia was established the proximal caecum was freed of its mesenteric attachments and excised with 5-7 cm of small intestine and 3 cm of proximal colon attached. The rabbit was subsequently euthanised with intracardiac pentobarbitone (125 mg/kg).

The excised caecum was placed immediately in a 41 × 15 × 7.5 cm L-shaped organ bath filled with Earle's Hepes buffer solution (HBS) maintained at 37°C and oxygenated with a mixture of 95% O_2 and 5% CO_2 via a recirculation system. The HBS buffer had a pH of 7.35 and the following composition in mM: NaCl 124.0, KCl 5.4, $MgSO_4$ 0.8, NaH_2PO_4 1.0, $NaHCO_3$ 14.3, Hepes 10.0, $CaCl_2$ 1.8 and glucose 5.0.

The attached sections of ileum and colon were each cannulated 2-3 cm away from the sacculus rotundus and ampulla coli, respectively. The base of the caecum was secured via the cannulae so that the junction of the ampulla caecalis with the base of the caecum lay on one margin of the mapped video field and the sacculus rotundus on the opposite margin (Fig. 3-1).

In specimens that were used to examine the overall motility of the caecal body, the tip of the caecal appendix was incised and a 0.5 cm diameter cannula inserted and tied firmly in place. This provided an exit point for coarse digesta during flushing with saline solution via the ileal cannula and also afforded a means by which the distal tip of the caecum could be secured in the organ bath. In other specimens, the portion of the body of the caecum that was situated distal to the fifth turn of the spiral indentation was excised. The walls of the lumen at the line of excision were subsequently gathered and secured around a similar cannula to that used in the appendix and the base secured in a similar orientation to preparations with intact caeca, i.e. via the ileal and colonic cannulae.

In both cases, digesta was flushed from the caecum via the caecal cannula by oxygenated saline perfused via the colonic and small intestinal cannulae.

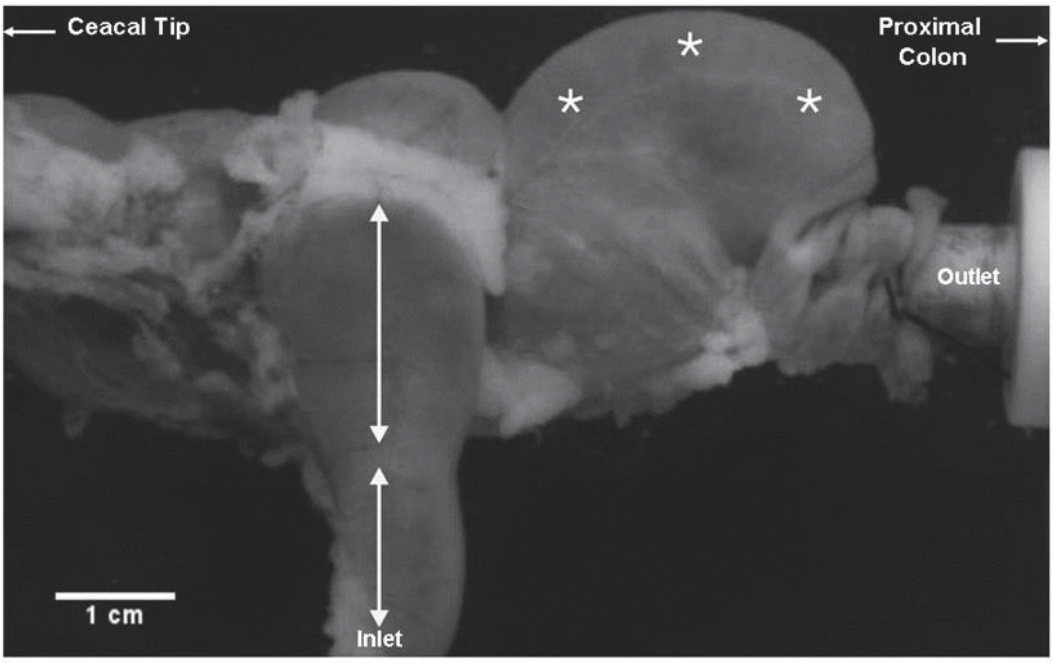


Fig. 3-1. Showing the orientation of the structures at the base of the caecum in the video frames used for spatiotemporal mapping. The bulk of the base of the caecum, which is deflated in this frame as it was taken early in the perfusion procedure, was situated beneath the sacculus rotundus. The bulk of the body of the caecum lies to the left and the cannulated end of the proximal colon lies to the right. The lower line shows the location of the LOI on the ileum that was used for SR mapping, its distal limit being the site of the slight transverse constriction. The upper line shows the location of the LOI on the sacculus rotundus that was used for SR mapping. The three asterisks mark the outer limit of the ampulla caecalis that was used for R mapping. See also Fig. 7c for a diagrammatic representation.

The caecal cannula was held via a clamp so that its outlet was maintained at 10 cm above the surface of the organ bath. The small intestinal cannula was secured to a syringe pump that delivered oxygenated saline at a constant rate of 1 ml/min and the colonic cannula secured in a position so that its outlet was maintained at 10 cm above the surface of the organ bath. The positions of the distal and proximal cannulae were adjusted so that the length of the caecum was under no longitudinal tension. A similar adjustment was also applied to the preparations of the resected proximal end of the caecum.

Hence perfusion of the preparation caused the ileal segment to function as a Trendelenberg preparation (Trendelenburg 1917) and exhibit distension induced contraction generating pulsatile inflow such as would occur *in vivo*.

Image acquisition and processing

A video camera (Basler scA1000-20fc, Ahrensburg, Germany) with a zoom lens (Cosmicar 12.5-75 mm) was mounted 450 mm above the organ bath. The position of the video camera was adjusted to allow the body of the caecum, the sacculus rotundus and the ampulla caecalis to be visualised. The IEEE 1394b output from this unit was connected to a PC which captured monochrome images of 1032 × 512 pixels at a rate of 5 frames per second and wrote these to hard disk as uncompressed TIF format files. This procedure yielded the high quality images necessary for generating high-fidelity maps with one pixel corresponding to 0.13 mm. Each image sequence, comprising of around 30,000 frames, was processed using a custom image processing program written in the Delphi language, which generated a number of maps to visualise the motility of the caeca.

Spatiotemporal mapping

The motility at various sites in the caecum and attached structures was assessed by a range of spatiotemporal mapping techniques. Where possible diameter, radial or strain rate (D, R or SR) maps were used as they are based on finite changes in the dimensions of the structure under study. In the case of investigating contractile activity on the surface of the caecum, the boundaries of the structure did not reflect contractile events taking place within it, and hence, we relied on spatiotemporal maps of the intensity of locally reflected light (see below) and separately assessed the movements on two associated structures in order to avoid any local error. The use of this technique allowed the frequency and rate of propagation of the contractions to be quantified but did not allow their amplitude to be determined.

All results regarding frequency and propagation velocity are presented as mean ± S.E. or as an absolute range if more appropriate, with n referring to the number of measurements.

Diameter and radial maps

The technique used to generate diameter and radial maps (D & R maps) was an extension of that used by other researchers (Hennig et al. 1999) and has been described previously (Lentle et al. 2007; Lentle et al. 2008). The D maps were compiled with the long axis of the caecum displayed in the horizontal direction and with time running downwards starting from the top of the map.

Briefly, each image was thresholded to separate the segment under study from the background, i.e. edge detection. The threshold level was set to the minimum value of the smoothed intensity histogram. The upper and lower edges of the outline of the structure were traced on the thresholded image using a simple search algorithm. Where the behaviour of a single boundary of a structure was being imaged, a straight line was drawn through the long axis of the structure at a distance from the boundary. Hence, the height of the pixel column between the separating line and the boundary gave the vertical distance, which was analogous to the R map of the colon (Lentle et al. 2008).

The diameter and radial maps were always compiled with the long axis of the structure under study in the horizontal direction and with successive scans stacked sequentially in the vertical (y) dimension, i.e. with run time increasing downwards from the top of the map. Thus each row of the D or R map corresponded to a single frame with the intensity of each map pixel at a given point along the length corresponding to the diameter or radius at that point. A smaller diameter or radius was represented by a lighter pixel shade and a larger one by a darker shade.

A number of parameters were derived from D and R maps. Velocities of propagation of contractile events were determined from their slopes on the maps whilst their frequency was calculated from the vertical distance (y) between successive events.

Strain rate maps

The contraction and relaxation of structures in a particular direction were determined using a modified version of the strain rate mapping (SR maps) methods described by Janssen et al. (2009), which were an improvement on earlier methods (Lentle et al. 2007). In these methods, cross-correlation between successive frames is used to quantify the displacement of a point on the preparation based on the movement of surface textural pattern. In this

work, the movements of points equally spaced along a user-specified line of interest (LOI) were determined by considering a 21 × 21 pixel square surrounding each point. As successive frames were captured at regular time intervals, the movements of these points represented the local velocity. The components of movement in the direction of the LOI were used to generate an array of velocities. Each row of the array was numerically differentiated with respect to the position along the LOI yielding the strain rates in the direction of the LOI. Repeating the differentiation procedure for every row in the velocity array yielded a strain rate map on which regions undergoing shortening are represented by a lighter shade and those undergoing extension by a darker shade.

Intensity maps

When a section of a preparation moves in the direction of the camera's line of sight, it is not possible to evaluate the movement using edge detection as employed in generating R and D maps, or cross-correlation as used in SR maps. However, the contraction can often be seen in a sequence of images because the movement changes the angle of the preparation surface and hence, the intensity of light reflected towards the camera. The rows of a spatiotemporal intensity map simply correspond to the pixel intensities along a user-specified LOI for sequential images. An intensity map does not provide any information about the amplitude of contractions but can be used to estimate their frequency and propagation velocity.

Coordination between motility events at different sites

Coordination between contractions in the various components of the caecum, colon and small intestine was assessed by directly comparing relevant synchronous portions of spatiotemporal maps and by graphic assessment of the profiles of synchronous transects from the respective spatiotemporal maps.

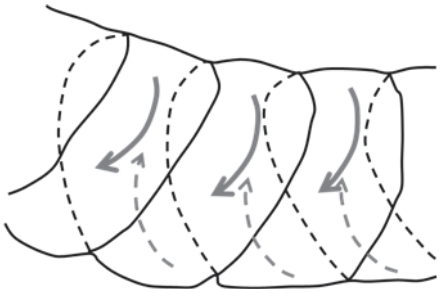
3.2.4 Results

Caecal 'ladder' contractions

Sustained sequences of low amplitude phasic contractions of individual axially-oriented bundles of circular muscle were seen progressing distally from the caecal base to the tip of the caecum traversing successive turns of the interspiral domain (Fig. 3-2).

These were termed ladder activity. On two occasions ladder contractions were seen progressing from tip to base but this was short-lived. Intensity mapping showed that distally progressing ladder activity occurred at a frequency of 18.7 ± 0.5 cycles/min with a velocity of 0.22 ± 0.03 mm/s (3 preparations mean total duration 22.6 min). The two proximally progressing ladder contractions occurred at around the same frequency and progressed at a similar velocity to those progressing distally. Intensity maps of successive turns of the interspiral domain (Fig. 3) showed that the propagation of ladder contractions across individual muscle bundles was always consistent, progressing sequentially from the superior or inferior border of each turn at a similar velocity. Consistency of frequency was assessed on transversely-`orientated intensity maps of successive turns of the interspiral domain by performing a series of plots of transects of these maps at a point halfway between the proximal and distal limit of each turn of the interspiral domain over the same time period (Fig. 3-3).

A)



B)

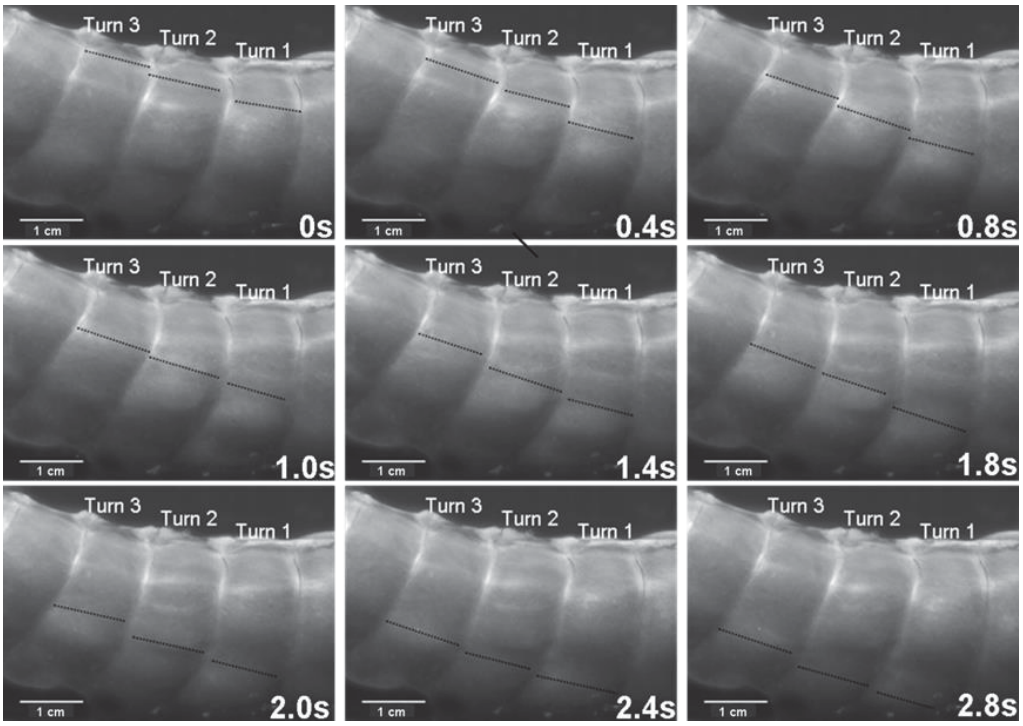


Fig. 3-2. Showing progression of ladder contractions across the body of the rabbit caecum.

The caecum is oriented with its distal tip to the left and a series of contractile events are moving distally along interspiral domain of the caecum (A). Outpouchings are seen in all visible turns of the interspiral domain each resulting from the contraction of a bundle of axially-oriented circular muscle fibres (B). Dotted lines have been superimposed on a single outpouching in each turn so as to allow their distal progression to be followed in successive frames taken 0.4 seconds apart. A video version is available on a disc accompanying this thesis.

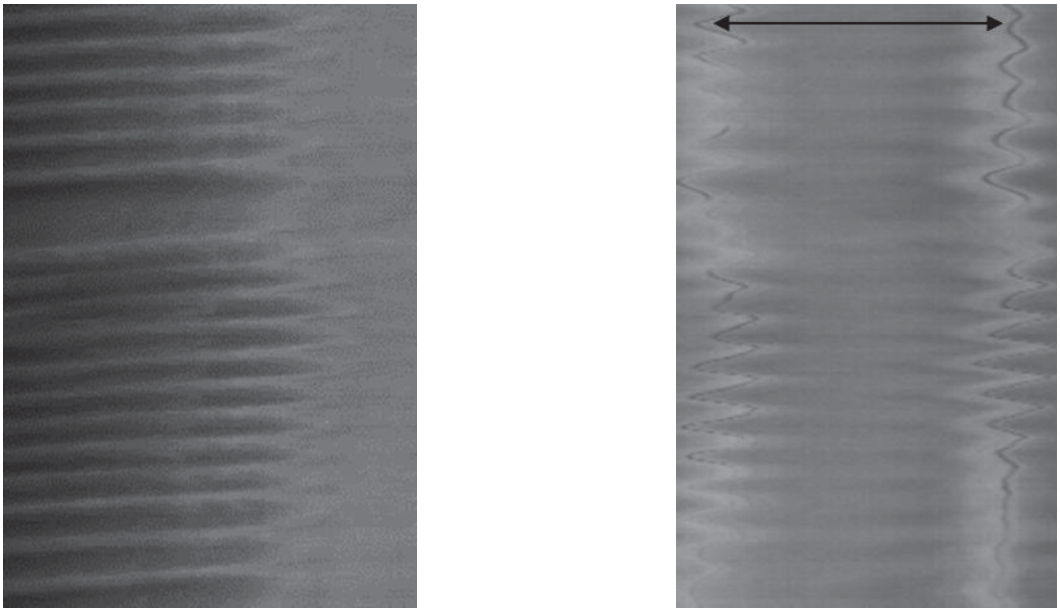


Fig. 3-3. Intensity maps of the body of the caecum showing progression of ladder contractions.

Maps generated from transversely (left) and axially (right) orientated LOIs showing the transit of a continuous series of ladder contractions and the shortening of interspiral distance (respectively) over one turn of the interspiral domains shown in Fig. 2. The transverse map shows the pattern of variation in image intensity as successive ladder contractions move down the visible portion of a turn of the interspiral domain. The contractile events are of constant frequency and their uniformly oriented slopes indicate they are progressing from base to tip at a constant velocity. The axial map shows the pattern of variation in the distance (black arrow) between the points at which the span of circular muscle across the intersprial domain intersects with the bounding spiral folds. The length of the line is seen to oscillate at the same frequency as that of local image intensity. Similar and concerted patterns were observed in consecutive turns of the spiral.

These plots showed that the frequencies of the ladder contractions were similar across all turns of the interspiral domain. Further the ladder contractions in successive turns that were nearer to the base of the caecum occurred in synchrony whilst contractions that were nearer the tip of the caecum and whose diameter increased progressively from tip to base, showed increasing phase lag in the profiles from successive turns (Fig. 3-4). Inspection of the unmapped half of the caecum during the times of recording showed that ladder contractions progressed similarly across the corresponding interspiral domain so as to arrive at the border of the next proximal or distal mapped interspiral domain. Thus during ladder activity successive individual muscle bundles contracted across the interspiral domain in consecutive spiral turns travelling either in a consistent clock-wise or anti-clockwise spiral viewed from the tip to base.

A series of axially-orientated intensity maps taken at the upper border of (Fig. 3-3) successive interspiral distances showed that the contraction of individual bundles of circular muscle each caused the corresponding interspiral distance to be locally shortened and the corresponding portions of each of the two longitudinal spirals to be drawn slightly inwards, indenting their profile. The frequency of this shortening on the axially-oriented intensity map was found to be identical to that of circular muscle bundle contraction on the transversely-orientated intensity map. Hence a series of transverse longitudinal constrictions of identical frequency to that in the vertical transects similarly progressed spirally along successive turns of the interspiral domain from tip to base. The speed of progression of the ladder contraction across successive circular bundles did not vary with the degree of distension of the caecum or with the distance of the interspiral turn from the tip or base.

SR maps (not shown) showed there was no significant lengthwise shortening of the longitudinal profile of the spiral fold either during or between ladder contractions. Hence, it appeared there was no concurrent contractile activity of the longitudinal muscle that formed the spiral fold during the progression of ladder contractions.

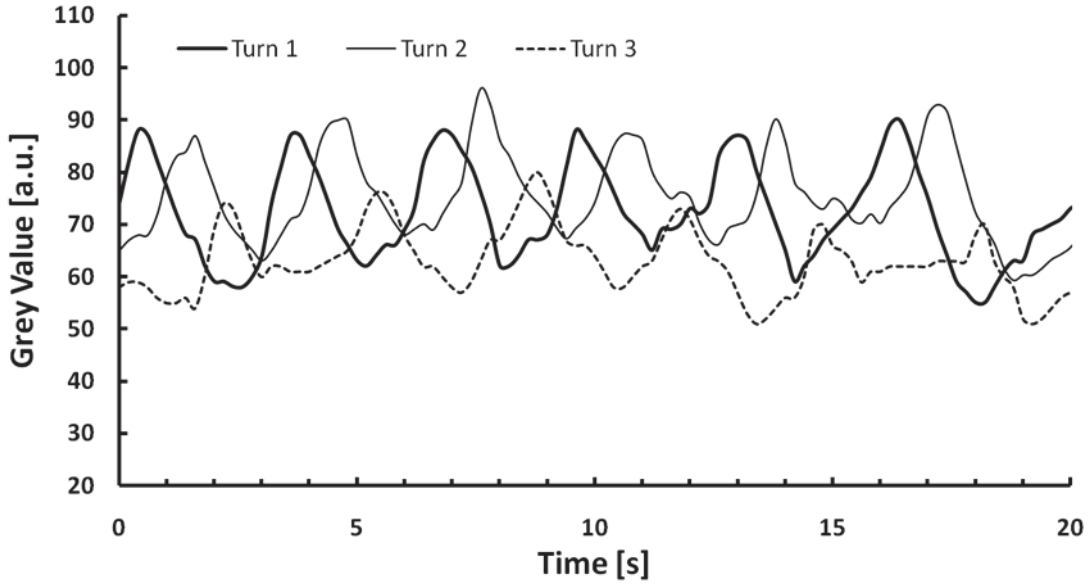
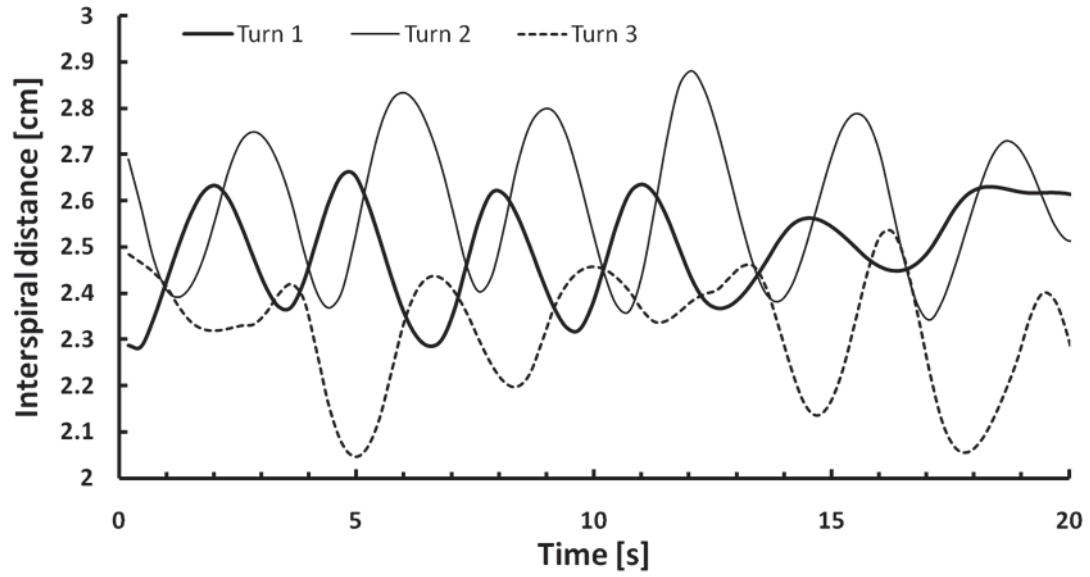


Fig. 3-4. Profiles of transects through caecal maps showing the relative timing of ladder contractions in three successive turns of the interspiral domain.

(A) Vertical transects of intensity maps generated from axially-orientated LOIs show the variation in image intensity at a fixed locus on the gut in three consecutive interspiral domains.



(B) Variations in the distances between neighbouring turns of the spiral fold compared for three successive turns of the rabbit caecum. In each graph the frequencies are similar across the three turns of the interspiral domain but with a distally increasing phase lag.

Mass Peristalsis

More-sustained and rapidly-progressing contractions of the axially-oriented circular muscle bundles occurred at irregular and unpredictable intervals ranging from 30 seconds to 10 minutes between events, notably when the caecum was distended with fluid. These were termed mass peristalses. The pattern of bundle contraction in mass peristalsis differed from that during ladder activity in that a region of near simultaneous contraction of muscle bundles that was distributed over several adjacent turns of the interspiral domain, rapidly progressed distally or proximally along the body of the caecum (Fig. 5). A somewhat greater proportion of mass peristalses propagated from the distal to the proximal end of the caecum (63% of 49 events in four preparations) than from proximal to distal. Further, the contraction of circular muscle was accompanied by significant shortening of the spiral groove indicating longitudinal muscle contraction was occurring (Fig. 5). The effect of simultaneous contraction of the bundles of circular muscle in an extensive region of the interspiral domain along with constriction of the associated longitudinal muscle spiral generated a propagating asymmetric shortening, which caused the body of the caecum to twist about the axis between its tip and base.

On six occasions, successions of mass peristalses occurred, notably when the caecum was distended with fluid. The maximal frequency of mass peristalses during these bursts was 0.42 cycles/min.

Generally, an individual mass peristaltic event was seen on spatiotemporal mapping to consist of a sequence of rapid tip to base, i.e. pro-peristaltic, and base to tip. i.e. anti-peristaltic (Ehrlein and Ruoff 1982) contractions (Fig. 3-6). The component contractions of mass peristaltic events, i.e. the zones of contraction of axially-oriented muscle bundles, each also occupied from two to three successive turns of the interspiral domain (Fig. 3-5) progressing either from tip to base (24.5 ± 5.5 mm/s; 3 preparations) or from base to tip at similar velocities (20.6 ± 3.6 mm/s; 3 preparations).

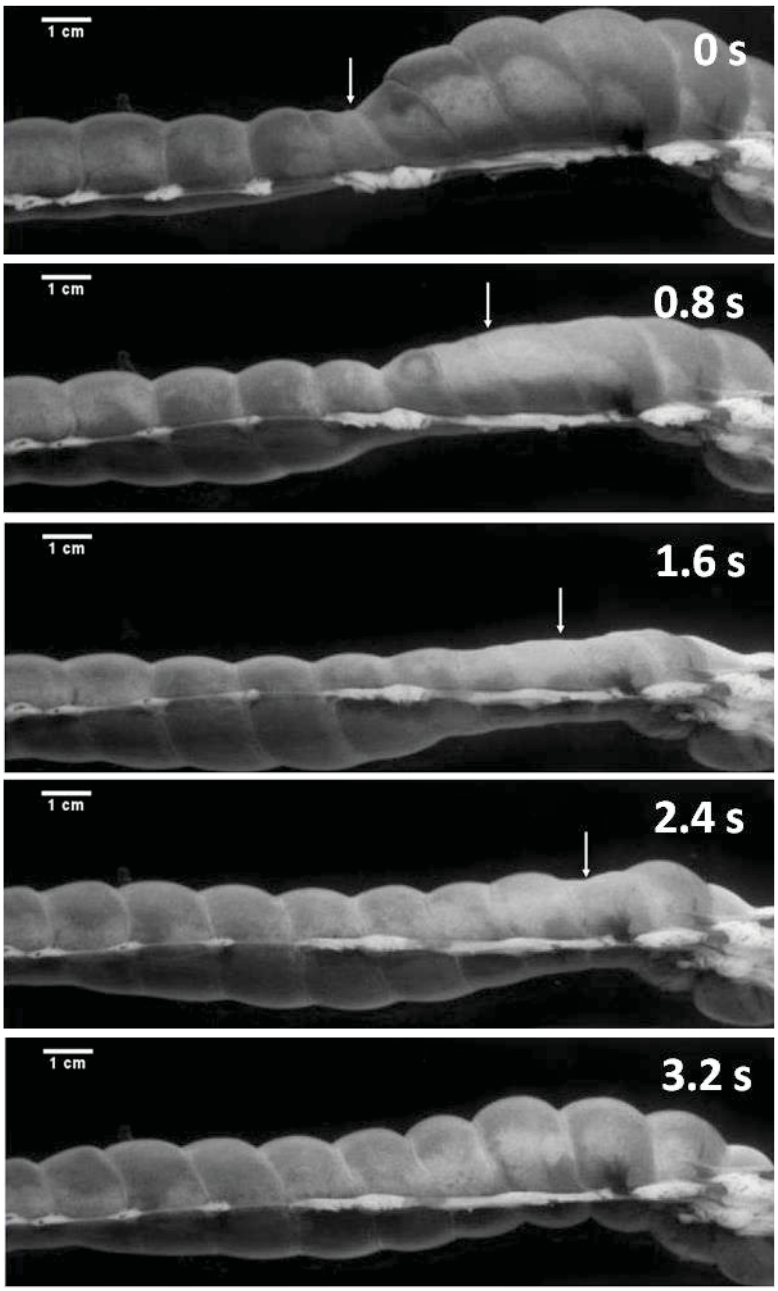


Fig. 3-5. Mass caecal peristalsis.

Frames taken at intervals of 0.8 seconds. The leading edge of a propagating mass peristaltic event which is propagating proximally toward the base of the caecum is marked with an arrow. Note the simultaneous constriction of all axially-oriented muscle bundles spanning several turns of the interspiral domain and the synchronous shortening of the spiral groove and associated longitudinal muscle. The event stops propagating in frame 4 and is extinguished in frame 5.

SR maps along the main axis of the caecum (Fig. 3-6) showed that during mass peristalsis an increase in strain rate occurred in successive turns of the interspiral domain (Fig. 3-6). The intensity, breadth and sequence of the component bounds again indicated that mass peristalsis comprised sequential synchronised contractions of large arrays of axial bundles of circular muscle.

A)

B)

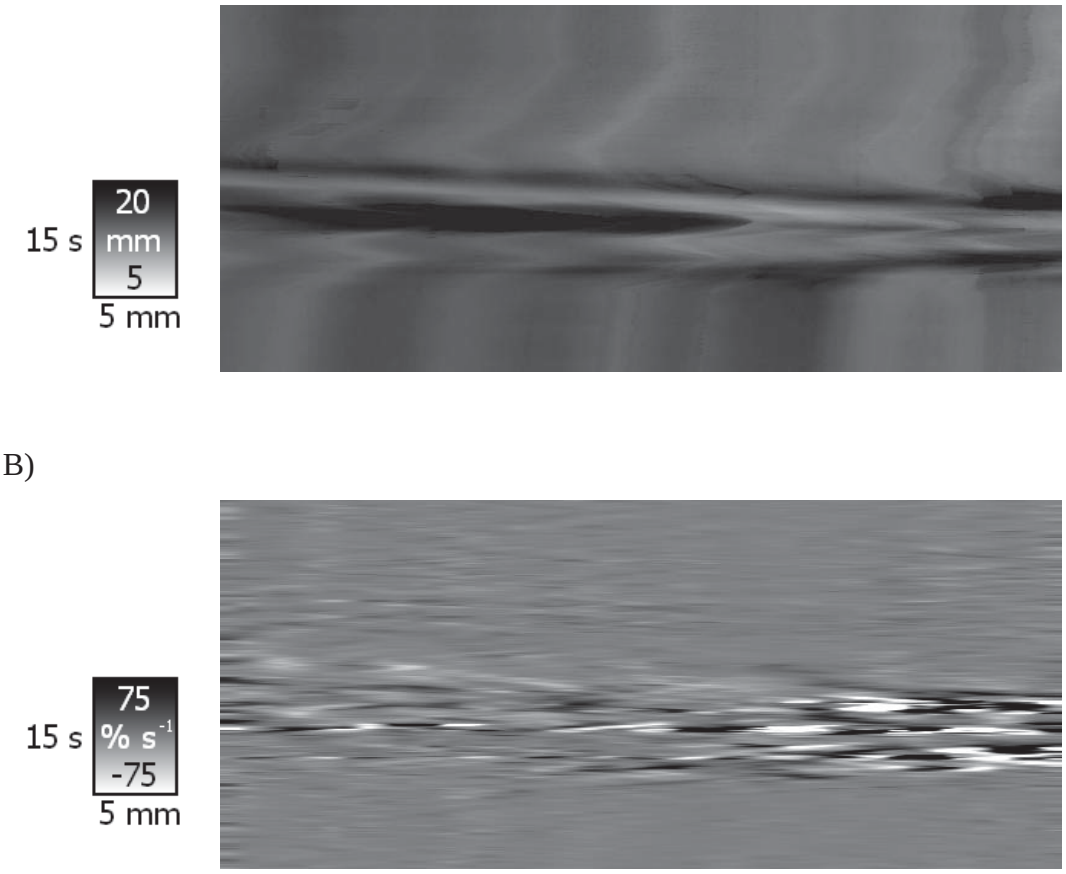


Fig. 3-6. Spatiotemporal maps of the progression of mass peristalsis across the body of the caecum.

The appendix lies to the left and the base of the caecum to the right. The R map (A) shows an initial rapid and uniform progression of a contractile event along the entire length of the caecum (light stripe) with preceding darker area of distension. A further component contraction then commences at the base and propagates toward the appendix, again with an area of distension in advance. The SR map generated from an axially-orientated LOI (B) shows that the axial contraction occurs in the interspiral domain during the initial contractile event with corresponding extension at the rapidly displaced spiral fold, presumably as a result of synchronous longitudinal constriction bringing about widthwise expansion. Hence alternate light and dark bands are seen along the longitudinal axis.

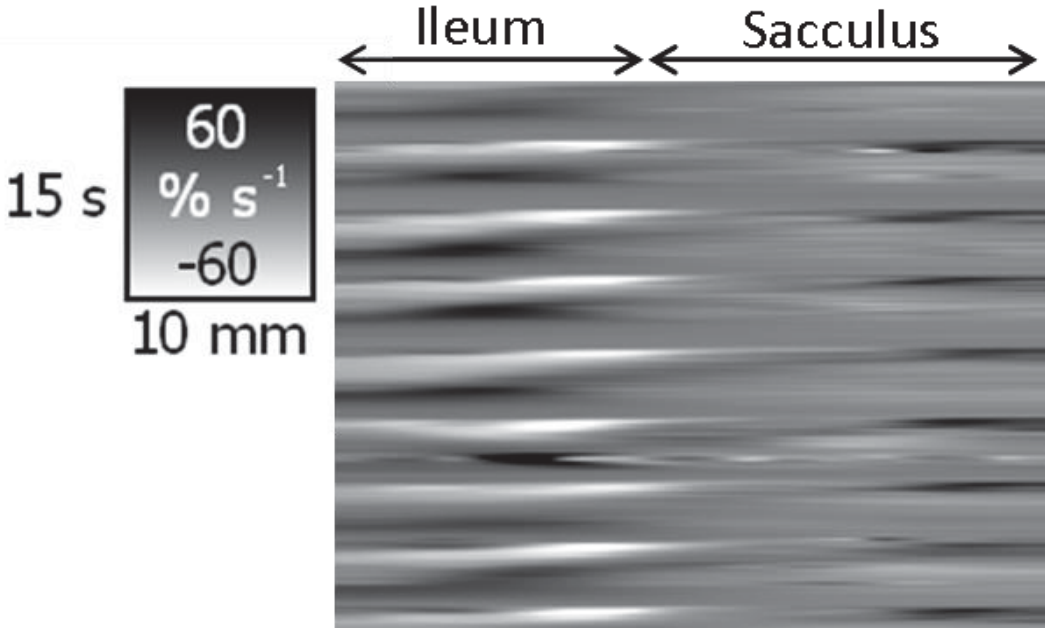
A zone of dilatation preceded the initial contractile event (Fig. 3-6). On occasion, a further zone of dilatation was interposed between successive antiperistaltic and properistaltic component contractions and a zone of dilatation also followed the final properistaltic event of the series. Hence, the component contractions within each mass peristaltic events both propelled and retropulsed the lumen contents.

Contractions of the terminal ileum and the sacculus rotundus

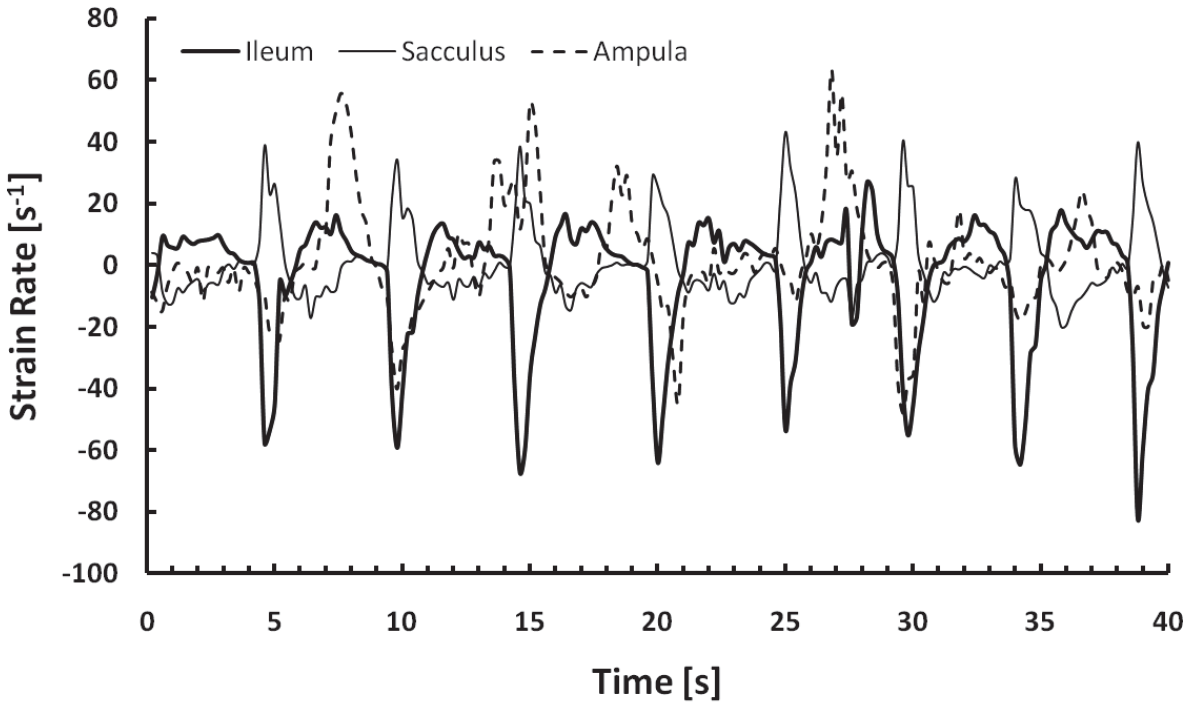
SR maps (Fig. 3-7) showed that axial and transverse contractions in the region of the terminal ileum originated at the junction with the sacculus rotundus and propagated orad at a steady frequency (11.8 ± 0.8 cycles/min n = 7 preparations) and velocity (5.8 ± 1.8 mm/s n = 7 preparations) throughout the period of observation.

Axial expansion of the most distal part of the sacculus rotundus also occurred regularly at a similar frequency (11.2 ± 2.3 cycles/min n = 7 preparations) to that of the terminal ileum (Fig. 3-7). However, the horizontal orientation of relaxation events in the distal part of the SR map of the sacculus indicated that this occurred instantaneously. Further, the peaks in strain rate in the sacculus were 90° out of phase (Fig. 3-7) with those in the ileum. Moreover, spatiotemporal analysis indicates that the strain rate of the sacculus remained almost entirely positive through successive cycles (see Fig. 3-7B) hence unlike the ampulla (see below) it showed no significant contractile activity following distension. Together these results indicate that ileal contraction caused concurrent distal hydraulic saccular expansion, the proximal progression of fluid from orally-progressing ileal contraction being prevented by the infusing cannula.

A)


Ileum
Sacculus
60
% s-1
-60
15 s
10 mm

B)


Ileum
Sacculus
Ampula
Strain Rate [s-1]
80
60
40
20
0
-20
-40
-60
-80
-100
0
5
10
15
20
25
30
35
40
Time [s]

C)

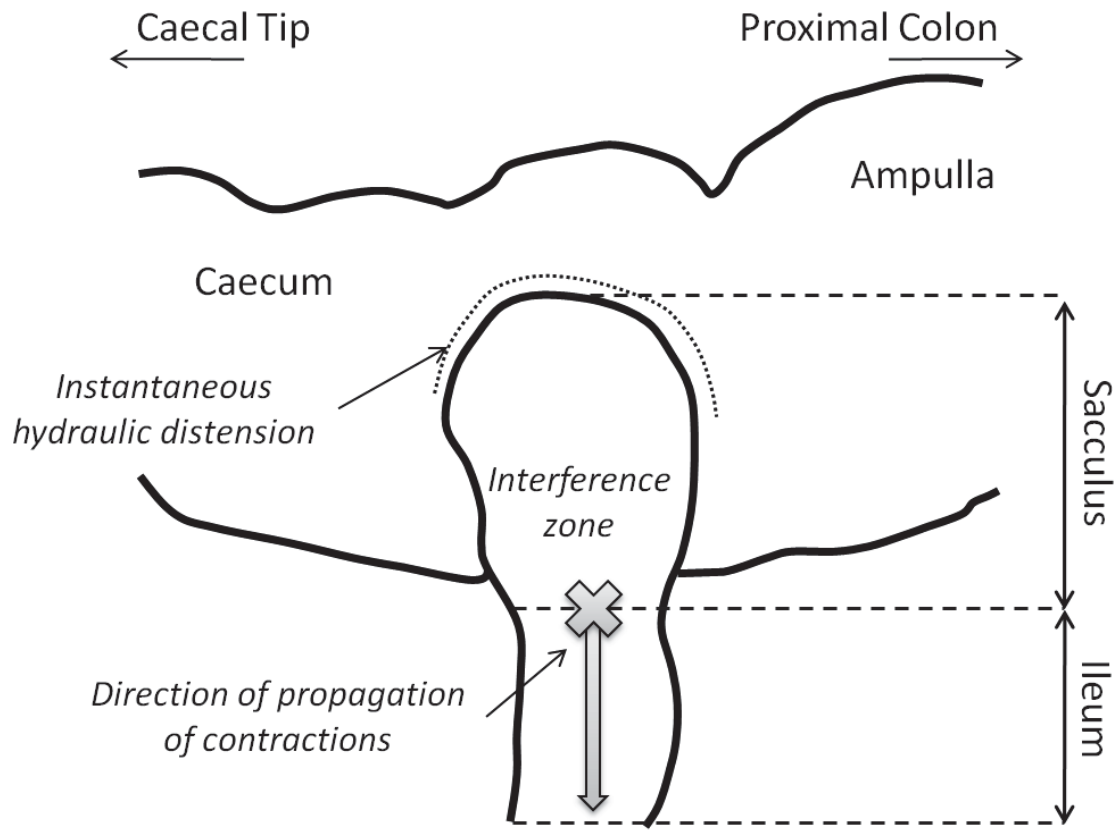


Fig. 3-7. SR map of the distal ileum and the sacculus rotundus.

A SR map generated for a LOI that runs axially along the ileum and sacculus (A) shows phasic contractions of the longitudinal muscles of the ileum (lighter shade) progressing orally at a constant rate (slope). This occurs synchronously with the regular global expansion of the distal part of the sacculus (horizontal darker areas), i.e. the two events are 180° out of phase. Transects of the SR map at the two dashed lines (B) confirms the phase relationship of the relative motility in the ileum and the sacculus (C).

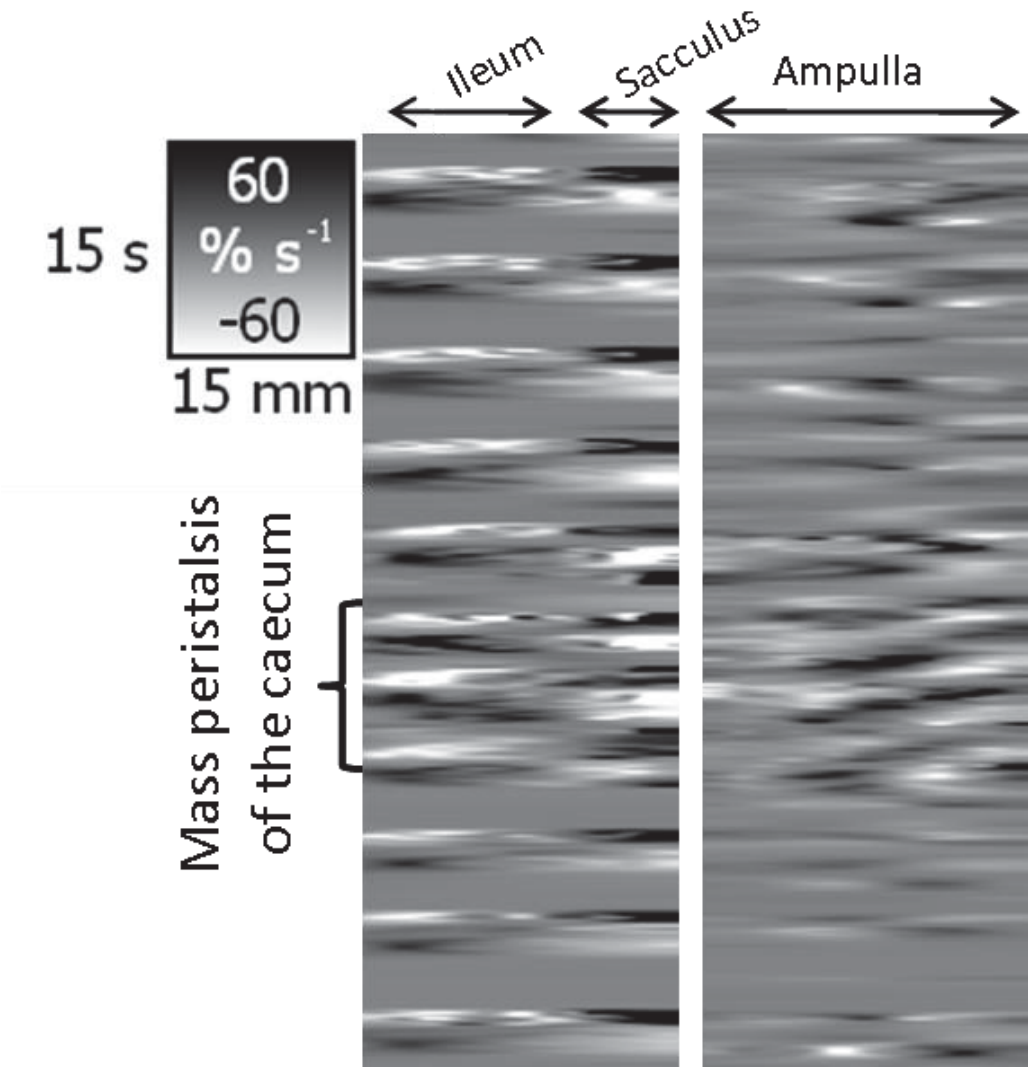


Fig. 3-8. SR maps of motility in the distal ileum, sacculus rotundus and ampulla caecalis during a mass peristaltic event in the body of the caecum.

Mass peristalsis of the caecum did not disturb the synchronisation between the ileum and the sacculus. However, during mass peristalsis their frequency of contraction increased of a factor 1.5.

The frequency of contraction of the ileum, and hence that of the sacculus, increased by a factor of approximately 1.5 during mass peristalses but the phase lag between ileal and saccular distension was preserved (Fig. 3-8). There was a similar increase in the frequency of ampullary contractions (see below).

Contractions of the ampulla caecalis

R maps taken along the upper border of the ampulla caecalis at its junction with the base of the caecum showed that contractions could propagate in either an orad or an aborad direction at a frequency of 11.0 ± 0.3 cycles/min. Comparisons of the patterns of concurrent variation in the magnitude of the strain rates at sites on the ileum, sacculus and ampulla in SR maps taken from LOIs orientated axially with respect to the gut segments (Fig. 3-7) showed broad synchrony of contraction of the ileum and the ampulla though with some variation in the magnitude and timing of peaks and troughs. Hence, there was a degree of synchrony of contraction and distension activity in the terminal ileum, sacculus and ampulla caecalis.

3.2.5 Discussion

Contractile activity in the caecal body

This paper is the first to report and to quantify the sequential propagation of 'ladder' contractions in axially-orientated bundles of circular muscle within the interspiral domain of intact ex vivo caecum of the rabbit. Other workers have noted similar sequential propagation of contractions in bundles of circular muscle fibres across haustral domains in the caecum of the guinea pig (Schulze-Delrieu et al. 1996) but did not quantify the characteristics of the contractile process. These workers referred to the propagation of these contractions as 'haustral rolling', a term which is inappropriate in the rabbit caecum where the boundaries are between successive turns of a fixed spiral of longitudinal muscle rather than occurring along a tubular section of gut bounded by the mobile borders of haustra (Lentle et al. 2008). In the rabbit caecum, ladder contractions of axially-oriented circular muscle bundles arise at a steady frequency with each contraction propagating in a consistent direction either from the distal to the proximal or from the proximal to the distal

limit of the interspiral domain. These contractions generally propagate along the length of the caecum from tip to base or from base to tip rather than travelling limited distances and dying out.

It is difficult to reconcile the spatiotemporal characteristics of progression of ladder contractions around the interspiral domain with reported patterns of progression of electrophysiological activity across implanted electrodes (Ehrlein and Ruoff 1982; Ruckebusch and Hörnicke 1977), which were reported as 'caecal contractions'. Indeed, the temporal characteristics of the reported electrophysiological events resembled those of mass peristalses more than those of ladder contractions (see below).

The shifting in the phase of ladder contractions between neighbouring turns of the interspiral domain may indicate that their timing originates in an endogenous neural or ICC network that is limited to the convoluted domain and does not cross the spiral fold itself. It is interesting to note that the rolling contractions in the rabbit colon is uncoordinated between adjacent intertaenial domains in the ex vivo proximal colon of the rabbit (Lentle et al. 2008) indicating a similar discontinuity across the taeniae. At all events the findings indicate that smooth contractions can propagate in a consistent direction across large distances.

The role of ladder contractions is currently unclear given the localised nature of component contractions and their low amplitude in comparison to the diameter of caecal lumen. In the taeniform caecum of the guinea pig the amplitude with which individual circular muscle bundles constrict in relation to the relatively low lumen diameter is reported to cause the contents of a haustrum to be shifted to adjacent haustra and to 'shake (solid) contents off the haustral wall' and to 'whirl them around the lumen to settle' (Schulze-Delrieu et al. 1996) but this structure is of a much lower diameter (Snipes 1982). The action of caecal ladder contractions is more similar to that ripple contractions in the proximal colon of the rabbit (Lentle et al. 2008) a structure of larger diameter in which the amplitude of displacement of a single contracting bundle of circular muscle is of lower amplitude than that during colonic mass peristalsis or haustral progression and hence would similarly be likely to bring about relatively little displacement of digesta.

The characteristics of the contractile activity that was termed mass peristalsis in this study were similar in temporal character to the 'caecal peristaltic waves', electrophysiological

events recorded in vivo from rabbits with chronically implanted electrodes (Ehrlein and Ruoff 1982). Hence, the speed of propagation (35.1 ± 2.9 mm/s) of component contractions of mass peristaltic events was of a similar order of magnitude to that shown by Ruckebusch and Hornike (15 mm/s interpolated from their diagram) (Ruckebusch and Hörnicke 1977) as was the maximum frequency of successive mass peristaltic events, i.e. 0.42 cycles/min compared to 0.5-1.2 cycles/min (Ruckebusch and Hörnicke 1977). Moreover, they were of a similar complex and protracted form (Ruckebusch and Hörnicke 1977).

The concerted constrictions in axial and transverse directions that are characteristic of mass peristaltic events bring about a significant local reduction in volume over a number of spiral turns. This and the rapid successive alternation in the axial direction of propagation and consequent movement of digesta suggest that this activity may be associated with mixing as well as the mass evacuation of caecal contents into the colon for subsequent voiding and coprophagy (Björnhag 1972). Which of these two outcomes occur may depend upon the synchrony of mass peristalsis with the direction of propagation of contractions in the ampulla caecalis and colon (see below). The rapid alternation in the direction of propagation of mass peristalses also suggests that subsidiary mass peristaltic events can be triggered by stretch receptors as well as by neurogenic stimuli.

Contractile activity at the caecal inlet and outlet

This paper is the first to report the pattern of volumetric change in the distal ileum and the sacculus rotundus in an ex vivo preparation. The rate of phasic contractions in the proximal ileum of the preparation was within the range reported in vivo by previous workers (Ruckebusch and Hörnicke 1977) (11.5-17.5 cycles/min) albeit with propagation in the orad direction.

Our studies show coordination between ileal and ampullary contractile activity but show that instantaneous distension of the distal sacculus rather than constriction accompanies ileal contraction. This finding conflicts to some extent with previously reported electrophysiological findings which show varying synchrony between the various components of the caecum. Hence, it is reported there is occasional synchrony in aboradly propagating contractile activity across the terminal ileum, the sacculus rotundus and the ampulla caecalis (Ruckebusch and Hörnicke 1977). It is also noteworthy that our studies showed incomplete coordination of orally propagating ileal contractions with caecal

ampullary contractions but it is possible that aborally propagating ileal contraction could more completely entrain ampullary activity at this site.

Ruckebusch and Hörnicke (1977) also reported electrophysiological events that were said to show that descending contractions of the caecum were 'activated' by contractions in the sacculus rotundus and the last 10 cm of distal ileum. No such coordination was shown in the current study although the frequency of contractile activity in the ileum and distension of the sacculus was accelerated during mass peristalsis.

In sum, our findings showed no clear concerted progression of contractions from the ileum across the sacculus rotundus to the ampulla caecalis. This situation is broadly analogous to the loose coordination of phasic contractile activity in the gastric antrum with that in the duodenum, which has led workers to postulate that contractile activity in the latter is induced by hydraulic effects of the former (Pallotta et al. 1998; Wang et al. 2005).

In conclusion, it appears that the ex vivo caecum and its associated components function autonomously to the extent that inflow of digesta augmented by ileal contraction generates instantaneous dilatation of the sacculus rotundus and may trigger contractile activity in the caecal ampulla. Such concerted activity may allow the bulk of ileal digesta to flow into the colon. However, excessive inflow and any backflow resulting from reversal of colonic and ampullary peristalsis may, as occurred in our preparations, be diverted into and generate distension of the body of the caecum. Further, it appears that such distension may engender autonomous activity in the latter of two types: ladder activity, which commence when the caecum is moderately distended and may serve to separate fine from course digesta particles; and mass peristalses, which commence when the caecum is more distended that, may serve to eject or to mix caecal contents. Whilst these autonomous functions may serve to interlink the processes of enzymatic and fermentative digestion and backflow from the colon, it is likely that they are modified in the intact animal to include coordination with colonic activity during caecotrophy (Ruckebusch and Hörnicke 1977).

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

The material in this section was not provided in Hulls et al. (2012). The following are presented: the design of the tissue bath used, layout of experimental apparatus and additional details of the spatiotemporal techniques used.

3.3.1 Organ bath design

A tissue bath was constructed to allow the caecum and sections of terminal ileum and proximal colon of the rabbit to be studied. The bath was designed to allow an unrestricted, 180° view of the preparation. The tissue bath was constructed to fulfil the following requirements:

A) The excised caecum must be maintained alive and viable at a temperature of 37°C and immersed in recirculated carboxygenated Earle-Hepes (HBS) solution.

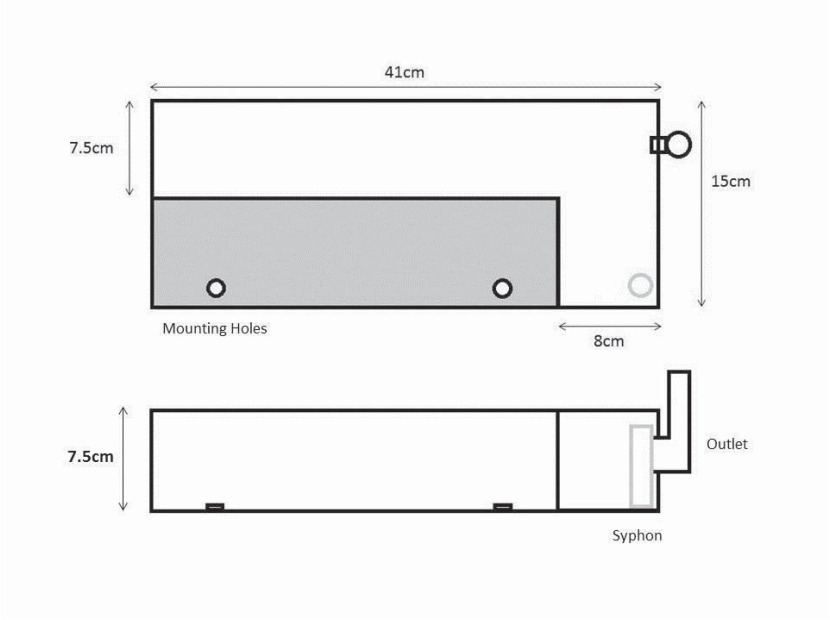
B) The excised caecum and its associated structures must be positioned in the optical planes of two visualizing cameras. There should also be some method of maintaining the organ position in the vertical and horizontal directions so as to allow as much of the surfaces of the organ as possible to remain within the optical plane of the recording cameras.

3.3.2 Organ bath

The caecum organ bath was constructed of 6mm Perspex in an 'L' shape (Fig. 3-9.). The Perspex bath was filled with oxygenated HBS via an inlet and fluid was tube driven by a peristaltic pump. The bath drained via an internal tubular damn which maintained the fluid at a constant level. HBS was recirculated. Flow of oxygenated HBS was maintained at approximately 400ml/min.

Three cannulae were installed in the caecum. The first was at the tip of the appendix. The second and third cannulae when positioned in the terminal ileum and proximal colon respectively.

The clamp stand that held the appendix cannula was able to be positioned so that the caecum was held with sufficient tension as to prevent the body of the caecum swinging out of focus.



. **Fig. 3-9. A**

Schematic of rabbit caecum organ bath with the associated dimensions. Top diagram is a "birds-eye" view of the organ bath, the image below the horizontal.

3.3.3 Layout of experimental apparatus

The general experimental setup can be seen in Fig. 3-10. Cameras were mounted both vertically and horizontally to capture images of the dorsal surface and one horizontal surface respectively.

The perfusion pump, heating apparatus and the computer for capturing and storing recorded images were placed on separate tables from the table on which organ bath was mounted. Hence, the excess HBS overflow from the organ bath was recirculated to a HBS reservoir near the experimental setup (Fig. 3-11.).

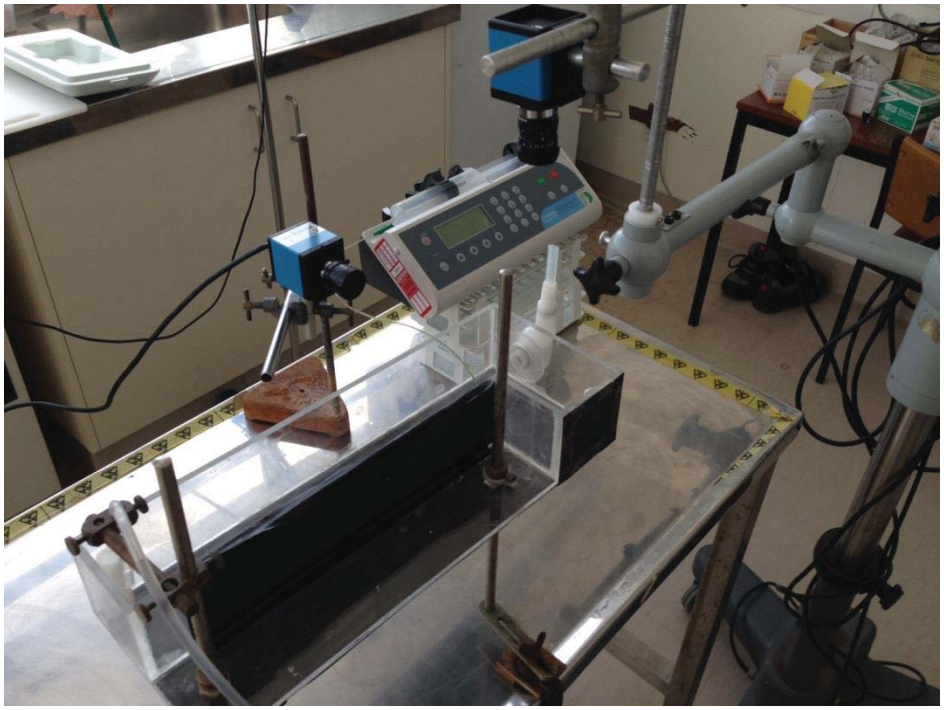


Fig. 3-10. General experimental setup. The 'L' shaped organ bath secured in place with the relative positions of cameras used to capture HD video.

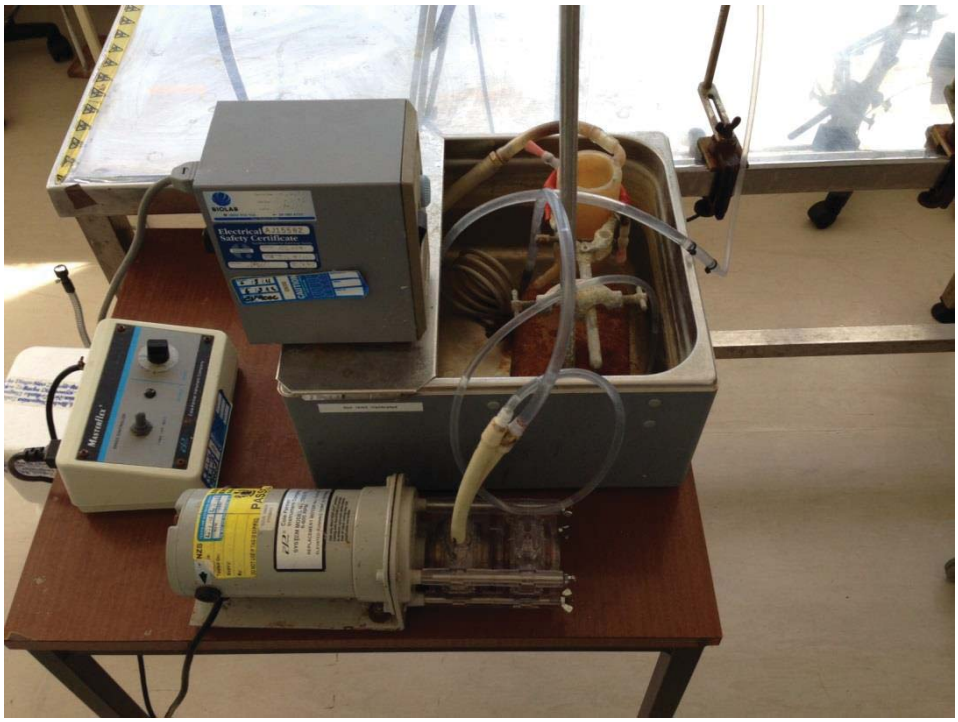


Fig. 3-11. Recirculating HBS system. The table holding the water bath and associated systems to maintain and pump the HBS at 37°C. A small glass reservoir for storage of HBS can be seen inside the water bath. The peristaltic pump (at bottom of picture) and flow rate control unit (left of picture) can also be seen in picture.

3.3.4 Additional details on the spatiotemporal mapping technique

The development of computational image processing (Jähne 2004; Russ 2006) has greatly increased our ability to quantify motility in the GI tract. Bernard et al. (1997) was the first to use ST mapping and to measure specific movements within the rat intestinal wall and development of the method has further increased its applications (Hennig et al. 1999; Bercik et al. 2000; Berthoud et al. 2002; Bogeski 2005; Janssen et al. 2007; 2009; Lentle et al. 2007; 2008; 2010).

ST mapping of contractile movement relies on the fixed position of the video camera or other imaging system in relation to the sample so that variations in the positions of the features within the image can be tracked frame by frame through time. This allows the rate movement of a defined point or an entire edge to be determined (Janssen and Lentle 2013). Through diametric and longitudinal ST mapping techniques information on local amplitude, frequency, speed, and direction of propagation of phasic contractions can then be measured.

Information, methods and techniques in ST mapping presented in this section have been taken from Janssen and Lentle (2013) and other references are provided on exception.

3.3.4.1 ST Mapping Using the Boundaries of Gut Segments: 'D' and 'R' Maps

ST maps are generated by identifying the boundaries of the gut segment in each image. The D type ST map represents measurements derived from the boundaries of a gut segment as a function of time and a spatial parameter. The easiest way to do this is to covert the original greyscale captured individual images of a film to a binary image by thresholding. For *ex vivo* preparations, this can be facilitated by placing a black background behind the organ to increase the contrast between the lighter organ and dark background.

3.4.4.1.1 D Maps

The first ST map type generated by the use of image thresholding was a diameter map or 'D map'. It can readily be performed on tubular segments of gut mounted in an organ bath with their long axis orientated horizontally across the images. After thresholding each image, the upper and lower boundaries of the organ segment are located. The algorithm

then steps along successive pixel columns of the image and calculates the distance in pixels between the upper and lower boundaries, which can be taken as the diameter of the segment at that position. The diameter estimate in each pixel column is translated into a single map pixel shaded from dark (i.e., distension) to light (i.e., constriction) in proportion to the chosen scaling factors. A row of pixels of the D map is compiled from successive diameter estimates along the long axis of the segment for each frame and hence each sampling time. Row summaries derived from successive images are stacked sequentially in the vertical dimension of the map, i.e. with run time increasing downwards from the top of the map.

D Maps allow the identification and parameterisation of a traveling diametric constriction. Hence, peristaltic events travelling along the length of a tubular segment of gut can be seen as an angled band of lighter shade. The angle of the band to the horizontal plane of the map enables its speed and direction of movement to be calculated. The width of the band in the horizontal direction reflects the length of the tissue that is undergoing constriction while the vertical width indicates the duration of that constriction. A regular sequence of contractions will appear as a series of lighter bands stacked vertically and their frequency of occurrence can be determined by counting the number of bands that traverse a vertical reference line of defined length (time), which corresponds to a particular location along the length of the intestine.

3.4.4.1.2 R Maps

Where the upper and lower boundaries of a segment of gut are likely to be moving independently of each other, the construction of radial maps or 'R maps' can be used to determine the degree to which they are coordinated. In a similar manner to that used by D maps, each image is thresholded and the upper and lower boundaries of the gut segment are traced on the thresholded image. A demarcation line is then positioned midway along the long axis of the gut component so as to separate it into upper and lower halves, the position of this separating line being programmed to be constant between successive frames. The algorithm is adjusted to determine the number of pixels in each subsidiary column above and below the line. The upper and lower R maps are each compiled in a similar manner to D maps with the long axis of the gut segment displayed in the horizontal

direction and with scans derived from successive images stacked sequentially in the vertical dimension.

3.3.4.2 Strain Rate Mapping Using Cross-Correlation

It is possible to determine relative longitudinal displacement of two defined reference points on a line of interest during a contraction by cross correlation between successive frames (Lentle et al. 2007). Thus, a measure of 'strain', and 'strain rate' can be calculated. Strain is a measurement of the relative displacement between two points and strain rate is the rate of change in that strain with respect to time. The movement of a reference point is typically detected in a 21 x 21 pixel square surrounding it. A detailed description of the correlation function can be seen in Janssen and Lentle (2013) but its simplest terms, cross correlation is the measurement of displacement on the x and y axis of pixels between the current and subsequent frames.

For a tubiform segment, mounted in the organ tank so that its long axis is oriented horizontally across the images, the horizontal component of the local velocity represents movement in the longitudinal direction and is used for further calculation while the vertical component is discarded. The above cross-correlation procedure is repeated at multiple reference points that are evenly spaced, typically 10 pixels apart, along the length of the intestine generating an array of longitudinal velocity values each with a sign indicating the direction of movement. A 3 x 3 or 5 x 3 median filter can then be applied to the array of longitudinal velocity values to eliminate spurious values. Each row of the filtered array is numerically differentiated with respect to the position along the length of the gut component yielding the longitudinal strain rate. Each interpolated strain rate value yields one map pixel with its intensity scaled to indicate whether the longitudinal muscle is undergoing shortening (lighter shade) or lengthening (darker shade), i.e. muscle motility. Repeating the procedure for every row in the velocity array yields a map of the change in longitudinal strain rate (L map) over time. L maps allow the direction and speed with which changes in strain propagate along a length of intestine to be determined.

3.3.4.3 Intensity Maps

The use of D and L maps depends on the identification of movements of areas that reflect light differently to others, for example, the edges or boundaries of the gut wall or distinctive

and repeatedly recognisable features or structures on its surface. When a section of a preparation moves in the direction of the camera's line of sight, these techniques are unsuitable. However, the contraction can often be seen in a sequence of images because the movement changes the angle of the organs surface, and hence, the intensity of light reflected towards the camera. The rows of an intensity map simply correspond to the pixel intensities along a user-specified LOI for sequential images. An intensity map does not provide any information about the amplitude of contractions but can be used to estimate their frequency and propagation.

In conclusion, ST maps of gut wall movement offer the ability to gain greater understanding of the temporal and morphological disposition of contractions. Hence, the patterns in which forces are applied to digesta within a gut segment and the degree of general or local mixing that they cause can be quantified.

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

4.1 Foreword

This chapter details the study of the electrophysiological and spatiotemporal analysis of the motility in the base of the rabbit caecum. The detailing of the work and the results presented are in the form of a published peer-reviewed paper. This will be followed by a presentation of additional information on the measurement and interpretation of electrophysiological recordings.

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

This chapter has been submitted for peer-review (at time of thesis submission). Thus, the main format of the submitted article is reproduced in this chapter section with its format and content maintained. All references cited in section 4.2 are listed in a separate sub-section after the main body of the article. These references would be reproduced in the main bibliography of this thesis only should they be used elsewhere beyond this section (i.e. of this published article). All further work beyond this section will be citing the work presented in this section where appropriate

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

4.2.1 Abstract

We examined the coordination between contractile events at different sites in the basal portion of the rabbit caecum and its associated structures that were identified by electrophysiological recordings with simultaneous one dimensional, and a novel two dimensional, spatiotemporal mapping technique. The findings of this work provide evidence that the caecum and proximal colon/ampulla coli act reflexly to augment colonic outflow when the caecum is distended and mass peristalsis instituted, the action of the latter overriding the inherent rhythm and direction of haustral propagation in the adjacent portion of the proximal colon but not in the terminal ileum. 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.

Key words:

Caecum, Rabbit, Contractile Activity, Coordination, Spatiotemporal

4.2.2 Introduction

The junction between the small and large intestine constitutes a potential barrier to the orderly transit of digesta along the gastrointestinal tract. In a number of mammals, including man, the mean diameter of the small intestine is smaller than that of the colon (Cronin et al 2010; Silva et al 2009; Ogata et al 1996). Hence, it can be assumed that if occluding peristaltic contractions were to progress from the former to the latter structure, the relative intraluminal volume displacement would differ in proportion to the radius of each component. However, a bulk of evidence indicates that such progression does not occur and that the origin, propagation and characteristics of peristaltic or mass peristaltic contractions

differ between the two segments as do their frequencies, amplitudes and speeds (Maslennikova 1961; Grasa et al 2004; Ehrlein and Scheman 2006; Lentle et al 2008). Nevertheless, volumetric discrepancies may still occur as digesta flows between the two segments. Indeed it seems reasonable to posit that diverticulae, such as the caecum, originate at the junctions of tubular structures of unequal capacity and arise as a means of accommodating volumetric discrepancies in the flow of digesta into and out of the structure that result from incoordination of pumping in the large and small intestine.

It remains then to determine the manner and extent to which the functions of the three components, the distal small intestine, the caecum and the proximal colon, can act to correct volumetric discrepancy as well as allowing orderly on flow. Two alternatives are possible. First, that a single centre coordinates the contractile activities of all three structures. Secondly, that a hierarchy exists between the normally independent centres that control the activities of the three sites such that each can function independently within a certain level of volumetric discrepancy, but that the function of one may become subservient to that of another when these discrepancies as exceeded so as to reduce them. To date no single centre governing contractile activities of all three components has been identified. Rather, separate centres have been described in both the small (Shafik et al 2001) and large intestine (Ehrlein and Ruoff 1982; Pluja et al 2001). Moreover, the finding that slow wave activity is generated separately in the colon and the small intestine (Pluja et al 2001) mitigates against the existence of a single centre. Again, the evolution of a single centre would be beset by ontogenic difficulties given emerging evidence regarding the order and direction of development of neuronal elements of the autonomic nervous system (Burns and Thepar 2006). Hence, it is difficult to imagine how nerves may radiate into all three structures from a common origin given their separate ontogeny. Thus, of the two alternatives, coordination between the independent control sites in the three structures is most likely.

Little is known regarding the levels of hierarchy; however candidate component reflexes that depend upon hydrostatic events have been identified. Thus distal ileal pressure (Corazziari et al 1991: Kellow and Phillips 1987; Kerlin et al 1983; Quigley et al 1984) and ileal propagating contractions (Dinning, Bampton, Kennedy, Cook 1999) have been associated with caecal pressure waves and the caecum has been observed to spontaneously

evacuate following distension (Fioramonti and Ruckebusch 1978; Dinning et al 2008; Hulls et al 2012). Again tone in the human ileocolic sphincter is augmented by caecal distension (Dinning, Bampton, Kennedy, Kajimoto et al 1999) although migrating motor complexes (MMCs) have only rarely been recorded to pass from the distal ileum to the caecum (Quigley et al 1984). Further, ripple contractions, sequential propagation of smooth muscle contraction around the spiral portion of the caecum, are suspended during mass peristalsis (Lentle et al 2008). Although retropulsive contractions of the colon (Ehrlein et al 1983) are undoubtedly important in the passage of nutrient dense fine particles from the colon to the caecum for further fermentation (Bjornhag 1972), there is little evidence as to whether colonic retropulsion can modulate caecal activity (Bjornhag 1981). Ruckebusch and Hornicke (1977) describe the direction of flow and propagation of contractions across the base of the caecum and the progression of myoelectrical activity down the body of the caecum into the sacculus rotundus, and from the colon and the sacculus rotundus distally into the body of the caecum, suggesting that the former activates ileal contraction whilst the latter inhibits that of the terminal ileum. Conversely, it has been postulated that, in human subjects, propagating contractions originate in the caecum and travel distally into and down the colon (Dinning, Bampton, Kennedy, Kajimoto et al 1999).

In this paper, we detail the results of experiments on the junction of the rabbit caecum with the terminal ileum and colon in which these organs, or portions of them, were maintained in an organ bath; the object being to determine the extent to which contractile movements and electrophysiological events were coordinated and whether any hierarchical rules governed their interaction and the functional outcomes of their concerted activities.

4.2.3 Method

Caecum Preparation.

All procedures were approved by Massey University Animal Ethics Committee (MUAEC approval no 12/01) and complied with the New Zealand Code of Practice for the Care and Use of Animals for Scientific Purposes.

Eight domesticated dwarf rabbits (4 male, 4 female) of between 2.1 and 3.3 kg body weight, were each anaesthetised in an induction chamber with 5% halothane in 33% oxygen and 66% nitrous oxide. They were subsequently maintained on 1.5% halothane in oxygen and nitrous oxide via a face mask attached to a Bain's circuit during the surgery. Once surgical anaesthesia was established, the proximal caecum was freed from its mesenteric attachments and excised along with 5-7 cm of attached small intestine and 5 cm of proximal colon. Each rabbit was subsequently euthanised with intracardiac pentobarbitone (125 mg/kg).

The excised caecum was placed immediately in a 41 × 15 × 7.5 cm L-shaped organ bath filled with Earle's Hepes buffer solution (HBS) maintained at 37°C and oxygenated with 95% O_2 and 5% CO_2 via a recirculation system. The HBS buffer (7.35 pH) had the following composition in mM: NaCl 124.0, KCl 5.4, $MgSO_4$ 0.8, NaH_2PO_4 1.0, $NaHCO_3$ 14.3, Hepes 10.0, $CaCl_2$ 1.8 and glucose 5.0.

The attached sections of ileum and colon were each cannulated; the former 2-3 cm proximal to the sacculus rotundus, the latter 2-3cm distal to the ampulla coli. The base of the caecum was secured via the cannulae so that the junction of the ampulla caecalis with the base of the caecum lay on one border of the mapped video field and the sacculus rotundus on the opposite border (Fig. 4-1.). The tip of the caecal appendix was incised and a 0.5 cm diameter cannula inserted and tied firmly in place. This provided an exit point for digesta during flushing of the caecal cavity with oxygenated physiological saline solution via the ileal cannula and a means by which the distal tip of the caecum could be secured in the organ bath. The caecal cannula was secured to a stopcock that could be opened and closed to atmosphere and was held by a clamp with its outlet was maintained in a position 3cm above the surface of the organ bath. The positions of the distal and proximal cannulae were adjusted so that the length of the caecum was under no longitudinal tension.

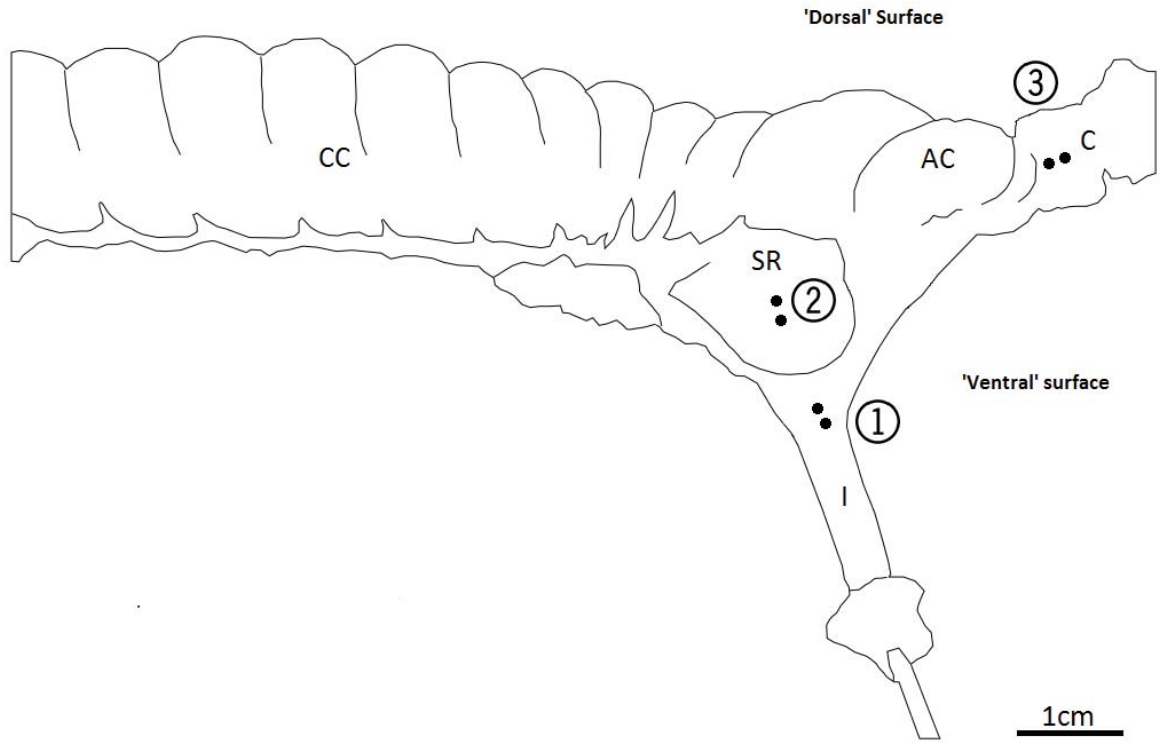


Fig. 4-1. Showing the morphology of the base of the rabbit caecum and the placement of electrodes.
The wall of the caecal base includes that below the sacculus rotundus (SR), that between the base of the ileum (I) and colon (C) and that around the ampulla caecalis (AC). The corpus caeca (CC) lies to the left of the base with the appendix to the left (out of frame).The positions of pairs of electrodes sets on the ileum (1) sacculus rotundus (2) and colonic base (3) are indicated as paired black circles.

Three flow treatments were applied to each preparation in random order as well as a 'control state' in which no flow was instituted. In the first, oxygenated saline was pumped into the ileal cannula by a syringe pump at a constant rate of 1 ml/min and the colonic cannula secured in a position so that its outlet was maintained at 3 cm above the surface of the organ bath with its port open to the atmosphere. In treatment two, saline was pumped at the same rate into the caecal cavity via the ileal cannula but the port of the colonic cannula was closed and that of the caecal outlet opened. In the third treatment, oxygenated saline was again pumped at the same rate into the caecal cavity but via the colonic cannula with the caecal cannula remaining open and the port on the ileal cannula closed. In the

'control' treatment, the port on the caecal cannula was closed, the colonic cannula open and the ileal cannula secured to the syringe pump but with no saline being infused.

Myoelectric data acquisition and analysis.

Myoelectrical activity was recorded with three pairs of multi-stranded, stainless steel wire electrodes (Cooner Wire, Chatsworth California) inserted into the smooth muscle at the relevant points and sutured to the serosa. The first pair was located on the terminal ileum 1.5 cm proximal to the sacculus rotundus. The second was located around the midpoint of the sacculus rotundus. The third was located on the proximal colon 1 cm distal to the ampulla caecalis. A common grounding electrode was positioned in the fluid of the organ bath.

Each electrode pair was connected via a Bioamp (ADInstruments) to a Powerlab data acquisition system (Maclab /8s ADInstruments Serial # M8556) and raw myoelectric data recorded using LabChart 7 Pro v7.3.7 at a rate of 1kbytes/sec/channel and stored on a PC for future analysis.

Raw data from the ileum, sacculus rotundus and colon were filtered with a band-pass digital filter set between 0.2 and 40 Hz. Raw data was filtered in the manner described by Sarna (1986) in two frequency ranges to elucidate the different electrical activities. A low-pass filter (0-0.3 Hz) was used to separate out slow waves; and a band-pass filter (5-10 Hz) to separate out short spike bursts (Fig. 4-2.). Most of the signal power was in the range of 5-10 Hz. Spike bursts were identified by computer based analysis using a method described by Lester et al (1992). Data were integrated using an absolute value and a time decay constant of 0.1 sec and a value was selected to reflect definite spike-burst activity (limit 1). A second value (limit 2) that represented activity at the commencement or end of a spike burst was used to determine duration of the spike burst. During program execution, the integrated block values were read sequentially until a value exceeding limit 2 was detected. The preceding blocks were then re-read until a block value below limit 2 was found and the start of the spike burst was identified. The data file was then read sequentially until the end of the burst was detected. This final block time was used to calculate spike burst duration. Myoelectric parameters for data from the ileum, sacculus rotundus and colon for each

treatment were calculated in 600 sec data blocks using a customized peak analysis program in LabChart 7 Pro v7.3.7.

Data comparisons were conducted by repeated measures analysis of variance (ANOVA) and paired t-tests in the SYSTAT statistical suite (SYSTAT Software, Inc. 2009 Version No.13.00.05).

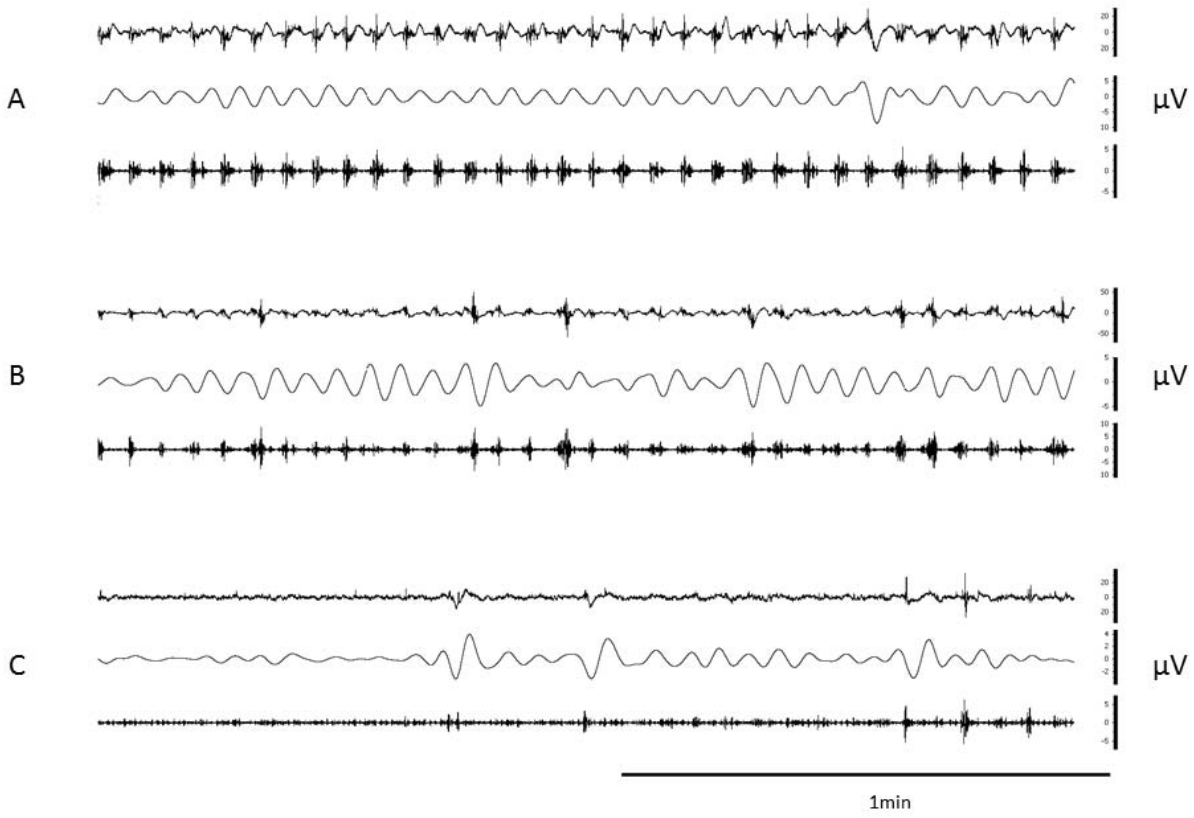


Fig. 4-2. Resting electrophysiological activity in the caecal base.

Activity was recorded in the (A) ileum (B) sacculus rotundus (C) and colon. *Top tracing* in all groups shows filtered raw signal (0.2 - 40Hz), *second tracing* shows slow waves (0 - 0.3Hz), and *third tracing* shows spike bursts (5 – 10Hz).

Image acquisition and processing.

A video camera (Basler scA1000-20fc, Ahrensburg, Germany) with a zoom lens (Cosmicar 12.5-75 mm) was mounted 450 mm above the organ bath. The position of the video camera was adjusted so that the body of the caecum, sacculus rotundus, ampulla caecalis and the attached lengths of ileum and colon all lay within the visual field. The IEEE 1394b output from this unit was connected to a PC, which captured monochrome images of 1032 × 512 pixels at a rate of 5 frames per second and wrote these to hard disk in uncompressed TIF file format. This procedure yielded the high quality images necessary for generating high-fidelity maps with one pixel corresponding to 0.11 mm. Each image sequence, comprising around 30,000 frames, was processed using a custom image processing program written in the Delphi language, which generated a number of maps that showed the pattern of motility over the bases of the caecum and associated structures.

Spatiotemporal strain rate maps.

The relative magnitudes of contraction and relaxation in the longitudinal direction were determined using a modified version of the strain rate mapping (SR maps) methods described by Janssen et al. 2009, which were an improvement on earlier methods (Lentle et al. 2007). In these methods, cross-correlation between successive frames was used to quantify the displacement of a given point on the preparation based on the local movement of the distinctive textural pattern on the surface. Hence, the movements of specific points equally spaced along a user-specified line of interest (LOI) were determined by considering a 21 × 21 pixel square surrounding each point. As frames were captured at regular time intervals, the relative movements of these points in successive frames represented the local velocity. The components of movement in the direction of the LOI were used to generate an array of velocities. Each row of the array was numerically differentiated with respect to the position along the LOI yielding the strain rates in the direction of the LOI. Repeating the differentiation procedure for every row in the velocity array yielded a strain rate map on which regions undergoing shortening were represented by a lighter shade and those undergoing extension by a darker shade.

Images of area strain rate.

Cross-correlation between successive frames was used to quantify the displacement of each point on a grid of equally spaced points within a rectangular region of interest (ROI). Differentiation of the x and y components of displacement with respect to position along the x and y directions, respectively, yielded the strain rates in the x and y directions for all the grid points within the ROI. The area strain rate (AS) for each grid point was determined from the strain rates in the x and y dimensions, (SR_x and SR_y), at that point using:

$$AS = (1 + SR_x)(1 + SR_y) - 1$$

The area strain rates were superimposed on the initial image using a lookup table (LUT) such that rapidly contracting areas appeared yellow whereas rapidly expanding areas appeared blue.

Coordination between contractile events at different sites.

Coordination between contractions in the various components of the caecum, ileum, sacculus rotundus and colon was assessed by directly comparing relevant synchronous portions of spatiotemporal maps and images of area strain rate, and by graphic assessment of the profiles of synchronous transects from the respective spatiotemporal maps. The activities of myoelectric data over a given period were compared directly with that of corresponding transects of spatiotemporal maps.

4.2.4 Results.

Electrophysiological recording of slow waves and contractile events.

Regular myoelectrical activity was recorded from all pairs of leads. The overall frequencies of slow waves, as evidenced by bouts of myoelectrical activity, varied significantly between segments on repeated measures ANOVA ($df=2$ $f= 45.13$ $P < 0.001$) but not with the site of perfusion (Table 4-1.). Slow wave frequency was significantly greater in the ileal segment

than in the colon (df=1 f= 49.29 P < 0.01), and greater in the sacculus rotundus than in the colon (df=1 f=56.23 P < 0.01), but those in the ileum and sacculus were not significantly different on repeated measures ANOVA.

The overall mean duration (Table 4-2.) of spike bursts also varied significantly between segments on repeated measures ANOVA (df =2 f=55.92 P < 0.01). The mean duration of spike bursts was significantly greater in the colon than in the ileum (df=1 f= 409.32 P<0.001), and that in the colon was greater than that in the sacculus (df=1 f=56.53 P<0.01). There was no significant difference in the mean duration of spike bursts in the ileum and sacculus.

Table 4-1. Effect of treatments on slow wave frequencies in the ileum, sacculus rotundus, and colon.

Treatment Type	Ileum	Sacculus Rotundus	Colon
Control	15.0 ± 1.13	17.1 ± 0.33	12.9 ± .92
Treatment 1 (Perfuse Ileum/ Closed Caecum/ Open Colon)	15.0 ± 0.72	15.9 ± 0.62	12.1 ± 0.92
Treatment 2 (Perfuse Ileum/ Open Caecum/ Closed Colon)	15.3 ± 0.75	16.2 ± 0.13	12.0 ± 0.58
Treatment 3 (Perfuse Colon/ Open Caecum/ Closed Ileum)	15.9 ± 0.64	16.8 ± 0.47	12.6 ± 0.21
Mean	15.3 ± 0.22	16.5 ± 0.28	12.4 ± 0.20

Values are means ± SE in CPM for all observations in all animals (n=8).

Table 4-2. Effect of treatments on spike burst duration in the ileum, sacculus rotundus, and colon with site of perfusion.

Treatment Type	Ileum	Sacculus Rotundus	Colon
Control	2.38 ±0.06	2.21 ±0.18	3.51 ±0.14
Treatment 1 (Perfuse Ileum/ Closed Caecum/ Open Colon)	2.54 ±0.04	2.22 ±0.16	3.79 ±0.18
Treatment 2 (Perfuse Ileum/ Open Caecum/ Closed Colon)	2.57 ±0.03	2.19 ±0.11	3.84 ±0.09
Treatment 3 (Perfuse Colon/ Open Caecum/ Closed Ileum)	2.48 ±0.03	2.13 ±0.17	3.46 ±0.05
Mean	2.49 ±0.04	2.19 ±0.02	3.65 ±0.1

Values are means ± SE in seconds for all observations in all animals (n=8).

The inter spike-burst period (Table 4-3.) was significantly longer on repeated measures ANOVA in the colon than in either the ileum (df= 1, f= 73.46 P<0.01) or the sacculus (df= 1, f= 52.09 P<0.01). However, there were no significant differences in mean spike burst period between the ileum and sacculus.

Table 4-3. Effect of treatments on inter spike-burst period in the ileum, sacculus rotundus, and colon with site of perfusion.

Treatment Type	Ileum	Sacculus Rotundus	Colon
Control	3.69 ±0.07	3.61 ±0.13	4.71 ±0.23
Treatment 1 (Perfuse Ileum/ Closed Caecum/ Open Colon)	4.15 ±0.21	3.83 ±0.24	4.98 ±0.19
Treatment 2 (Perfuse Ileum/ Open Caecum/ Closed Colon)	4.07 ±0.19	3.87 ±0.17	4.97 ±0.17
Treatment 3 (Perfuse Colon/ Open Caecum/ Closed Ileum)	3.89 ±0.16	3.66 ±0.12	4.8 ±0.09
Mean	3.95 ±0.10	3.75 ±0.06	4.87 ±0.07

Values are means ± SE in seconds for all observations in all animals (n=8).

The slow wave frequencies in the ileum, sacculus and colon did not differ significantly from those in the non-perfused control on paired t-test when they were being perfused by any of the three routes, nor did mean durations of spike burst activity (Fig. 4-3A. & B.). However, the mean inter spike-burst period of colonic activity was significantly higher (paired t-test, df=3 P<0.01) when the caecum was perfused via the ileum (Treatment 1) when compared to the un-perfused control (Fig. 4-3C.).

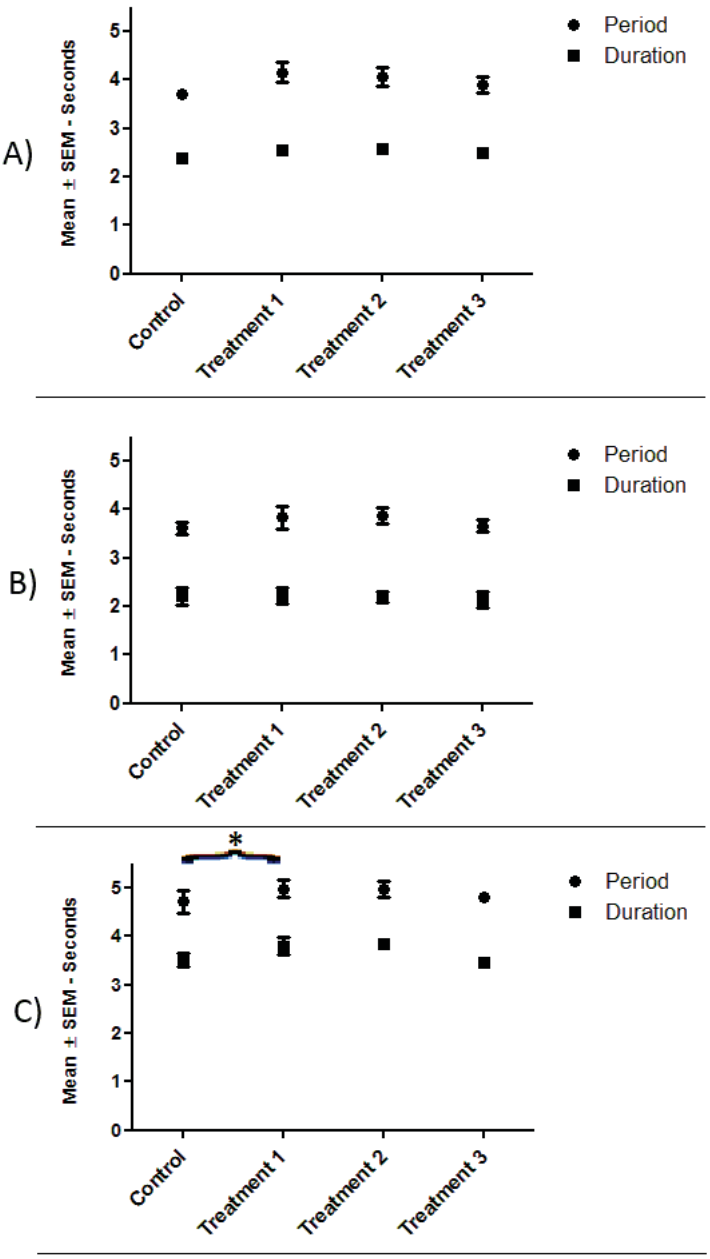


Fig. 4-3. Variation with treatment in mean durations and periods of spike bursts in A) Ileum, B) Sacculus rotundus, and C) Colon.

'Control' no perfusion with closed caecum and open colon; 'Treatment 1' perfusion via ileum with closed caecum and open colon; 'Treatment 2' perfusion via ileum with open caecum and closed colon; 'Treatment 3' perfusion via colon with open caecum and closed ileum. (*Indicates differences between pairs on t-test between control and treatment type of $P < 0.01$). Error bars indicate ±SEM.

Spatiotemporal strain rate maps.

The directions of the slopes of the lines on spatiotemporal maps of ileal strain rates during normal contractile activity, i.e. between mass peristalsis (Fig. 4-4A.), indicated that axial contractions propagated distally from the terminal ileum to the sacculus rotundus.

The slopes of the lines of strain rates of the colon (Fig. 4-4B.) on spatiotemporal maps taken during normal contractile activity showed the haustral contractions propagated distally during regular contractile activity. The flow of liquid into and distension of the proximal colon was often associated with an increase in colonic haustral contractile activity. During mass peristalsis, the direction of the slopes of the lines of strain rate (Fig. 4B.) on the spatiotemporal maps of the colon varied, haustral propagation occurring either in an orad or aborad direction.

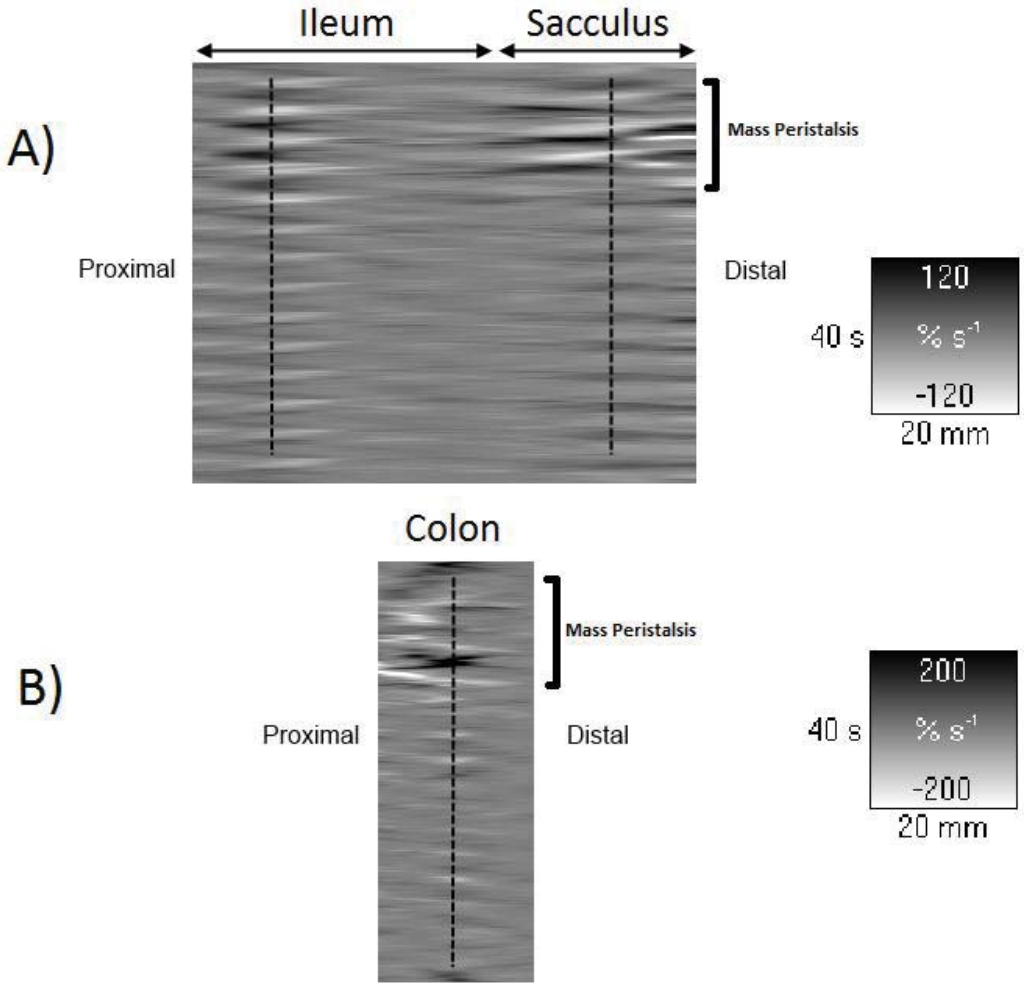


Fig. 4-4. Spatiotemporal maps of longitudinal contractile activity in the distal ileum and sacculus rotundus (A) and colon (B) during and after a mass peristaltic event.

The temporal relationship between longitudinal contractions in the ileum and the sacculus is not altered by mass peristalsis although the amplitudes of the component contractions and intervening relaxations are increased. The temporal pattern of longitudinal contractions in the colon is disrupted, contractions of larger amplitude and of varying speed and direction occurring in the colon. The regular sequence of proximal to distal contractions of lower amplitude is reinstated when the mass peristalsis has terminated in the colon. Dashed lines indicate the points at which transects shown in Fig. 4-8 were taken.

Mass peristaltic contractions occurred at irregular intervals on occasions when the caecum became fully distended with fluid. The direction of propagation of the initial mass peristalsis was either from the distal end to the base or from the base to distal end of the caecum.

The frequencies of mass peristalses in the distended caecum were not influenced by the site of inflow (Fig. 4-5.). The frequencies of mass peristalses were greater when pumping was continuing than those when the distended caecum that was not being perfused, i.e. in the control state, (paired t-test, df=3 $P<0.05$) regardless of the site of pumping. There was no significant variation in the frequency of mass peristalses with the site of pumping. Together these results suggest that the mean frequency of mass peristaltic events was governed by caecal distension regardless of site of perfusion and that the rate of change in volume was important in the frequency of their initiation.

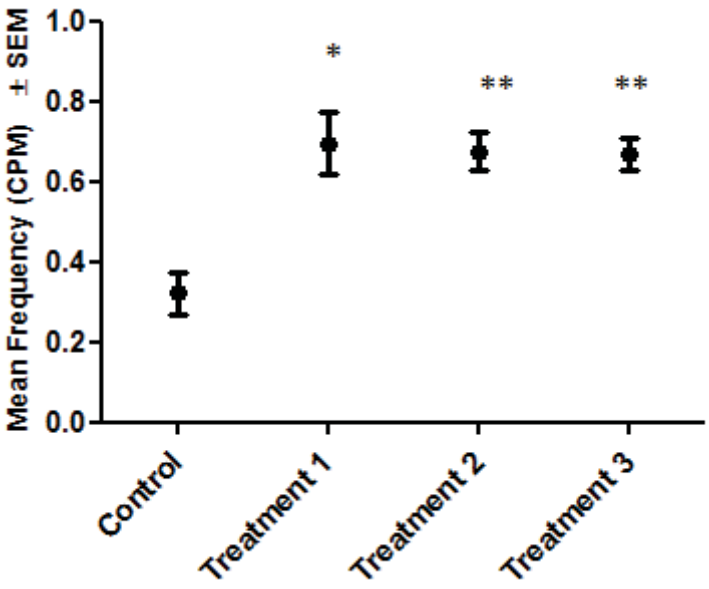


Fig. 4-5. Effect of treatments on the mean frequency of mass peristalses.

'Control' no perfusion with closed caecum and open colon; 'Treatment 1' perfusion via ileum with closed caecum and open colon; 'Treatment 2' perfusion via ileum with open caecum and closed colon; 'Treatment 3' perfusion via colon with open caecum and closed ileum. (*Indicates differences between pairs on t-test between control and treatment type of $P < 0.05$ and $P < 0.01$ **). Error bars indicate ±SEM

Area strain rate images.

Sequences of area strain rate images indicated that the propagation of contractions over the base of the caecum were broadly similar when the caecum was perfused via the ileum to those when perfusion was via the colon (Fig. 4-6.). At the beginning of a contractile cycle a contraction of the right ventral edge of the body of the caecum (0.4s in Fig. 4-6A.) commenced shortly after the arrival of an ileal contraction at the junction with the sacculus rotundus (time 0.2sec in Fig. 4-6A.). The subsequent contraction of the sacculus rotundus (time 0.6s -1.0s in Fig. 4-6A.) was followed by commencement of contraction in the dorsal edge of the body of the caecum (1.0-1.6s in Fig. 4-6A. and B.) which propagated distally into the proximal colon and into the caecum toward the vermiform appendix (1.2-1.4 seconds Fig. 4-6A.). A similar cycle operated when pumping was via the colon save that the contraction of the dorsal edge was of greater amplitude (1.0-1.4s in Fig. 4-6B.).

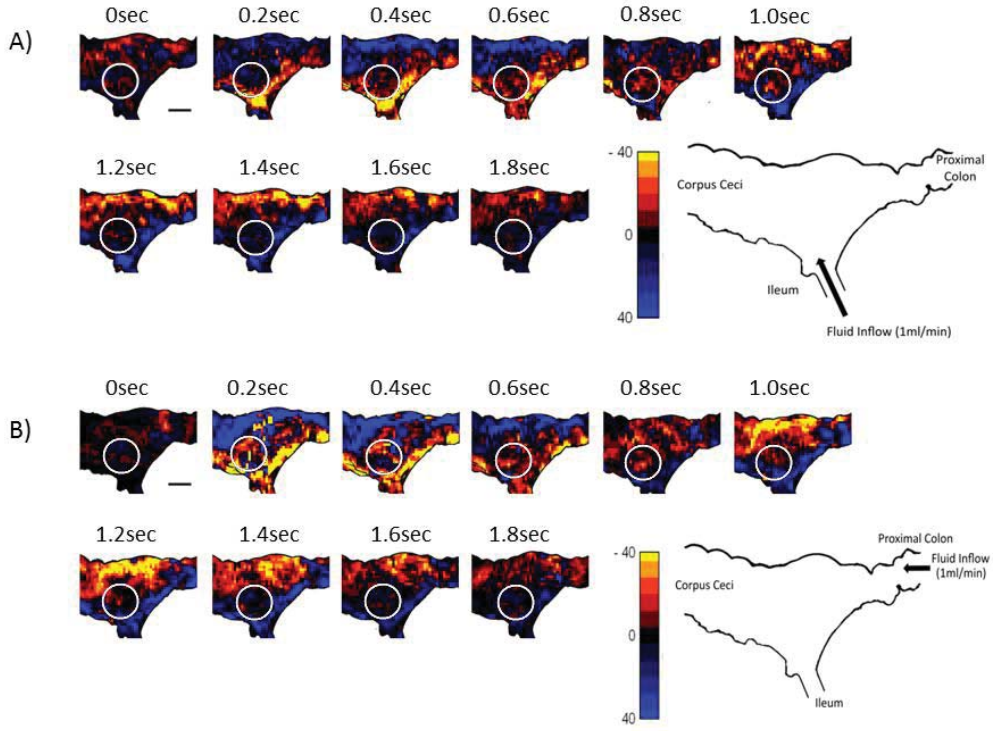
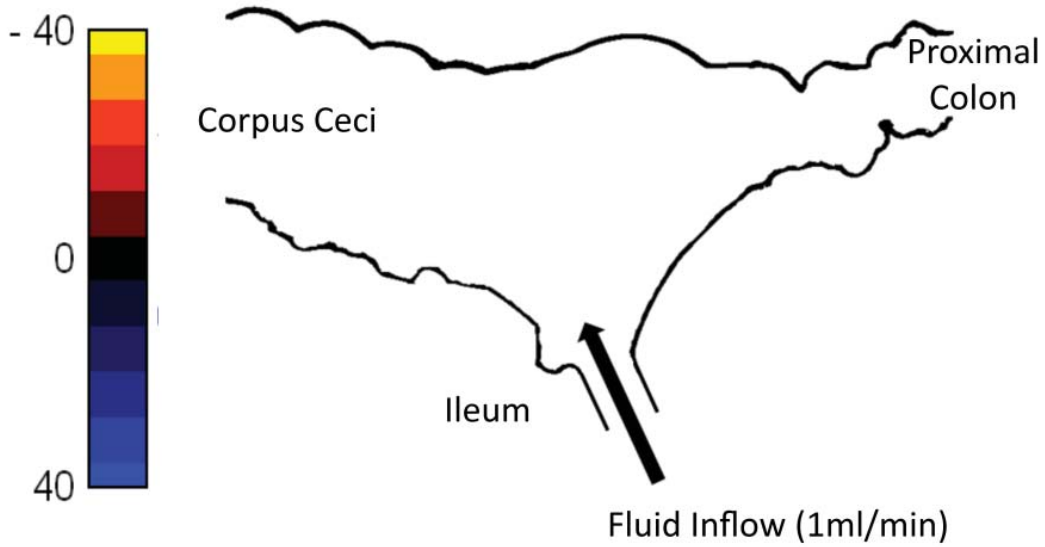


Fig. 4-6. Sequences of images of rate of change of area during normal contractile activity in the caecal base and associated structures with perfusion via the ileum (A) or the proximal colon (B).

Each frame is 0.2 seconds with sequence running left to right, top to bottom. The location of the sacculus rotundus is indicated by a white circle. The scale bar in the first frame of each sequence represents 10 mm. Area strain rate key has units of %/s.

The occurrence of distally propagating mass peristaltic events and subsequent retrograde mass peristaltic events had little effect on the initial part of the normal sequence of contractile events at the base of the caecum. Hence, contraction of the sacculus rotundus was followed by contraction of the musculature on the ventral right edge regardless of the arrival and departure of mass peristaltic events (Fig. 4-7.). However, during mass peristalsis the subsequent contraction on the dorsal side of the caecal base was accentuated and propagated only briefly into the colon. When a mass peristaltic contraction originated in the caecal base, contraction in the dorsum of the caecal base was more widespread than in the normal sequence and the subsequent propagation of this accentuated contraction travelled toward the caecal appendix. Often a retrograde peristalsis (See Fig. 4-7. RGP1) followed soon after initial mass peristalsis (Fig. 4-7. MP1) and was accompanied by dilatation of the ampulla coli (e.g. frame 11sec in Fig. 4-7. see arrow). Hence, isolated mass peristaltic events did not occur often, either propagating from base to tip (MP 1, 2 etc.) or from tip to base (RGP 1, 2). Rather, a sequence of both contributed to turbulent mixing of the contained digesta and its propulsion into the proximal colon.



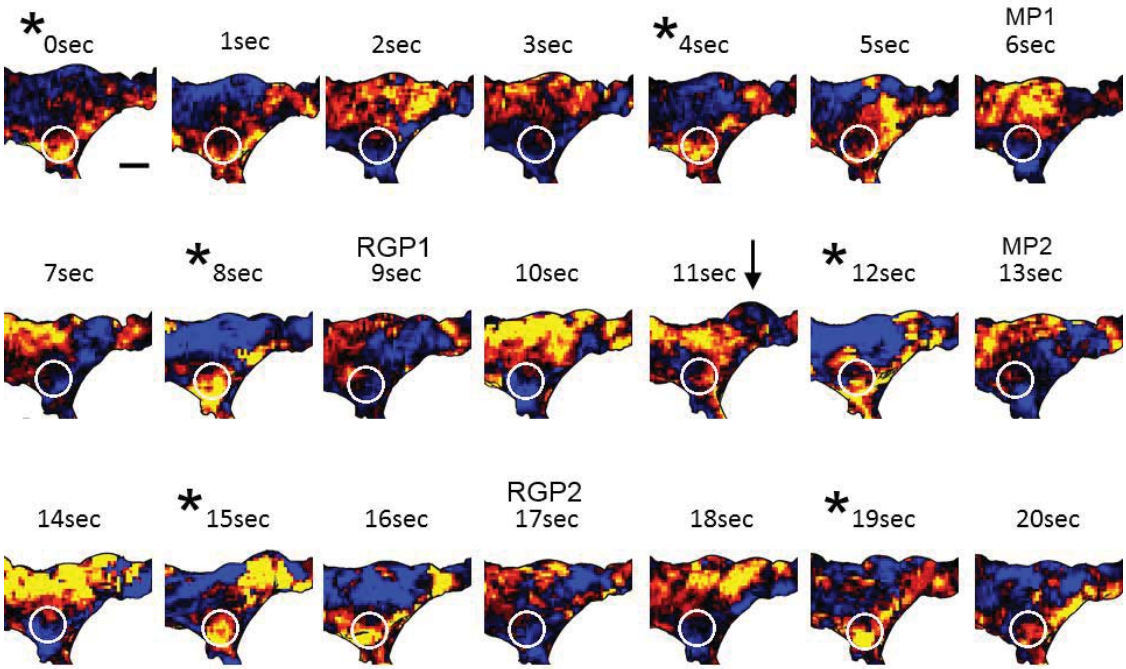


Fig. 4-7. Sequence of images of rate of change of area in the caecal base and associated structures during repeated episodes of mass peristalses with perfusion via the ileum.

Each frame is 1 second. The topographical location of the sacculus rotundus is indicated by a white circle. The initial mass peristaltic event (MP1) begins in the body of the caecum and travels proximally toward the vermiform appendix. A subsequent retrograde peristalsis (RGP1) travels in the opposite direction causing distension of the ampulla caecalis (arrow on frame 11sec). A second mass peristaltic event (MP2) again originates in the body of the caecum and travels from base to tip. A second retrograde peristalisis (RGP2) travelling tip to base enters the ICJ in frame 17sec. The scale bar in frame 0sec represents 10mm. *Asterisks* (*) denote the repeated and regular contraction in the sacculus rotundus during a mass peristaltic event. Area strain rate key has units of %/s.

Correlation between electrophysiological and spatiotemporal contractile events.

Comparison of the timing of contractions on transects of ST maps of longitudinal strain (Fig. 4-8A.) with electrophysiological events (Fig. 4-8B.) during ileal perfusion showed that whilst there was synchrony of integrated spike burst activity in the ileum and sacculus rotundus, ileal contractions on transects of ST maps were 90^0 out of phase with those of the sacculus. However, it was less easy to determine whether the integrated spike burst activity of the colon was synchronised with contractile activity in transects from ST maps of the colon.

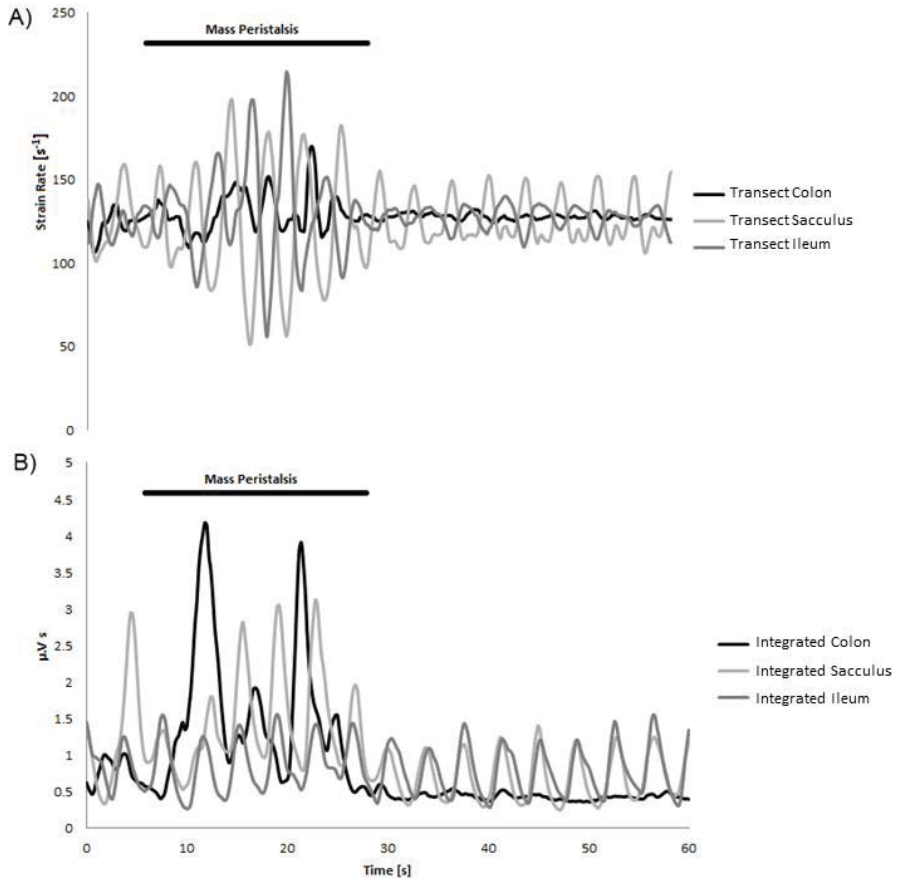


Fig. 4-8. Temporal profiles of transects from ST maps of the ileum, sacculus rotundus and colon (A), and the corresponding integrated spike burst activity (B) during and after a mass peristaltic event.

The duration of the (single) mass peristaltic event is shown as a dark line. The amplitudes of longitudinal contractions are increased at all sites during the event (note the peak for the sacculus rotundus is 90^0 out of phase to the ileum). The amplitude of the integrated spike burst activity at the three sites are similarly increased during the event but synchronous activity is restored during the subsequent resting phase.

Mass peristalsis augmented both integrated spike burst activity and contractile activity on ST transects (Fig. 4-8A. and 4-8B.) in the ileum, sacculus and colon but the peaks in ileal and saccular activity were not synchronised with those in the colon. Hence, the peaks in colonic strain rate in transects of ST maps taken during normal contractile activity were lower in frequency than those in the ileum and sacculus.

4.2.5 Discussion.

The results of this study indicate that the basal rhythm of contraction in the ileum is not affected by contractile events in the caecum. Further, the basal patterns of contraction of the walls of the caecum are similarly unaffected by contractions in the wall of the colon and small intestine. The basic sequence of contraction of the basal surface of the caecum adjacent to the sacculus rotundus and subsequent contraction of the dorsal portion adjacent to the ampulla caecalis also continues during and after proximally and distally progressing mass peristalses. However, our observations show that the amplitudes of these contractions and those of the ampulla coli increase. Further, the direction of haustral propagation changes momentarily to progress proximally and the electrophysiological interval between the successive spike bursts that signify haustral contractions also increases. Hence it appears that the proximal colon and ampulla coli is sensitive to caecal luminal overpressure or to mass peristalsis and institutes changes in amplitude and direction of propagation of contractions.

The manner in which contractions in the basal walls of the caecum are coordinated with contractions in the ileum and sacculus rotundus is not straightforward. Hence, the electrophysiological tracings indicate that slow waves propagate as far as the ampulla coli (Fig. 2A and 2B) but they do not appear to be coincident with the contraction of the sacculus rotundus. Rather, the 90^{0} phase lag between the contraction of the sacculus and the contraction of the ileum in spite of the synchronicity of slow waves in the two components suggests that contraction of the latter results from distension by chyme displaced from the terminal ileum. The lag in activation raises the question as to whether contraction in the upper caecal wall results from electrophysiological consequences of this event or again from hydrostatic effects, i.e. distension.

The linear progression of contraction along the lower basal wall of the caecum prior to the contraction of the sacculus rotundus and subsequent spread along the upper basal wall during and after its contraction rather than a steady outward radial progression from around the sacculus rotundus suggest that propagation in this direction is facilitated by some means. Given that slow waves are conducted across the base of the caecum as far as the ampulla coli they could act to propagate caecal wall contraction in spite of the delay in ampullary contraction.

Conversely, it is possible that mechanoreceptors in the mucosa bordering the sacculus and ileocaecal orifice and the associated folds (Snipes 1978) could sense shear during ampullary distension from inward flow of digesta to mechanoreceptors in the adjacent or opposite wall of the base of the caecum.

In the absence of mass peristalsis, it is apparent that incoming material from the ileum could be pumped toward the ampulla coli and subsequently distally along the spiral portion of the caecum toward the vermiform appendix. Similarly the pattern of basal contraction will cause any material that is retro-pulsed from the colon to the caecum to move distally along the body of the caecum toward the appendix. However, in spite of the evident dyssynergy between contractions in the colon and ampulla caecalis with those in the ileum which has also been reported in pigs (Hipper and Ehrlein 2001), it is likely that a significant fraction of digesta will be propelled into the colon when the distal portion is distended with digesta and that this percentage will be increased during mass peristalsis as was discussed hitherto.

In summary, the findings of this work indicate that control of volume discrepancies in the flow of digesta through the caecal base and its adjoining structures results from alteration of the normal inherent rhythm of the caecum, subsequently overriding the inherent rhythms of the colon, but not the small intestine, to augment colonic outflow, a behaviour that constitutes an hierarchical adjustment between three normally independently functioning structures. Further, the findings suggest that the action of the sacculus rotundus may result from its distension with chyme from the action of ileal peristalsis and that the subsequent propagation of contraction along the basal wall of the caecum toward the colon may be augmented by local distension.

4.2.6 Journal Article References

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4.3 Additional details on the measurement and interpretation of electrophysiological recordings and 2D spatiotemporal maps in the previous chapter

The material in this section was not provided in the submitted paper. Information presented here expands on the method for collection and interpretation of electrophysiological recordings, as well 2D spatiotemporal mapping techniques.

4.3.1 Electrophysiological recording of Muscle Contractions

Electromyography (EMG) is the study of muscle electrical signals. Muscle tissue conducts electrical potentials similar to the way nerves do and the name given to these electrical signals is muscle action potential. The development of EMG began with Francesco Redi in 1666 which his description of highly specialised muscle of the electric ray fish which generated electricity (Basmajian and de Luca 1985). It wasn't until 1890 that the first recording of electrical activity of a voluntary muscle contraction was made by Marey, who also introduced the term *electromyography* (Cram et al. 1998). The capability of detecting electromyographic signals improved steadily from the 1930's through the 1950's and researchers began using improved electrodes for the study of muscles (Shahid 2004). It was not until the middle 1980's that integration techniques in electrodes had sufficiently advanced to allow production of the required small and lightweight instrumentation and amplifiers (Reaz et al. 2006). Today EMG is used in many types of research and has both clinical and biomedical applications.

Modern electrophysiological techniques involve the mapping of the transit of electrical potentials across the surface of the gut with patterns containing multiple electrodes. In the work conducted on the base of the caecum I was endeavouring to trace the transit of an electrical potential across the surface of the component structures (small intestine, caecum, and colon) and hence the use of such arrays was not practicable. Hence, myoelectric activity was recorded with single wire electrodes. In *ex vivo* studies, single or multi-strand insulated flexible wire electrodes are usually employed. The electrode is inserted into the serosa of

the muscle with the aid of a small gauge needle. The needle may be passed through the seromuscular layer with the flexible wire passed through the lumen of the needle, and the needle is pulled back along with the wire. The wire is secured in place with sutures. Two wires, 5-10mm apart, are implanted this way for bipolar recordings. The electrode wire has a bare length of 1-3mm near its end. The wire is positioned so that the bare portion is embedded in the seromuscular layer as well as a 1-2mm length of insulation.

The EMG signal is picked up at the electrode and amplified. An unfiltered and unprocessed signal detecting the superposed MUAPs is called a *raw* EMG Signal. Typically, an EMG signal that has not been amplified has charges between a few microvolts and 2-3 millivolts. There are many dependent factors that can affect the recording of an EMG since the signal is susceptible to noise interference such as hum, signal acquisition such as clipping and baseline drift, movement artefacts, processing errors, and interpretation problems. For example, the contact of electrode to the tissue could distort a recording signal. Because of this, electronic filtering with a computer is essential to precisely and objectively measure the parameters of the electric signal.

The frequency of electrical control activity in the gastrointestinal tract of humans and most mammalian species studied thus far lies in the range of 2-40 cycles/min (Sarna 1989). The frequency response potentials within a burst of electrical response activity lie in the range of 0.9-10 Hz (Sarna et al. 1980; Sarna et al. 1981). This separation of frequency bands allows the use of electronic filters to separate these signals. As previously described, raw myoelectric data from the rabbit ileum, sacculus rotundus and colon were filtered with a band-pass digital filter set between 0.2 and 40 Hz. Band pass filtering gives a clearer electrical response activity signal without significantly attenuating its amplitude. Raw data was filtered again in the manner described by Sarna (1986) in two frequency ranges to elucidate the different electrical activities. A low-pass filter (0-0.3 Hz) was used to separate out slow waves; and a band-pass filter (5-10 Hz) to separate out short spike bursts. Low and high pass filters are used because noise can be broadly classified as low frequency drift and high frequency interference.

Recorded raw EMG offers valuable information but in a particularly useless form. The information is only useful if it can be quantified. Advances in technologies of signal

processing and mathematical models have made it practical to develop advanced EMG detection and analysis techniques. Various mathematical techniques include wavelet transform, time-frequency approaches, Fourier transform, statistical measures, and higher-order statistics. The information derived from EMG after mathematical analysis includes standard amplitude parameters, such as mean, peak, minimum value, area and slope and Fourier Transforms can be used to analyse and estimate the frequency contents of EMG.

In summary, there is depolarization of electrical activity underlying every phasic contraction in gastrointestinal smooth muscle. With the use of EMG recording it is possible observe the control mechanisms that act in concert to control the spatial and temporal patterns of contractions, both of which are important in the control of gastrointestinal motility.

4.3.2 2D Spatiotemporal Mapping

The cross-correlation technique of spatiotemporal mapping derives the displacement of reference points in two dimensions but only one of these is used in the L map technique described in Chapter 3. However, a two dimensional strain map is more useful for tracking contractile movement over the surface of large organs. In this variation, the movements of points equally spaced along a user-specified line of interest (LOI) are determined by considering a 21 x 21 pixel square surrounding each point. In the generalised case, the components of movement in the direction of the LOI are used to generate the array of velocities.

In this method, the local movement of the distinctive visual textural features between successive frames was used to quantify the area displacements i.e. changes in surface area between reference points on a grid of equally spaced points within a rectangular region of interest (ROI). The area strain rate (ASR) for each reference point was determined from the local displacement rates. ASRs were expressed as the percentage change in muscle area per unit time, e.g. %/s. Local ASRs were superimposed on the video images of the ileocaecal junction to enable the patterns of motility on its surface to be visualised. The area of superimposition was limited by a user-specified ellipsoidal mask, which excluded sites that were close to the edge of the organ profile where artefacts from rotation of the organ could occur, in this case an area 5mm from the edges of the caecum. The ASRs were color-coded

such that rapidly contracting areas appeared yellow (-ve ASR), more slowly contracting areas appeared red and expanding areas appeared blue (+ve ASR). A further parameter was derived to quantify the extent of contractile activity within the elliptical mask at a particular time. This was termed the contraction index (CI) and can be viewed as the average area contraction rate of all the grid points within the mask and has units of %/s.

Chapter 5- General Discussion

5.1 Contractile motility of the caecum

In the herbivore, the rate of fermentative digestion is generally slower in comparison to the rate that which they may undergo enzymatic digestion (Van Soest 1994). In a number of mammals, including man, the mean diameter of the small intestine is smaller than that of the colon (Cronin et al. 2010; Silva et al. 2009; Ogata et al. 1996). Where the diameter of hindgut is larger there is also a corresponding longer residence time of nutrient rich elements of digesta where fermentative digestion is taking place (Stevens and Hume 1995). In an animal such as the rabbit, enzymatic and fermentative digestion in successive segments must be integrated. However, where fermentative digestion follows enzymatic digestion in successive tubular components, a generalised contractile physiology and velocity of propulsion would push digesta at a similar rate through both components. Also, it can be assumed that if peristaltic contractions were to progress from the former to the latter structure, the relative intraluminal volume displacement would differ in proportion to the radius of each component. This would be disadvantageous as digesta traveling through the higher bored component is needed to be slowed to allow the absorption of nutrients through the gastrointestinal mucosa. Again, a reduction in the rate at which digesta moves through the two adjoining segments may cause congestion at the junction of the two components and necessitate the development of an ancillary structure that can temporarily accommodate excess flow from the enzymatic to the fermentative compartment. The rabbit processes a large and structurally complex caecum (Snipes 1978) which can accommodate on flow from the small intestine as well as backflow of fine nutrient rich particles from the proximal colon so their residence time can be extended for ongoing microbial fermentation (Björnhag 1981, 1987). Indeed it seems reasonable that the caecum originates at the junctions of tubular structures of unequal capacity and arise as a means of accommodating volumetric discrepancies in the flow of digesta into and out of the structure that result from incoordination of pumping in the large and small intestine.

Thus remains then to determine the manner and extent to which the functions of the three components, the distal small intestine, the caecum, and proximal colon, can act to correct volumetric discrepancy as well as allowing orderly on flow.

High definition radial, strain rate and intensity spatiotemporal mapping, which was also supplemented by electrophysiological recordings, was used to quantify contractile movements of the body and associated structures of the rabbit caecum.

The findings of this work indicate that control of volume discrepancies in the flow of digesta through the caecal base and its adjoining structures results from alteration of the normal inherent rhythm of the caecum, subsequently overriding the inherent rhythms of the colon, but not the small intestine, to augment colonic outflow, a behaviour that constitutes an hierarchical adjustment between three normally independently functioning structures. It was concluded that the functions of the three components, the distal small intestine, the caecum and the proximal colon, can act to correct volumetric discrepancy as well as allowing orderly on flow. Hence, these structures appear autonomous and can function without higher levels of control. Further, the findings suggest that the action of the sacculus rotundus may result from its distension with chyme from the action of ileal peristalsis and that the subsequent propagation of contraction along the basal wall of the caecum toward the colon may be augmented by local distension. This may allow digesta to transit from the terminal ileum, across the base of the caecum, and into the proximal colon. The proximal colon and ampulla caecalis can pump fine particulate digesta into the caecum without disturbing motility at the base of the caecum, and the corpus ceci can accommodate this material and mix it within its lumen.

5.2 Possible further work into the motility of the ileocaecal junction

Novel spatiotemporal techniques and electrophysiological recordings allowed us to quantitatively evaluate the characteristics of caecum contractions (tonic and phasic contractions), and to characterise subtle patterns of contraction and dilation associated with displacement and accommodation of digesta in a specific way not yet reported in the literature. Future work may seek to further develop more sophisticated models that better approximate the intestinal environment. Below is a list of some of the modifications and features that could be incorporated into future experiments. Such experiments would provide a useful tool for the food and pharmaceutical industry and offer some insight into

the assessment of motility disorders such as idiopathic ileus, diverticulitis, and stasis in the human caecum.

1. It would be of interest to use a non-newtonian fluid (e.g. Guar Gum) instead of water as an infusion agent. Lentle (et al 2007) showed that watery and viscous perfusates altered longitudinal and radial movements during spontaneous contractions of isolated segments of terminal ileum of the brushtail possum. Perfusate type has also been shown to modify contractile motility in the proximal rabbit colon (Lentle and Janssen 2008). Whether this perfusate driven change in contractile motility seen in the distal ileum and proximal colon and how it affects the inherent rhythm of the ileocaecal junction and caecum is unknown.
2. It would be of interest to examine how the size of digesta particles affects contractile motility at the ileocaecal junction. While the transit of food particles larger than 1mm from the stomach to the small intestine is restricted (Meyer et al 1981), this restriction is relative and there are ways in which larger particles may enter the small intestine (Itoh et al 1986). Consequently, while particular physiological mechanisms retain and concentrate certain size ranges in particular segments of intestine, the size distribution of digesta particles generally remains varied and dispersed (Lentle and Janssen 2011). Given the propensity of the proximal colon of the rabbit to act as a selective sorting mechanism, and the caecum itself to act as a reservoir, it would be of interest to see how contractile motility and function is affected by an infusion of monodisperse suspension compared to a highly polydisperse suspension.
3. It would be of interest to see how the ICC slow wave travels the rabbit ileocaecal junction. The sphincter formed in the ileocaecal junction divides two sections of gastrointestinal tract. Electrophysiologically, the terminal ileum is characterized by slow waves with a higher frequency than that of the proximal colon (Grasa et al. 2004; Lentle et al. 2008), and on which spike potentials are sometimes superimposed. In spite of the fact that the electrical activity of these two sections is different, there probably exists correlation in their motor activity, which guarantees rhythmic movement of digesta through the ileocaecal junction in a direction from ileum to colon. In the rabbit, a great variety of potentials existed in the ileocaecal region. The appearance of spike activity of the ileum and the proximal colon was

observed at certain time intervals. The appearance of spike activity of the ileum may lead to the activation of the proximal colon. In other cases colon activation was entirely independent. There may be also occur cases when both in the ileum and colon the electrical activity is characterised by spike potentials. However, it is difficult to determine the starting point of the spike activity. Further work would be required to identify if there is a pacemaker centre and also characterise, if any, slow wave and spike potentials in the corpus ceci.

4. It would be of interest to examine the effect of pharmacological agents on the motility of the *ex vivo* rabbit caecum. In experiments on unanaesthetized rabbits, myoelectric activity of distal ileum, caecum, and proximal colon has shown that nonselective blockade of pre- and postsynaptic alpha-adrenoceptor cause's excitation suggesting alpha-adrenergic innervation of the ileocaecal junction. Evidence also exists for an excitatory, vagal (cholinergic) innervation (Berezina and Ovsiannikov 2005). The spatiotemporal mapping analysis of pharmaceutical manipulation of the motility of the ileocaecal junction has not been reported.

5.3 Overall context of the work

Overall, it should be noted that the factors that have been identified in this thesis, which could promote co-ordinated motility, transfer of digesta, and mixing in the ileocaecal region of the hindgut, have only been assessed in the ileocaecal junction of the rabbit. The information presented in this thesis is the first to spatiotemporally describe the concerted action of structures around the caecal base, and the first to describe unique contractile activity in the body of the rabbit caecum. This work indicates that the caecum could be more adapted for accommodating any excess inflow from an enzymatic to a fermentative compartment and for the processes of ongoing microbial fermentation. Also, components of the ileocaecal junction can correct volumetric discrepancy as well as allowing orderly on flow. The work also shows that diverticula that occur at the junction of two segments of different diameter can become adapted so that there is no need for hierarchical control i.e. an autonomous structure is found.

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