2007

Carprofen compromises the integrity and barrier function of the colonic mucosa of the dog

Catherine Alix Briere
Louisiana State University and Agricultural and Mechanical College, cbriere@vetmed.lsu.edu

CARPROFEN COMPROMISES THE INTEGRITY AND BARRIER FUNCTION OF THE COLONIC MUCOSA OF THE DOG

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The School of Veterinary Medicine
Through
The Department of Veterinary Clinical Sciences

by
Catherine Briere
DVM, University of Montreal, 2002
May 2007

DEDICATION

To Christiane and Daniel for believing in me

ACKNOWLEDGEMENTS

I had the fortune of working on this project with Dr. Giselle Hosgood whose help and contribution were immense. For guiding me every step of the way, for reading and editing draft after draft and above all, for teaching me the respect for sound research, thank you.

To Dr. Cheryl S. Hedlund and Dr. Rebecca S. McConnico my other committee members thank you for your support and commitment to this project.

I would also like to thank Dr. Tim Morgan and Dr. Kevin M. Kleinow for their time and expertise and and Merrin Hicks, Catherine Koch and her assistants for their technical assistance. Thank you to the Charles E. Blass Foundation Fund for financial support.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Effects of carprofen on colon of dog have not been investigated.

Objectives

1) Measure conductance and permeability to mannitol of transverse, proximal descending and distal descending colonic mucosa of dog.

2) Measure conductance and permeability to mannitol of colonic mucosa of dog in presence of carprofen.

Design

In vitro experimental – nested, randomized block design

Animals

Colonic mucosa from 6 (objective 1) and 7 (objective 2) mature mixed-breed dogs.

Methods

Objective 1) Control - Three sections of mucosa from each region of colon were mounted in Ussing chamber units. Conductance was calculated every 15 min for 240 min. Flux of mannitol was calculated for three periods of one hour.

Objective 2) Carprofen - Methods based on results for objective 1. Sections of mucosa were prepared as in objective 1. Carprofen (400µg/ml) was added to bathing solution. Data for conductance and flux of mannitol was obtained as in objective 1.

Histologic examination of all sections was performed after experiment.

For both objectives, conductance was graphed against time for each chamber and area under each curve calculated. Conductance*time, flux of mannitol and frequency distribution of histologic categories were used for analysis.

Results

Objective 1) Mean +/- SEM conductance*time transverse colonic mucosa was higher than proximal and distal descending. Mean +/- SEM flux of mannitol increased from period 1 to period 3 for transverse colonic mucosa. Objective 2) Data from objective 1 was used as control for objective 2. Mean +/- SEM conductance*time carprofen group was higher than control group for all regions of colon. For carprofen group, mean +/- SEM flux of mannitol increased from period 1 to period 2 and from period 2 to period 3 for all regions of colon. There was higher proportion of sections with severe sloughing of cells and erosions involving more than 10% of epithelium in carprofen group compared to control.

Conclusion

Carprofen increases in vitro conductance and permeability to mannitol and causes sloughing of cells and erosions of colonic mucosa of dog which suggests compromise of integrity and loss of barrier function.

CHAPTER 1. BACKGROUND AND REVIEW OF LITERATURE

1.1 Colon

1.1.1 Macroscopic Anatomy

The colon of dog accounts for almost the entire length of the large intestine. It is bordered proximally by the ileocolic orifice and sphincter and distally by the rectum. The colon lies in the dorsal abdomen where it forms an arc around the root of the mesentery. The colon is suspended by the mesocolon which maintains the colon's position while allowing some mobility (Evans 1993).

The colon is divided into three anatomical regions. The ascending colon is the most proximal region. The ascending colon lies to the right of the root of the mesentery. It is the shortest region and begins at the ileocolic sphincter, extends cranially and terminates at the right colic flexure. The right colic flexure marks the junction between the ascending and transverse colon. The transverse colon courses from right to left, cranial to the root of the mesentery. The transverse colon terminates at the left colic flexure, which marks the junction between the transverse and descending colon. The descending colon, the longest and most distal of the three regions, lies to the left of the root of the mesentery. The descending colon courses caudally and terminates at the pelvic inlet (Evans 1993).

The arterial blood supply to the proximal two thirds of the colon is via branches of the cranial mesenteric artery: the colic branch of the ileocolic artery supplies the ascending colon; the right colic artery runs in the mesocolon to the right colic flexure and supplies the distal ascending and proximal transverse colon; the middle colic artery runs in the mesocolon to the left colic flexure and supplies the distal transverse and proximal descending colon. The left colic branch of the caudal mesenteric artery supplies the distal descending colon. The caudal

mesenteric vein carries blood from the colon into the portal vein. Autonomic innervation to the colon is by the cranial and caudal mesenteric plexuses (Guilford, Center et al. 1996),(Evans and deLaHunta 1996).

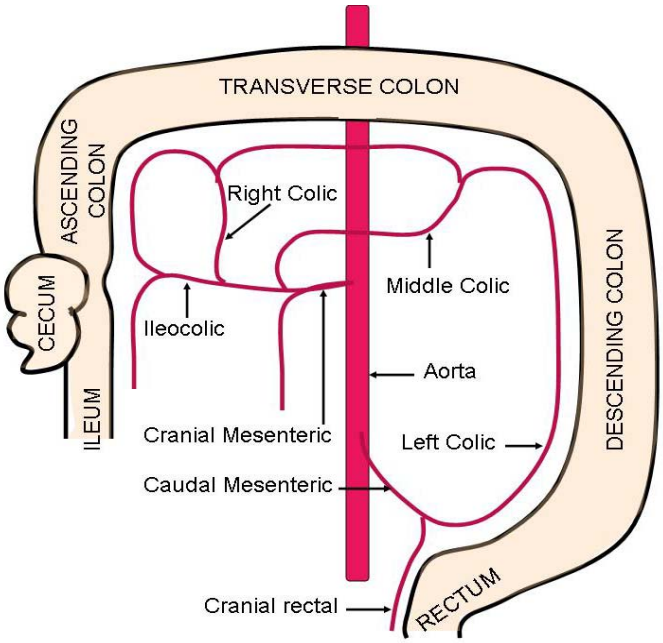

Figure 1.1 Arteries of the Colon of the Dog

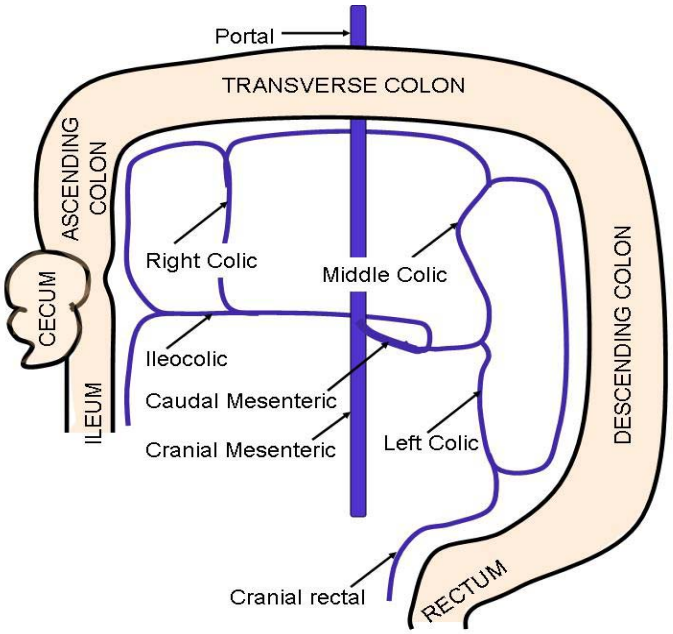

Figure 1.2 Veins of the Colon of the Dog

1.1.2 Microscopic Anatomy

The wall of the colon of the dog is composed of four distinct layers: the mucosa, the submucosa, the muscularis and the serosa. The mucosa lines the lumen of the colon. It is flat without villi and consists of a single layer of cells, mostly columnar epithelial cells with interspersed goblet cells that secrete mucus. The epithelium of the mucosa is folded inwards on itself to form strait tubular mucosal glands that extend away from the lumen towards the muscularis mucosae. The muscularis mucosae is a thin layer of smooth muscle located at the base of the mucosa, adjacent to the submucosa. A loose connective tissue called the lamina propria occupies the space between the epithelium and the muscularis mucosae. The organization of the mucosal glands in the mucosa is very dense and leaves little place for the lamina propria. Epithelial cells found at the surface of the mucosa (surface epithelial cells) are distinct from those found within the mucosal glands. It has been suggested that surface epithelial cells are primarily for absorption of water and electrolytes whereas epithelial cells within the mucosal glands are primarily for secretion of water and electrolytes. More recent studies have shown that in epithelial cells within the mucosal glands, absorption is constitutive and secretion is regulated by neurohumoral agonists, such as cyclic adenosine monophosphate and carbachol, released from cells of the lamina propria (Heitzmann, Warth et al. 2000),(Mall, Bleich et al. 1998).

The submucosa is a layer of dense fibroelastic connective tissue. The submucosa contains blood vessels, lymphatics and nervous fiber plexuses. The thick muscularis consists of smooth muscle arranged in an inner circular and an outer longitudinal layer. The serosa consists of a layer of loose connective tissue covered by a single layer of mesothelial cells, the visceral peritoneum. This is the outer layer of the colon (Fawcett 1994; Guilford, Center et al. 1996; Gartner and Hiatt 1997).

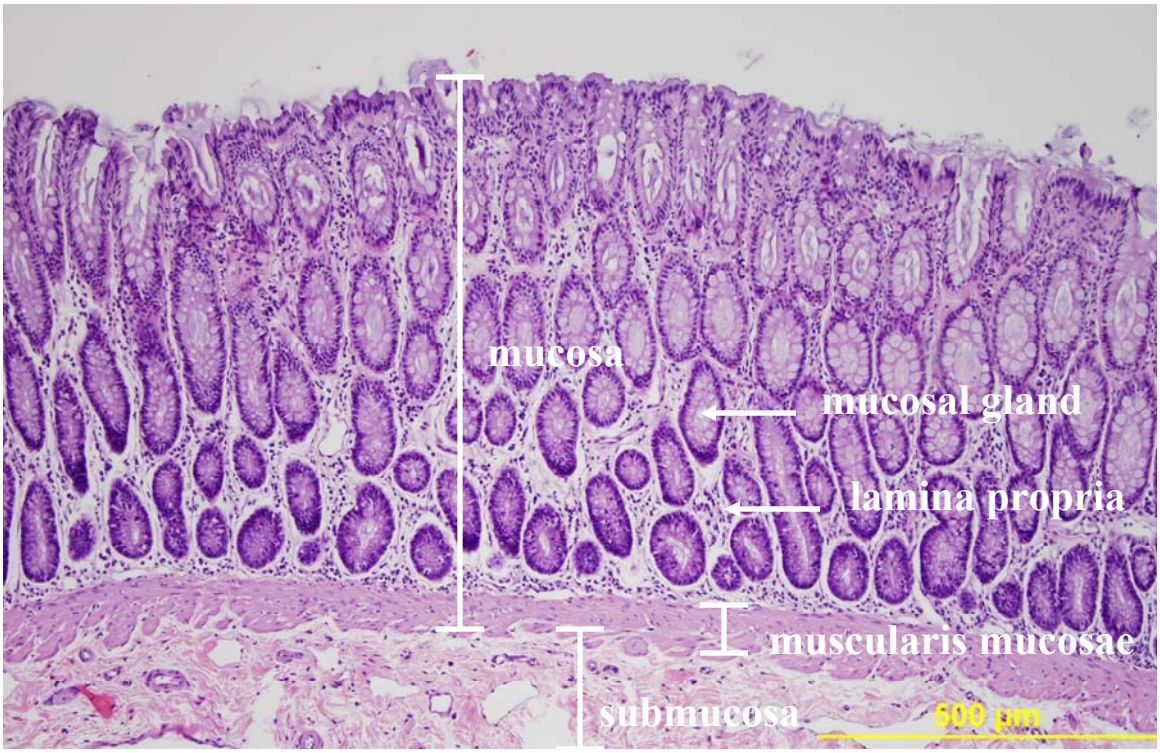

Figure 1.3 Microscopic Anatomy of the Colon of the Dog

1.1.3 Epithelium of the Mucosa

The colon serves as a conduit and reservoir for gastrointestinal contents and is an important site of absorption of fluid and electrolytes. The epithelium of the mucosa functions as a selectively permeable barrier between the luminal environment and the interstitium allowing simultaneous segregation and exchange (Guilford, Center et al. 1996). Cells of the epithelium of the mucosa are tightly held into a sheet-like ultra structure by intercellular junctions. Anchoring junctions mechanically attach cells to one another, to the basement membrane and to the extracellular matrix while intercellular tight junctions seal adjacent epithelial cells together. Intercellular tight junctions consist of trans-membrane proteins that form a belt around the apical extremity of every cell in the epithelium (Alberts, Bray et al. 1994.; Amasheh, Schmidt et al. 2005). The presence of intercellular tight junctions allows for segregation of plasmic membrane proteins and thus, the formation of two distinct plasmic membrane domains. The first domain is

the apical membrane which is in contact with the luminal environment. The second domain is the baso-lateral membrane which is in contact with the interstitial space. The polarity of these membranes is very important for absorption of molecules against a gradient of concentration (Alberts, Bray et al. 1994).

Transport across the epithelium of the colonic mucosa can occur via transcellular or paracellular transport. Transcellular transport is a directional flux of molecules through the epithelial cells and relies on polarity of the epithelium. Paracellular transport is a passive flux of small molecules through the intercellular tight junctions along a gradient of concentration (Alberts, Bray et al. 1994.; Guilford, Center et al. 1996; Amasheh, Schmidt et al. 2005).

With transcellular transport, proteins of the apical membrane actively transport selected molecules from the lumen into the cell against a gradient of concentration. These molecules then passively diffuse along a gradient of concentration from the intracellular space to the interstitial space. Occlusion of the paracellular space by intercellular tight junctions prevents diffusion of molecules back into the lumen along a gradient of concentration. From the interstitial space, molecules are absorbed into blood vessels (Alberts, Bray et al. 1994).

While transcellular transport depends on the impermeability of intercellular tight junctions, paracellular transport depends on their permeability. The seal formed by intercellular tight junctions is relative and despite their name, intercellular tight junctions are in fact the most permeable element of the epithelium of the mucosa. This allows passive movement of small molecules across the epithelium of the mucosa along a gradient of concentration. The impermeability of intercellular tight junctions to molecules varies considerably from one epithelium to another (Alberts, Bray et al. 1994.; Guilford, Center et al. 1996; Amasheh, Schmidt et al. 2005).

The polarity of the membrane and the transport of molecules with an electrical charge across the epithelium of the colonic mucosa results in electrophysiologic parameters that can be quantified including transepithelial voltage, short-circuit current, resistance and conductance (Hongyu, Sheppard et al. 2004).

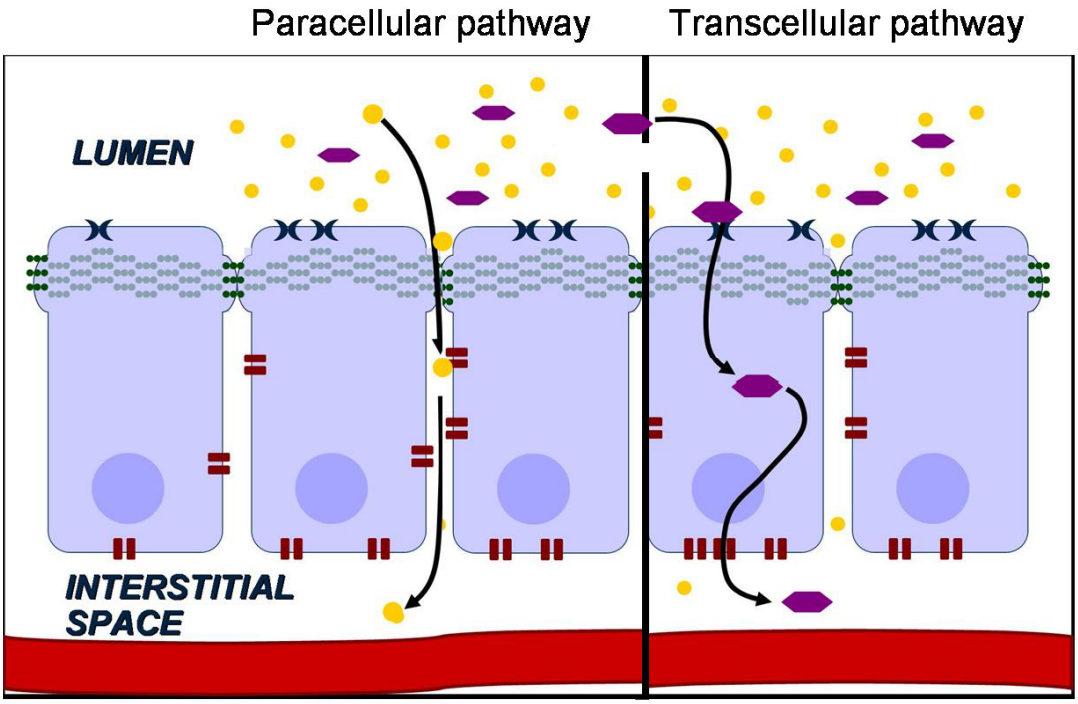

Figure 1.4 Transport Across the Epithelium of the Colonic Mucosa

1.1.4 Trans-epithelial Ion Transport

The main ions transported across the epithelium of the colonic mucosa of mammals are sodium ions (Na), chloride ions (Cl), potassium ions (K), bicarbonate (HCO_3) and short chain fatty acids (Binder, Rajendran et al. 2005). Ion transport systems across the epithelium of the colonic mucosa are complex and our knowledge of those transport systems is expanding as new transporter molecules, modulator and inhibitor molecules, pathways and interactions are discovered. Transport systems for sodium ions, chloride ions, potassium ions, bicarbonates and short chain fatty acids in the colon of mammals are well described.

The two main transport systems for absorption of sodium ions from the lumen of the colon into the interstitial space are electroneutral sodium-chloride absorption and electrogenic sodium absorption. During electroneutral sodium-chloride absorption, sodium is transported across the apical membrane of the epithelial cell by the transport protein sodium-proton exchange-3 (NHE-3) isoform. During electrogenic sodium absorption, sodium is transported across the apical membrane of the epithelial cell by the amiloride sensitive epithelial sodium channel (ENaC) (Shull, Miller et al. 2000; Gawenis, Hut et al. 2004). These two transport systems for absorption of sodium ions occur in the surface epithelial cells and not in the crypt epithelial cells. A third and less important transport system for absorption of sodium ions occurs in the crypt epithelial cells. Sodium ions are transported across the apical membrane of the crypt epithelial cell by the chloride-dependant sodium-proton exchange transport protein. This chloride-dependant transport system for absorption of sodium ions is present to a lesser extent in the surface epithelial cells. Sodium-potassium adenosine triphosphatase (Na-K ATPases) of the basolateral membrane maintain gradient and electroneutrality necessary for functioning of those three transport systems for absorption of sodium ions. Once into the epithelial cell, sodium ions diffuse passively across the basolateral membrane into the interstitial space along a gradient of concentration (Sellin and DeSoignie 1987; Smith and Benos 1991; Palmer 1992; Guilford, Center et al. 1996; Gawenis, Hut et al. 2004).

Chloride ions are absorbed from the lumen of the colon into the epithelial cells by one of three transport systems of the apical membrane known as chloride-bicarbonate exchange system, chloride-hydroxide exchange system or chloride-short chain fatty acids exchange system. Once into the epithelial cell, chloride ions are transported across the basolateral membrane into the interstitial space by chloride-potassium cotransport (where a membrane protein transports together one chloride ion and one potassium ion) or through chloride channel-2 (ClC-2).

Absorption of chloride ions occurs mostly in surface epithelial cells and to a lesser extent in crypt epithelial cells (Gawenis, Hut et al. 2004).

Chloride ions are secreted from the interstitial space into the lumen of the colon by a secretion system stimulated by cyclic adenosine monophosphate (cAMP). This system requires an apical membrane transport protein, called cystic fibrosis transmembrane conductance regulator (CFTR) and three different basolateral membrane transport proteins including one sodium-potassium adenosine triphosphatase, one sodium-potassium-chloride-chloride (NKCC) transporter and one or more potassium channels (Hass 1994; Heitzmann, Warth et al. 2000; Day, King et al. 2005; Mayol, Alarma-Estrany et al. 2005; Schultheiss, Siefjediers et al. 2005).

Potassium ions are absorbed from the lumen of the colon into the epithelial cell by passive diffusion, along a gradient of concentration or by an active system requiring a proton-potassium adenosine triphosphatase (H, K-ATPase) at the apical membrane and a potassium-chloride cotransport system at the basolateral membrane (Shull, Miller et al. 2000).

Potassium ions are secreted from the interstitial space into the lumen of the colon by a potential dependant potassium secretion system or by an active potassium secretion system. In the colon of normal mammals, the two potassium transport systems sum up to an overall low rate of potassium ion secretion (Rechkemmer, Frizzell et al. 1996; Binder, Rajendran et al. 2005).

Bicarbonate ions are secreted from the interstitial space into the lumen of the colon by a chloride-dependant bicarbonate secretion system, a cyclic adenosine monophosphate-induced bicarbonate secretion system or a short-chain fatty acid-dependant bicarbonate secretion system. Secretion of bicarbonate ions occurs mostly in surface cells but also to a lesser extent in crypt cells (Vidyasagar, Rajendran et al. 2004; Binder, Rajendran et al. 2005).

Short-chain fatty acids are the primary anion in the lumen of the colon. Butyrate, propionate and acetate are the most abundant short-chain fatty acids. These short-chain fatty

acids are synthesized in the lumen of the colon by resident bacteria. Short-chain fatty acids are absorbed from the lumen of the colon into the interstitial space by a transport system requiring an apical membrane short-chain fatty acid-bicarbonate exchange protein, linked in parallel to a sodium-proton exchange protein and a chloride-short-chain fatty acid exchange protein; and a basolateral membrane short-chain fatty acid-bicarbonate exchange protein distinct from the apical one (Sellin 1999).

1.1.5 Ussing Chamber System

The Ussing chamber is a system used to study molecule transport across epithelia. Since its first description in 1951 by Hans Ussing, it has been used in a broad range of applications and has improved our knowledge of permeation and absorption of epithelia. The system consists of a chamber unit connected to an electrical circuit (Hongyu, Sheppard et al. 2004).

The chamber unit is composed of 2 symmetrical halves consisting of a "U" shaped reservoir system connected to an acrylic hemi-chamber via polyethylene tubes. Both hemi-chambers are mounted with their lumen communicating to form one unique chamber. During an experiment, the epithelium to be studied is mounted between the two hemi-chambers. The only communication from one hemi-chamber to the other is through the epithelium. The tissue is bathed in the experimental solution contained in each of the reservoirs. The reservoirs and chambers are designed to minimize hydrostatic pressure on the tissue. The reservoirs are water jacketed for a temperature controlled environment. Gas, usually 95% oxygen / 5% carbon dioxide, is delivered into the reservoirs to oxygenate the experimental solution and also to stir the solution in the reservoir. The specific design of the chamber unit allows measurement of permeability of epithelia to molecules soluble in the bathing solution (Hongyu, Sheppard et al. 2004).

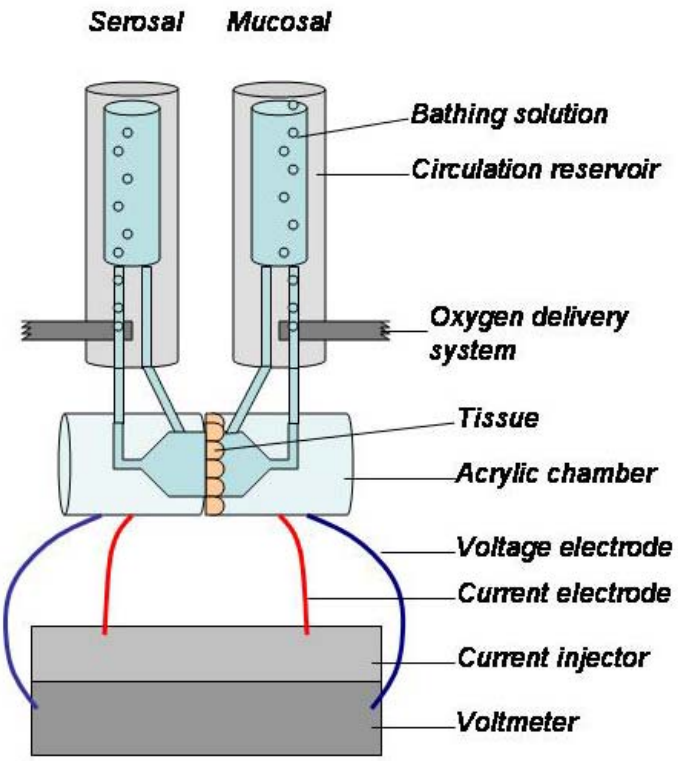

Figure 1.5 Ussing chamber

Each hemi-chamber is connected to an electrical unit via a voltage and a current electrode. The silver chloride electrodes connect to plastic cartridges designed to be filled with a conducting gel, usually agar. The electrodes are connected to a preamplifier. This circuitry allows measurement of transepithelial potential difference. Recordings are performed in current-clamp (open-circuit). Short-circuiting of the tissue, which means bringing the transepithelial potential difference to zero, allows measurement of the short-circuit current, defined as the charge flow per time when the tissue is short circuited. Transport of chloride, sodium and potassium ions accounts for most of the in vitro short-circuit current across the colonic mucosa (Rechkemmer, Frizzell et al. 1996). Electrical conductance is a measure of how easily ions flow through the tissue (Somasundaram, Sigthorsson et al. 2000). Electrical conductance can be calculated from transepithelial potential difference and short-circuit current using Ohm's law (Hongyu, Sheppard et al. 2004).

Ohm's law states that the electrical potential difference or voltage drop between two points of an electrical circuit is equal to the product of the current flowing through it and the electrical resistance of the conductor.

$$V = R \times I$$

where

- V is the potential difference (V, volt)
- I is the current (A, ampere)
- R is the electrical resistance of the conductor (Ω, ohm) (Somasundaram, Sigthorsson et al. 2000)

Using this relation, transepithelial potential difference across and short-circuit current through the section of colonic mucosa can be used to calculate its resistance. Electrical resistance is a measure of the degree to which a conductor opposes the passage of electrical current. In an electrical circuit, electrical resistance does not depend on the amount of current flowing or the voltage applied. Rather, the electrical resistance of the conductor determines the amount of current flowing for any given voltage applied. Electrical conductance is the reciprocal of electrical resistance such that

$$G = 1 / R = I / V$$

Where

- G is the conductance (S, siemens)
- R is the resistance (Ω, ohm)
- V is the potential difference (V, volt)
- I is the current (A, ampere) (Somasundaram, Sigthorsson et al. 2000)

Using this relation, transepithelial potential difference across and short-circuit current through the section of colonic mucosa can be used to calculate its electrical conductance.

1.1.6 Electrophysiology and Permeability in Function of Region of Colon

Differences in electrophysiologic parameters (such as transepithelial potential difference, short-circuit current, electrical resistance and conductance) and permeability of the mucosa exist between anatomical regions of the intestinal tract. Polentarutti and co-workers showed that transepithelial potential difference of the intestinal mucosa of rats is highest in the colon, followed by duodenum, jejunum and ileum. In their study, short circuit current was highest in the duodenum, followed by jejunum, ileum and colon. Electrical resistance was higher in the colon than the small intestine. Their study also showed regional variation in the permeability of the intestinal mucosa. The permeability to propanolol was greatest in the duodenum, followed by jejunum, ileum and colon while the permeability to mannitol was greater in the small intestine than in the colon. In their study the colon was treated as a single anatomical region (Polentarutti, Peterson et al. 1999).

Sellin and DeSoignie studied the transport of sodium and chloride ions in the colonic mucosa of people in vitro. Electrical conductance was lower in the proximal descending colon, compared to the transverse and distal descending colon but there were no other differences in basal electrophysiologic parameters between anatomical regions (Sellin and DeSoignie 1987). Charney and co-workers reported a positive transepithelial potential difference across the epithelium from lumen to interstitial space of similar magnitude in the colonic mucosa of the proximal and distal colon of mice. Short-circuit current in the proximal colon and the distal colon were not compared. Their study showed the electrical conductance of the colonic mucosa to increase from proximal to distal. Transport of sodium ions was similar in the proximal and the distal colon, but absorption of chloride ions occurred at a higher rate in the distal colon than in the proximal colon (Charney, Egnor et al. 2001). Sellin showed that electrical conductance of the colonic mucosa of rabbits increases from proximal to distal (Sellin and DeSoignie 1984).

Differences in electrophysiologic parameters and permeability between anatomical regions of the colonic mucosa of normal dogs have not been investigated.

1.2 Non Steroidal Anti-inflammatory Drugs and Colon

Non steroidal anti-inflammatory drugs (NSAIDs) can adversely affect the colon. In people, one third of life-threatening gastrointestinal complications due to non steroidal anti-inflammatory drugs involve the lower gastrointestinal tract (Lanas, Panes et al. 2003). A wide spectrum of side effects have been reported in people, including isolated colonic ulcers, diffuse colonic ulceration with or without bleeding and perforation, diffuse colitis (Hall, Petty et al. 1983; Pierrugues and Fontes 1994; Kurahara, Matsumoto et al. 2001). and reactivation of inflammatory bowel disease (Hall, Petty et al. 1983; Pierrugues and Fontes 1994; Evans, McMahon et al. 1997; Kurahara, Matsumoto et al. 2001).

1.2.1 Prostaglandin Synthesis

Non steroidal anti-inflammatory drugs exert their anti-inflammatory effects through inhibition of prostanoid synthesis. Non steroidal anti-inflammatory drugs inhibit the action of fatty-acid cyclooxygenase (COX) enzymes. Fatty-acid cyclooxygenase enzymes catalyse the synthesis of prostaglandins and thromboxanes from arachidonic acid. Arachidonic acid is also the substrate for the synthesis of leucotrienes via lipooxygenase (LOX) enzymes. At least two isoforms of fatty-acid cyclooxygenase enzymes have been identified. Cyclooxygenase-1 enzymes are present in most cells and are responsible for the synthesis of the majority of prostaglandins involved in normal homeostasis of the gastrointestinal tract. Cyclooxygenase-2 enzymes are present in inflammatory cells during inflammatory states. Cytokines, bacterial products, laparotomy and exposure to serotonin up-regulate the expression of cyclooxygenase-2 enzyme (Josephs, Cheng et al. 1999; Engelmann, Bindslev et al. 2002). In dogs, the messenger ribonucleic acid (mRNA) for cyclooxygenase-2 but not the enzyme itself, is found in all regions

of the gastrointestinal tract, spleen, liver, brain, kidneys, lungs and ovaries (Wilson, Chandrasekharan et al. 2004). Cyclooxygenase enzymes catalyse two reactions that transform arachidonic acid into prostanoids. The first is via an endoperoxidase synthase action that transforms arachidonic acid into cyclic endoperoxide prostaglandin G_2 (a reaction involving oxygenation and cyclisation of arachidonic acid). The second is via a peroxidase action that transforms prostaglandin G_2 to another cyclic endoperoxide, prostaglandin H_2. Further transformation of prostaglandin H_2 produces different metabolites depending on the cell type hosting the reaction. In platelets, prostaglandin H_2 is transformed into thromboxane A_2 (TXA_2). In vascular endothelium, prostaglandin H_2 is transformed into prostacyclin (PGI_2). In macrophages, prostaglandin H_2 is transformed into prostaglandin E_2 (PGE_2). In mast cells, prostaglandin H_2 is transformed into prostaglandin D_2 (Hardman, Limbird et al. 2001).

1.2.2 Prostaglandins and Normal Gastrointestinal Physiology

Prostaglandins are important in the normal physiology of the gastrointestinal tract. Endogenous prostaglandins modulate gastric acid secretion, mucus secretion and bicarbonate secretion in the stomach and duodenum (Reimer, Heim et al. 1992; Soll 1992) and influence blood flow to the enteric mucosa (Gana, MacPherson et al. 1988). In the colon, prostaglandins are mainly produced by the mononuclear cells of the lamina propria of the mucosa (Barrera, Lai et al. 1996; McCarn, Yursik et al. 2002). A study by Barrera and co-workers demonstrates that prostaglandin E_2 has an immuno-modulatory action on the resident lymphocyte T population of the colon during non-inflammatory states (McCarn, Yursik et al. 2002). Prostaglandins are also involved in the secretory physiology of the colonic mucosa and it is shown that prostaglandin E_2 stimulates secretion of chloride ions in several species (Dharmsathaphorn, Mandel et al. 1985; Rechkemmer, Frizzell et al. 1996; Sahi, Wiggins et al. 1996; Ahrens, Gabel et al. 2003; King, Haque et al. 2004). An in vitro study by McCarn and co-workers showed that prostaglandins

increase trans-epithelial electrical resistance in monolayers of human colonic epithelial cells suggesting a positive effect of prostaglandins on the barrier function of the colonic mucosa in people (McCarn, Yursik et al. 2002).

1.2.3 Prostaglandin and Gastrointestinal Inflammation

The inflammatory response is invariably accompanied by the synthesis of prostaglandins and other prostanoids, predominantly prostaglandin E_2 and to a lesser extent prostacyclin (Hardman, Limbird et al. 2001). In acute inflammation, cells from local tissues and blood vessels produce prostaglandin E_2 and prostacyclin. In chronic inflammation, macrophages and other mononuclear cells also produce prostaglandin E_2 and thromboxane A_2 (Hardman, Limbird et al. 2001). Prostaglandin E_2 and prostacyclin and other cytokines mediate the local inflammatory reaction which induces local pain but also modulates central nociception and fever (Hardman, Limbird et al. 2001).

In the gastrointestinal tract, prostaglandins are synthesized during acute and chronic inflammation. During acute inflammation, prostaglandins stimulate secretion of water and electrolytes in the intestine. King and co-workers showed that inhibition of cyclooxygenase enzymes by prostaglandins increases the effect of serotonin, a potent stimulant for the secretion of chloride ions in the rat distal colon (King, Haque et al. 2004). Ahrens and co-workers showed that prostaglandins increases the effect of histamine, a stimulant for the secretion of chloride ions in the pig proximal colon (Ahrens, Gabel et al. 2003). Schmitz and co-workers showed that prostaglandins E_2 increases the effect of tumor necrosis factor-α (TNF-α), a stimulant for the secretion of chloride and potassium ions in the distal colon of people (Schmitz, Fromm et al. 1996). In people, increased prostaglandin synthesis, mainly prostaglandin E_2, occurs in the colonic and rectal mucosa of patients with active inflammatory bowel disease (IBD). Additionally, prostaglandin concentration is increased in stool and venous blood of people with

active inflammatory bowel disease compared to that of healthy people (Rampton and Sladen 1984; Raab, Sundberg et al. 1995). Prostaglandin concentration correlates with disease activity and decreases when disease is controlled (Rampton and Sladen 1984).

1.2.4 Non Steroidal Anti-Inflammatory Drugs and Normal Colon

Non steroidal anti-inflammatory drugs can adversely affect both the normal and the diseased colon. In the normal colon, non steroidal anti-inflammatory drugs can cause diffuse colitis, isolated or diffuse ulcers, bleeding and perforation (Hall, Petty et al. 1983; Pierrugues and Fontes 1994; Kurahara, Matsumoto et al. 2001). There is debate over the pathophysiology of these complications and whether inhibition of prostaglandin synthesis plays a role. Jenkins and co-authors showed that non steroidal anti-inflammatory drugs increase permeability of the colonic mucosa in people. They propose that there is a relation between increased permeability of the colonic mucosa and adverse reaction to non steroidal anti-inflammatory drugs (Jenkins, Trew et al. 1991). Somasundaram and co-workers showed that non steroidal anti-inflammatory drugs uncouple oxidative phosphorylation in the mitochondria of epithelial cells of the intestinal mucosa. They propose that this alters the intercellular junctions and increases mucosal permeability (Somasundaram, Sigthorsson et al. 2000). Increased mucosal permeability would allow luminal substances such as ingesta molecules, bile acids and bacteria to enter the interstitial space and invoke an inflammatory reaction. Inflammation would further contribute to mucosal damage and loss of barrier function (Bjarnason, Hayllar et al. 1993; Lanas, Panes et al. 2003). A study from Whittle and co-workers suggests that colonic complications due to non steroidal anti-inflammatory drugs, such as erosions and ulcerations, are unlikely the result of inhibition of prostaglandin synthesis. Whittle and co-workers showed that as macroscopic damage to the intestinal mucosa develops in rats, the activity of fatty-acid cyclooxygenase enzymes have returned to values similar to those observed prior to administration of non

steroidal anti-inflammatory drugs. Further, they showed no relationship between the severity of damage to the intestinal mucosa and the decrease in synthesis of mucosal prostaglandins. Whittle and co-workers also showed that inhibition of 95% of prostaglandin synthesis did not induce macroscopic damage to the intestinal mucosa in rats (Whittle 1981).

In contradiction of Whittle's work, other studies suggest that inhibition of prostaglandin synthesis plays a role in the development of colonic complications due to non steroidal anti-inflammatory drugs. Redfern and co-workers documented intestinal erosions and ulcerations in rats in which prostaglandin synthesis was inhibited by induction of a fatty-acid cyclooxygenase enzyme antibody (Redfern and Feldman 1989). Seibert and co-workers showed that rats administered an overdose of a selective inhibitor of fatty-acid cyclooxygenase-2 enzymes (SC-58125) did not develop gross intestinal erosions or ulcerations. Rats administered an overdose of a non-selective inhibitor of fatty-acid cyclooxygenase enzymes (indomethacin) developed gross intestinal erosions and ulcerations (Seibert and Masferrer 1994).

1.2.5 Non Steroidal Anti-Inflammatory Drugs and Inflamed Colon

Non steroidal anti-inflammatory drugs can adversely affect the chronically inflamed colon. In people, non steroidal anti-inflammatory drugs can reactivate or exacerbate inflammatory bowel disease. This is observed with both non-selective and fatty-acid cyclooxygenase-2 enzyme selective non steroidal anti-inflammatory drugs (Kaufmann and Taubin 1987; Singh, Patil et al. 2004). Prostaglandins likely play an important role in the pathogenesis of inflammatory bowel disease (Bjarnason, Hayllar et al. 1993; Eberhart and Dubois 1995; Lanas, Panes et al. 2003). Higher concentrations of prostaglandins, mainly prostaglandin E_2, are found in the colonic and rectal mucosa of people with active inflammatory bowel disease compared to concentrations in healthy people (Rampton and Sladen 1984; Raab, Sundberg et al. 1995). This could result from up-regulation of the expression of fatty-acid

cyclooxygenase-2 enzymes causing increased synthesis of prostaglandins. Hendel and co-workers showed that the concentration of fatty-acid cyclooxygenase-2 messenger ribonucleic acid in the colonic mucosa of people with active inflammatory bowel disease increased with severity of disease (Hendel and Nielsen 1997). Singer and co-workers documented the presence of fatty-acid cyclooxygenase-2 enzymes in the colonic mucosa of people with active inflammatory bowel disease. They did not find fatty-acid cyclooxygenase-2 enzymes in the colonic mucosa of healthy people (Singer, Kawka et al. 1998). In both studies, concentrations of messenger ribonucleic acid for fatty-acid cyclooxygenase-1 enzyme and fatty-acid cyclooxygenase-1 enzyme were similar in the colonic mucosa of people with active inflammatory bowel disease and healthy people (Hendel and Nielsen 1997; Singer, Kawka et al. 1998). Otani and co-workers suggest that high concentrations of prostaglandin E_2 in the colonic mucosa of people with active inflammatory bowel disease are the consequence of both increased synthesis and reduced catabolism. Their work showed decreased concentrations of 15-hydroxyprostaglandin dehydrogenase, the key enzyme responsible for metabolic inactivation of prostaglandin E_2 (Otani, Yamaguchi et al. 2006). They suggest that high concentrations of prostaglandin E_2 promote wound healing in the inflamed colonic mucosa of people with inflammatory bowel disease. Thus, inhibition of prostaglandin synthesis by non steroidal anti-inflammatory drugs would result in exacerbation of clinical signs of inflammatory bowel disease (Otani, Yamaguchi et al. 2006). Reactivation or exacerbation of inflammatory bowel disease by non steroidal anti-inflammatory drugs becomes an important clinical problem as many people with inflammatory bowel disease have a concurrent condition, such as arthritis, requiring treatment with non steroidal anti-inflammatory drugs.

1.2.6 Carprofen

Carprofen is a non steroidal anti-inflammatory drug of the propionic acid class. Carprofen is available in oral and injectable formulations. Clark and co-workers reported a maximum plasma concentration of 16.9μg/ml after oral intake of 25mg carprofen in dogs. Maximum plasma concentration was reached within 30 min to 3 hours (Clark, Chieffo et al. 2003). At steady-state (25 mg, per os, every 12 hour), a maximum plasma concentration of 18.7μg/ml was reached within 30 min to 3 hours of intake (Clark, Chieffo et al. 2003). Maximum plasma concentration of 8μg/ml was reached within 1.5 to 8 hours of subcutaneous injection of 25mg carprofen. At steady-state (25 mg, subcutaneous, every 12 hour), a maximum plasma concentration of 14.7μg/ml was reached within 1.5 to 4 hours of injection (Clark, Chieffo et al. 2003). Plasma concentrations are proportional to dose. The drug is found in plasma mostly as an intact molecule. The carprofen molecule forms a strong bond with plasma proteins resulting in a high ratio of concentration in plasma relative to tissue. Metabolites are rapidly eliminated by biotransformation. The carprofen molecule is transformed into an ester glucuronide which determines the pharmacokinetics of the drug. In rats and dogs, the ester glucuronide of carprofen is eliminated predominantly by biliary secretion. Less than 5% of carprofen is eliminated as the intact molecule. The drug undergoes an extensive enterohepatic circulation in people but not in dogs. In dogs, 70% of an intra-venous dose is excreted in feces while 8-15% is excreted in urine (Rubio, Seawall et al. 1980).

Carprofen inhibits the activity of fatty-acid cyclooxygenase enzymes but its effect is reversible. Carprofen is a non-selective non steroidal anti-inflammatory drug in people but preferentially inhibits fatty-acid cyclooxygenase-2 enzymes in dogs. Wilson and co-workers showed that carprofen had a 6 times greater preference for inhibiting fatty-acid cyclooxygenase-2 enzymes in a whole blood assay in dogs (Wilson, Chandrasekharan et al. 2004).

1.3 Summary and Hypotheses for Present Studies

The colon of dogs is divided into three anatomical regions: the ascending colon, the transverse colon and the descending colon. The wall of the colon is composed of four layers: the mucosa, the submucosa, the muscularis and the serosa. Cells of the epithelium of the mucosa are held together by intercellular tight junctions. These allow the epithelium of the mucosa to function as a selectively permeable barrier between the lumen and the interstitial space. The presence of intercellular tight junctions forces transport across the epithelium of the colonic mucosa in one of two ways; 1) transcellular transport is a directional flux of molecules occurring through the epithelial cells against a gradient of concentration; 2) paracellular transport is a passive flux of small molecules occurring through the tight junctions along a gradient of concentration. The main ions transported across the epithelium of the colonic mucosa of mammals are sodium ions, chloride ions, potassium ions, bicarbonate ions and short chain fatty acids. There are multiple systems to transport each one of these ions across the epithelium of the colonic mucosa. Transport of ions across the epithelium of the colonic mucosa results in measurable electrophysiologic parameters. The Ussing chamber system can be used to determine electrophysiologic parameters and permeability of epithelia including the epithelium of the colonic mucosa.

Electrophysiologic parameters and permeability vary between anatomical regions of the colonic mucosa in people, rabbit, mice and rats. Differences in electrophysiologic parameters and permeability between anatomical regions of the colonic mucosa of the dog have not been investigated. The first objective of this study is to measure the in vitro electrical conductance and permeability to mannitol of the mucosa of the transverse, proximal descending and distal descending colon of the dog. Based on evidence in other omnivorous species we hypothesize that

the in vitro electrical conductance and permeability to mannitol are greater in the mucosa of the transverse colon compared to the proximal descending and distal descending colon of the dog.

Non steroidal anti-inflammatory drugs can adversely affect the colon of people and rats. Non steroidal anti-inflammatory drugs inhibit the activity of fatty-acid cyclooxygenase enzymes which in turn inhibits the production of prostaglandins. Prostaglandins participate in both the normal physiology of the colon as well as the development of acute inflammation. Prostaglandins may also play a role in chronic inflammatory diseases. Whether or not non steroidal anti-inflammatory drugs have an adverse effect on the colon due to inhibition of prostaglandin synthesis remains controversial.

It is not known if non steroidal anti-inflammatory drugs adversely affect the colon of the dog. The second objective of this study is to measure the in vitro electrical conductance and permeability to mannitol as well as describe the histologic appearance of the colonic mucosa of the dog in the presence of carprofen. We hypothesize that carprofen increases the in vitro electrical conductance and permeability to mannitol and causes deleterious effects to the histologic appearance of the colonic mucosa of the dog.

CHAPTER 2: MATERIALS AND METHODS

2.1 Objective 1. Control Group

2.1.1 Harvesting and Preparation of Sections of Colonic Mucosa

Six mature mixed breed dogs were used for objective 1. The dogs were placed under general anesthesia with thiopental and maintained with isoflurane. The entire colon was harvested immediately prior to euthanasia, cut open on the mesenteric side, placed in ice-cold Krebs-Ringer bicarbonate buffer solution and transported to the laboratory. The dogs were then euthanized for reasons unrelated to the study with an overdose of sodium pentobarbital. The time from harvest to mounting in the Ussing chamber was less than 30 minutes.

The colon was placed in stripping pans filled with 400 ml ice-cold oxygenated (95% oxygen / 5% carbon dioxide) Krebs-Ringer bicarbonate buffer solution. The colon was divided in three regions: transverse colon, proximal descending and distal descending. The transverse colon extended from the cecocolic junction to the middle colic vein. The descending colon extended from the middle colic vein to the pelvic inlet and was divided into two equal proximal and distal segments. The colonic mucosa was separated from the seromuscular layer using blunt and sharp dissection. Three sections of mucosa were obtained from each region of the colon. An additional section of mucosa was obtained from a randomly chosen region of the colon. That section was placed in neutral-buffered 10 % formalin and reserved for histologic examination.

2.1.2 Ussing Chamber Studies

2.1.2.1 Mounting

Each section of mucosa was randomly assigned to one of nine Ussing chamber units (3.14 cm^2 aperture). The mucosa was clamped as a flat sheet between the two halves of the acrylic chamber.

2.1.2.2 Solutions

Each hemi-chamber was filled with 15 ml Krebs-Ringer bicarbonate buffer solution at pH 7.4 containing (in nM) 118 NaCl, 4.7 KCl, 25 $NaHCO_3$, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 2.5 $CaCl_2$ and 10 dextrose. The Krebs-Ringer bicarbonate buffer solution was continuously oxygenated (95% oxygen / 5% carbon dioxide) and circulated in water-jacketed reservoirs. The temperature of the solution was maintained at 37ºC.

2.1.2.3 Electrical Measurements

Transepithelial potential difference (mV) was measured using agar bridges connected to silver/silver chloride voltage electrodes. If transepithelial potential difference was between -1.0 and 1.0 mV, tissues were current clamped at 100 μA for 5 seconds and transepithelial potential difference was recorded.

Transepithelial potential difference was short-circuited through the voltage electrodes using a voltage clamp that corrected for fluid resistance. Short-circuit current (μA) was measured using a separate pair of agar bridges connected to silver/silver chloride electrodes (current electrodes).

Transepithelial potential difference and short-circuit current were recorded every 15 minutes for 240 minutes. Electrical conductance was calculated from transepithelial potential difference and short-circuit current using Ohms law. Ohm's law states that the voltage drop (electrical potential difference) between two points of an electrical circuit is equal to the product of the current flowing through it and the electrical resistance of the conductor.

$$\mathbf{V = R \times I}$$

where

- V is the potential difference (V, volt)
- I is the current (A, ampere)

- R is the electrical resistance of the conductor (Ω, ohm)

Using this relation, transepithelial potential difference across and short-circuit current through the section of colonic mucosa were used to calculate its electrical resistance. Electrical conductance is the inverse of resistance.

$$G = 1 / R$$

Where

- G is the electrical conductance (S, siemens)
- R is the electrical resistance (Ω, ohm)

Using this relation, electrical conductance of the section of colonic mucosa was calculated.

2.1.2.4 Mannitol

Tritium-mannitol (3H-mannitol) as a solution containing 10 μCi/ml was added to the mucosal bathing solution 15 minutes after mounting. A sample (0.1 ml) was then collected from the mucosal solution 30 minutes after addition of ^{3}H-mannitol. Samples (0.5 ml) were collected from the serosal solution 60, 120, 180 and 240 minutes after addition of ^{3}H-mannitol. Samples were assessed for β emission (counts/min) and mucosal to serosal flux of mannitol was calculated for each of three periods: 60-120 minutes, 120 to 180 minutes and 180 to 240 minutes.

At the end of the experiment, sections of colonic mucosa were removed from the Ussing chambers. The sections were placed in neutral-buffered 10% formalin for later histologic examination.

2.1.3 Histologic Examination

Fixed sections of colonic mucosa were trimmed, embedded in paraffin and sectioned at a thickness of 5 μm. Tissue slices were mounted on slides and stained with hematoxylin and eosin.

Tissue slices were evaluated by a single, blinded evaluator with light microscopy for the presence of inflammation, edema, sloughing of cells from the surface epithelium, erosions and sloughing of epithelial cells within the mucosal glands. Any additional outstanding findings were noted. Inflammation was determined based on the percent surface area of lamina propria infiltrated by inflammatory cells (lymphocytes and plasma cells). Less than 20% of the lamina propria infiltrated by inflammatory cells was considered normal. Inflammation was considered mild if 20 to 40% of the surface area of lamina propria was infiltrated by inflammatory cells, moderate if 40 to 60% of the surface area of lamina propria was infiltrated by inflammatory cells and severe if more than 60% of the surface area of lamina propria was infiltrated by inflammatory cells. Edema was considered absent if the mucosal glands were not separated from each other by clear fluid, mild if the mucosal glands were less than 50 μm apart, moderate if the mucosal glands were 50 to 150 μm apart and severe if mucosal glands were more than 150 μm apart. Sloughing of cells from the surface epithelium was defined as detachment of surface epithelial cells without discontinuity of the surface epithelium. Sloughing of cells from the surface epithelium was considered minimal if <10% of the surface area was affected, mild if 10 to 20% of the surface area was affected, moderate if 20 to 50% of the surface area was affected, and severe if greater than 50% of the surface area was affected. Erosions were defined as discontinuity of the surface epithelium. Erosions were classified as absent, involving less than 10% of the epithelial surface, or involving more than 10% of the epithelial surface. Sloughing of epithelial cells within the mucosal glands was considered absent if no glands had detached epithelial cells in their lumen, mild if less than 5% of mucosal glands had detached epithelial cells in their lumen, moderate if 5 to 10% of mucosal glands detached epithelial cells in their lumen, and severe if greater than 10% of mucosal glands detached epithelial cells in their lumen.

2.2 Objective 2. Carprofen Group

Methods for objective 2 were based on results for objective 1. The methods specific to objective 2 are described.

2.2.1 Harvesting and Preparation of Sections of Colonic Mucosa - Seven mature mixed breed dogs were used for objective 2. Sections of colonic mucosa were harvested and prepared as in objective 1.

2.2.2 Ussing Chamber Studies

2.2.2.1 Mounting

Each section of mucosa was randomly assigned to one of nine Ussing chamber units (3.14 cm^2 aperture). Sections of colonic mucosa were mounted as in objective 1.

2.2.2.2 Solutions

Each hemi-chamber was filled with 15 ml Krebs-Ringer bicarbonate buffer solution at pH 7.4 as in objective 1. The Krebs-Ringer bicarbonate buffer solution was continuously oxygenated (95% oxygen / 5% carbon dioxide) and circulated in water-jacketed reservoirs. The temperature of the solution was maintained at 37ºC. Carprofen was added to the bathing solution at a concentration of 400µg/ml thirty minutes after mounting. The pH of the Krebs-Ringer bicarbonate buffer solution with carprofen was 7.4.

2.2.2.3 Electrical Measurements and Mannitol

Data for electrical conductance was obtained as in objective 1. Data for mucosal to serosal flux of mannitol was obtained as in objective 1.

2.2.3 Histologic Examination

At the end of the experiment, sections of colonic mucosa were removed from the Ussing chambers. The sections were placed in neutral-buffered 10% formalin for later histologic examination.

2.3 Statistical Analysis

2.3.1 Ussing Chamber Studies

2.3.1.1 Electrical Conductance

Data from 0 minute to 15 minutes were not used for analysis (equilibration period). Electrical conductance from 30 minutes to 240 minutes (end of experiment) was graphed against time for each chamber and the area under each curve was calculated using the trapezoid method. Sections from the same region of the colon within a dog were considered replicates. The area under the curve was the response variable used for the statistical analysis. The mean+/-SEM area under the curve (electrical conductance*time) for each region (transverse, proximal descending and distal descending colon) was calculated. The electrical conductance*time was normally distributed verified by failure to reject the null hypothesis of normality at $p \leq 0.05$ (Shapiro-Wilks'statistic). Data from objective 1 was first explored for an effect of region using a one-way analysis of variance (ANOVA) for repeated measurements with ad-hoc comparisons made with the Scheffe adjustment to maintain alpha at 0.05. Based on the results of objective 1, data from objective 2 was evaluated incorporating data from objective 1 such that the electrical conductance*time was compared across regions and treatments using a two-way analysis of variance (ANOVA) for repeated measurements with ad-hoc comparisons made with the Scheffe adjustment to maintain alpha at 0.05. Thus, where results are considered significant, $p \leq 0.05$ unless otherwise stated.

2.3.1.2 Mannitol

Mucosal to serosal flux of mannitol was calculated for three periods of one hour: 60 to 120 minutes, 120 to 180 minutes and 180 to 240 minutes. Sections from the same region of the colon within a dog were considered replicates. Mucosal to serosal flux of mannitol was the response variable used for the statistical analysis. The mean+/-SEM mucosal to serosal flux of

mannitol for each period and for each region of the colon (transverse, proximal descending and distal descending colon) was calculated. The data was normally distributed verified by failure to reject the null hypothesis of normality at $p \leq 0.05$ (Shapiro-Wilks'statistic). Data from objective 1 was first explored for an effect of region and period using a two-way analysis of variance (ANOVA) for repeated measurements with ad-hoc comparisons made with the Scheffe adjustment to maintain alpha at 0.05. Based on the results of objective 1, data from objective 2 was evaluated incorporating data from objective 1 such that the mannitol flux was compared across regions, treatments and periods using a three-way analysis of variance (ANOVA) for repeated measurements with ad-hoc comparisons made with the Scheffe adjustment to maintain alpha at 0.05. Thus, where results are considered significant, $p \leq 0.05$ unless otherwise stated.

2.3.1.3 Histology

The frequency distribution of histologic categories from the control group and the carprofen group across sections were compared using a Chi square analysis or Fisher's exact test. Where the frequency was 0 in a category for both groups, the category was deleted. Where there was 3 or less categories, a Fisher's exact test was performed. Where there were four categories, a Chi square analysis was performed with a 0.5 correction used where a single cell had a 0 frequency entry. The null hypothesis of similar distributions was rejected at $p \leq 0.05$ for both tests. PROC UNIVARIATE, PROC GLM and PROC FREQ were used for the analysis (SAS V.9.1).[1]

[1] SAS Institute, Cary, NC

CHAPTER 3. RESULTS

3.1 Objective 1. Control Group

3.1.1 Ussing Chamber Studies

3.1.1.1 Electrical Conductance

Mean +/- SEM electrical conductance*time for transverse, proximal descending and distal descending colon for the control group were 3115 +/- 304.8, 2367 +/- 147.0 and 2449 +/- 156.7 mS/cm^2*min respectively; mean +/- SEM electrical conductance*time for transverse colon was significantly higher than proximal descending and distal descending colon; mean +/- SEM electrical conductance*time for proximal descending and distal descending colon were not significantly different.

3.1.1.2 Mannitol

Mean +/- SEM mucosal to serosal flux of mannitol 60-120 min for transverse, proximal descending and distal descending colon for the control group were 0.18 +/-0.016, 0.15 +/- 0.018 and 0.15 +/- 0.016 μmol/cm^2*h respectively; mean +/- SEM mucosal to serosal flux of mannitol 120-180 min for transverse, proximal descending and distal descending colon were 0.21 +/- 0.014, 0.19 +/- 0.029 and 0.18 +/- 0.018 μmol/cm^2*h respectively; mean +/- SEM mucosal to serosal flux of mannitol 180-240 min for transverse, proximal descending and distal descending colon were 0.23 +/- 0.013, 0.20 +/- 0.020 and 0.19 +/- 0.018 μmol/cm^2*h respectively.

Mean +/- SEM mucosal to serosal flux of mannitol 60-120 min for transverse colon was significantly different from mean +/- SEM mucosal to serosal flux of mannitol 180-240 min for transverse colon; there was no significant difference between mean +/- SEM mucosal to serosal flux of mannitol periods for proximal descending and distal descending colon; there was no significant difference between mean +/- SEM mucosal to serosal flux of mannitol for transverse, proximal descending and distal descending colon for any of the flux periods.

3.1.2 Histologic Examination

3.1.2.1 Sections of Colonic Mucosa Obtained Prior to Mounting

Six sections of colonic mucosa (one from each dog) were examined. All sections had a normal amount of lymphocytes and plasma cells. None of the sections had edema. Sloughing of cells from the surface epithelium was minimal in 5 sections. One section had mild sloughing of cells from the surface epithelium. Erosions were absent in 5 sections. One section had erosions involving less than 10% of the surface epithelium. None of the sections had sloughing of epithelial cells within the mucosal glands.

3.1.2.2 Sections of Colonic Mucosa Removed from the Ussing Chamber

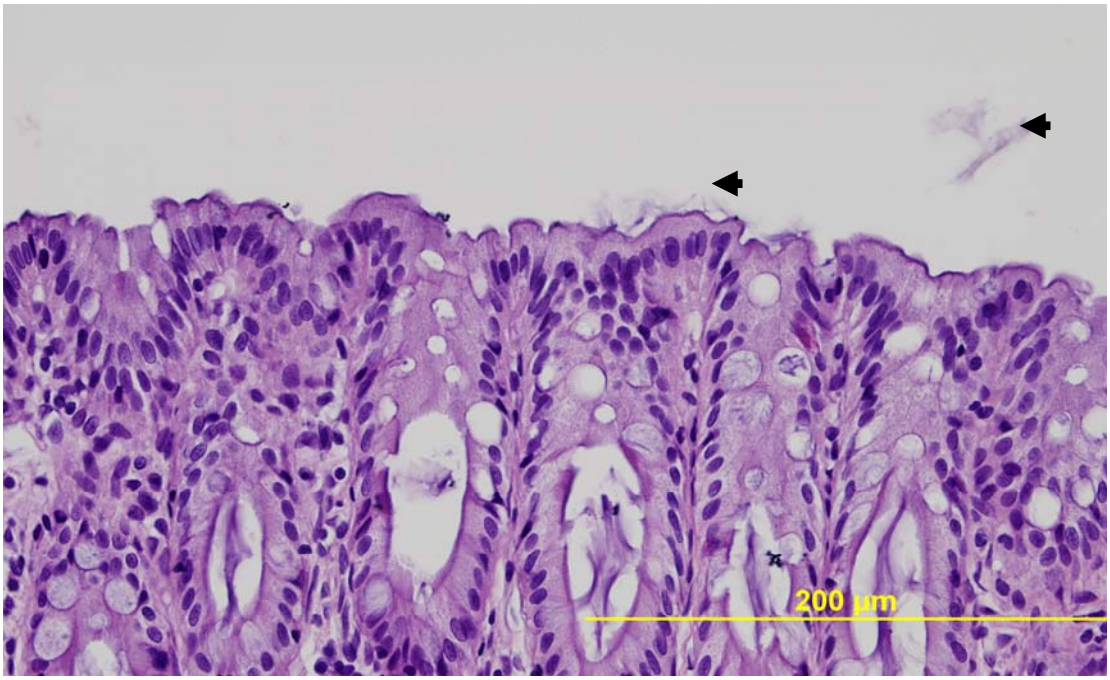

Figure 3.1 Light micrograph of section of colonic mucosa from control group at the end of the experiment. There is mild sloughing of cells from the surface epithelium (arrowhead) but no sloughing of epithelial cells within the mucosal glands or erosions. x40 magnification

Fifty-four sections of colonic mucosa (9 from each dog) were examined. All sections had a normal amount of lymphocytes and plasma cells. Edema was absent in 9/54 (16.7%) sections, mild in 25/54 (46.3%) sections, moderate in 14/54 (25.9%) sections and severe in 6/54 (11.1%)

sections. Sloughing of cells from the surface epithelium was minimal in 7/54 (13%) sections, mild in 23/54 (43%) sections, moderate in 16/54 (30%) sections and severe in 8/54 (15%) sections. Erosions were absent in 34/54 (63%) sections, involved less than 10% of the surface epithelium in 19/54 (35%) and involved at least 10% of the surface epithelium in 1/54 (2%) sections. Sloughing of epithelial cells within the mucosal glands was absent in 25/54 (46%) sections, mild in 20/54 (37%) sections, moderate in 6/54 (11%) sections and severe in 3/54 (6%) sections.

3.2 Objective 2. Carprofen Group

3.2.1 Ussing Chamber Studies

3.2.1.1 Electrical Conductance

Mean +/- SEM electrical conductance*time for transverse, proximal descending and distal descending colon for the carprofen group were 3902 +/- 232.5, 3829 +/- 309.6 and 3975 +/- 294.3 mS/cm^2*min respectively. Mean +/- SEM electrical conductance*time for the carprofen group was significantly higher than for the control group for all regions. In the carprofen group, mean +/- SEM electrical conductance*time for transverse, proximal descending and distal descending colon were not significantly different.

Table 3.1 Mean electrical conductance*time (SEM) (mS/cm^2*min) for control group and carprofen group for different regions of the colon. Means from control group with a * are significantly different from means from carprofen group ($p \leq 0.05$). Within each row, means with different superscripts are significantly different ($p \leq 0.05$).

	Transverse	Proximal descending	Distal descending
Control	3115^{a*} (304.8)	2367^{b*} (147.0)	2449^{b*} (156.7)
Carprofen	3902^{a} (232.5)	3829^{a} (309.6)	3975^{a} (294.3)

3.2.1.2 Mannitol

Mean +/- SEM mucosal to serosal flux of mannitol 60-120 mins for transverse, proximal descending and distal descending colon for the carprofen group were 0.10 +/- 0.016, 0.10 +/- 0.011 and 0.09 +/- 0.013 μmol/cm^2*h respectively; mean +/- SEM mucosal to serosal flux of mannitol 120-180 min for transverse, proximal descending and distal descending colon were 0.23 +/- 0.037, 0.21 +/- 0.027 and 0.22 +/- 0.037 μmol/cm^2*h respectively; mean +/- SEM mucosal to serosal flux of mannitol 180-240 mins for transverse, proximal descending and distal descending colon were 0.43 +/- 0.061, 0.38 +/- 0.039 and 0.36 +/- 0.056 μmol/cm^2*h respectively.

Mean +/- SEM mucosal to serosal flux of mannitol for transverse, proximal descending and distal descending colon significantly increased from period 60-120 mins to period 120-180 mins and from period 120-180 mins to 180-240 mins; there was no significant difference between mean +/- SEM mucosal to serosal flux of mannitol for transverse, proximal descending and distal descending colon for any of the flux periods.

Mean mucosal to serosal flux of mannitol for treated sections of transverse colon and distal descending colon was significantly lower than corresponding control sections at 60-120 mins; treated sections of proximal descending colon was not significantly different than control sections at 60-120 mins. The mean mucosal to serosal flux of mannitol for treated sections of transverse colon, proximal descending colon and distal descending colon were not significantly different than corresponding control sections at 120-180 mins; The mean mucosal to serosal flux of mannitol for treated sections of transverse colon, proximal descending colon and distal descending colon were all significantly higher than corresponding control sections at 180-240 mins.

Table 3.2 Mean (SEM) mucosal to serosal flux (µmol/cm^2*h) of mannitol for control group and carprofen group for different regions of the colon. Means from control group with a * are significantly different from the corresponding mean from carprofen group (p≤0.05). Mean fluxes within a region (column) with different superscripts are significantly different (p≤0.05).

		Transverse	Proximal descending	Distal descending	Comparison across regions
Control	Flux 60-120 min	**0.18^{a*}** (0.016)	**0.15^{p*}** (0.018)	**0.15^{x*}** (0.016)	NSD
	Flux 120-180 min	**0.21^{ab}** (0.014)	**0.19^{p}** (0.029)	**0.18^{x}** (0.018)	NSD
	Flux 180-240 min	**0.23^{b*}** (0.013)	**0.20^{p*}** (0.020)	**0.19^{x*}** (0.018)	NSD
Carprofen	Flux 60-120 min	**0.10^{a}** (0.016)	**0.10^{p}** (0.011)	**0.09^{x}** (0.013)	NSD
	Flux 120-180 min	**0.23^{b}** (0.037)	**0.21^{q}** (0.027)	**0.22^{y}** (0.037)	NSD
	Flux 180-240 min	**0.43^{c}** (0.061)	**0.38^{r}** (0.056)	**0.36^{z}** (0.056)	NSD

3.2.2 Histologic Examination

3.2.2.1 Sections of Colonic Mucosa Obtained Prior to Mounting

Seven sections of colonic mucosa (one from each dog) were examined. Six sections had a normal amount of lymphocytes and plasma cells. One section had a small focus of neutrophilic inflammation. None of the sections had edema. Sloughing of cells from the surface epithelium was minimal in 4 sections. One section had mild sloughing of cells from the surface epithelium. Two sections had moderate sloughing of cells from the surface epithelium. Erosions were absent in 4 sections. Two sections had erosions involving less than 10% of the surface epithelium. One section had erosions involving at least 10% of the surface epithelium. None of the sections had sloughing of epithelial cells within the mucosal glands. The distribution of all histologic features was not different between groups.

Table 3.3 Histologic examination of sections of colonic mucosa obtained prior to mounting: Inflammation, edema, sloughing of cells from the surface epithelium, erosion and sloughing of epithelial cells within the mucosal glands.* denotes a significantly different distribution across categories between control and carprofen groups.

Inflammation		**Absent**	**Mild**	**Moderate**	**Severe**
	Control (n=6)	6	0	0	0
	Carprofen (n=7)	6	1	0	0
Edema		**Absent**	**Mild**	**Moderate**	**Severe**
	Control (n=6)	6	0	0	0
	Carprofen (n=7)	7	0	0	0
Sloughing surface epithelium		**Minimal**	**Mild**	**Moderate**	**Severe**
	Control (n=6)	5	1	0	0
	Carprofen (n=7)	4	1	2	0
Erosion		**Absent**	**< 10%**	**≥ 10%**	
	Control (n=6)	5	1	0	
	Carprofen (n=7)	4	1	2	
Sloughing within mucosal glands		**Absent**	**Mild**	**Moderate**	**Severe**
	Control (n=6)	6	0	0	0
	Carprofen (n=7)	7	0	0	0

3.2.2.2 Sections of Colonic Mucosa Removed from the Ussing Chamber

Sixty-three sections of colonic mucosa (9 from each dog) were examined. Mild inflammation was present in one section. All the other sections had a normal amount of lymphocytes and plasma cells. The distribution of inflammation was not different between groups. Edema was absent in 16/63 (25.4%) sections, mild in 37/63 (58.7%) sections and moderate in 10/63 (15.9%) sections. The distribution of edema in the carprofen group was significantly different from the control group ($p<0.001$). Sloughing of cells from the surface epithelium was mild in 5/63 (8%) sections, moderate in 17/63 (27%) sections, and severe in 41/63 (65%) sections. The distribution of sloughing of cells from the surface epithelium in the carprofen group was significantly different from the control group ($p<0.001$) Erosions were absent in 1/63 (2%) sections, involved less than 10% of the surface epithelium in 27/63 (43%) sections and involved at least 10% of the surface epithelium in 35/63 (56%) sections. The

distribution of erosions in the carprofen group was significantly different from the control group ($p<0.001$) Sloughing of epithelial cells within the mucosal glands was absent in 39/63 (62%) sections, mild in 20/63 (32%) sections, moderate in 3/63 (5%) sections and severe in 1/63 (2%) sections. The distribution of sloughing of epithelial cells within the mucosal glands was not different between groups.

Table 3.4 Histologic examination of sections of colonic mucosa removed from the Ussing Chambers: Inflammation, edema, sloughing of cells from the surface epithelium, erosion and sloughing of epithelial cells within the mucosal glands.* denotes a significantly different distribution across categories between control and carprofen groups.

Inflammation		**Absent**	**Mild**	**Moderate**	**Severe**
	Control (n=54)	**54** (100%)	**0**	**0**	**0**
	Carprofen (n=63)	**62** (98.4%)	**1** (1.6%)	**0**	**0**
Edema		**Absent**	**Mild**	**Moderate**	**Severe**
	Control (n=54)	**9** (16.7%)	**25** (46.3%)	**14** (25.9%)	**6** (11.1%)
	Carprofen (n=63)	**16** (25.4%)*	**37** (58.7%)	**10** (15.9%)	**0**
Sloughing surface epithelium		**Minimal**	**Mild**	**Moderate**	**Severe**
	Control (n=54)	**7** (13.0%)	**23** (42.6%)	**16** (29.6%)	**8** (14.8%)
	Carprofen (n=63)	**0***	**5** (7.9%)	**17** (27.0%)	**41** (65.1%)
Erosion		**Absent**	**< 10%**	**≥ 10%**	
	Control (n=54)	**34** (63.0%)	**19** (35.2%)	**1** (1.9%)	
	Carprofen (n=63)	**1** (1.6%)*	**27** (42.9%)	**35** (55.6%)	
Sloughing within mucosal glands		**Absent**	**Mild**	**Moderate**	**Severe**
	Control (n=54)	**25** (46.3%)	**20** (37%)	**6** (11.1%)	**3** (5.6%)
	Carprofen (n=63)	**39** (61.9%)	**20** (31.7%)	**3** (4.8%)	**1** (1.6%)

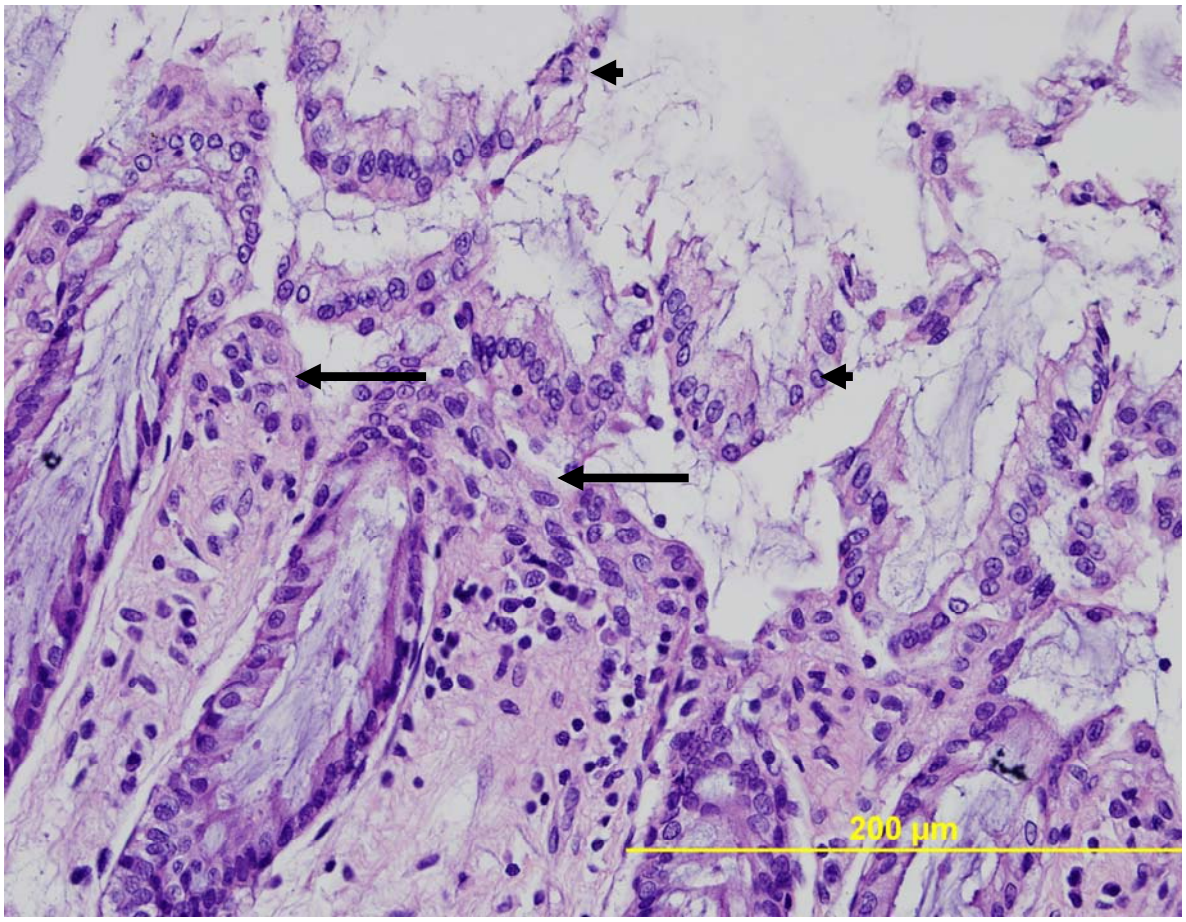

Figure 3.2 Light micrograph of section of colonic mucosa from carprofen group at the end of the experiment. There is mild edema, severe sloughing of cells from the surface epithelium (arrowhead) and erosions of the surface epithelium (arrow). x40 magnification.

CHAPTER 4. DISCUSSION

Carprofen increased the in vitro electrical conductance and permeability to mannitol of the colonic mucosa of the dog. Together, these findings suggest compromise of the integrity and loss of barrier function of the colonic mucosa.

Increased electrical conductance may reflect changes in transcellular and paracellular transport of ions across the epithelium of the colonic mucosa, or compromise of the functional integrity of intercellular tight junctions (Nedergaard, Larsen et al. 1999; Mlodzik-Danielewicz and Tyrakowski 2005). Carprofen increased electrical conductance of the colonic mucosa of the dog by a mechanism other than inhibition of prostaglandin synthesis. This can be explained by examining the normal physiology of prostaglandins in the colon. In the colon of mammals, under normal conditions, prostaglandins are important secretagogues, promoting mainly secretion of chloride ions into the lumen. Transport of chloride, sodium and potassium ions account for most of the in vitro short-circuit current across the epithelium of the colonic mucosa. Short-circuit current is referred to as positive for positively charged ions traversing the epithelium of the mucosa from the luminal side to the serosal side. Transport of negatively charged chloride ions from the serosal side to the luminal side consequently increases short-circuit current. According to Ohm's law, electrical conductance of the conductor (the colonic mucosa) increases proportionally with short-circuit current flowing through the conductor assuming transepithelial potential difference across the conductor is unchanged (Rechkemmer, Frizzell et al. 1996). By promoting secretion of chloride ions, prostaglandins increase electrical conductance across the epithelium of the colonic mucosa. These interrelations between prostaglandins, secretion of chloride ions, short-circuit current and electrical conductance are well demonstrated in a study by Rechkemmer and co-workers, in which exogenous prostaglandin E_2 increased secretion of chloride ions and electrical conductance in the distal colon of guinea-pigs (Rechkemmer, Frizzell

et al. 1996). In a study by Dharmsathaphorm and Pandol, prostaglandin E_1 potentiated the positive effect of carbachol on secretion of chloride ions and increased electrical conductance in a monolayer of well-differentiated human colonic cells (Dharmsathaphorn and Pandol 1986). Prostaglandins increase electrical conductance. Accordingly, inhibition of prostaglandins synthesis should decrease electrical conductance. If inhibition of prostaglandin synthesis was the mechanism by which carprofen affected electrical conductance of the colonic mucosa of the dog in our study, electrical conductance would have been lower in the carprofen group compared to the control group. Therefore, inhibition of prostaglandin synthesis does not explain how carprofen increased electrical conductance of the colonic mucosa.

Carprofen could have increased electrical conductance of the colonic mucosa of the dog by compromising the functional integrity of intercellular tight junctions. Somasundaram and co-workers showed that oral administration of indomethacin, a non-steroidal anti-inflammatory drug, uncouples oxidative phosphorylation in mitochondria of epithelial cells of the intestinal mucosa of rats. This results in decreased production of adenosine tri-phosphate by mitochondria, which in turn causes release of calcium from cytoplasmic storage vesicles. The release of calcium affects the functional integrity of intercellular tight junctions (Somasundaram, Sigthorsson et al. 2000). This was shown in a study by Tai and co-workers in which increased intracellular concentration of calcium ions in a monolayer of human colonic cells compromised the functional integrity of intercellular tight junction. A simultaneous increase in electrical conductance was observed (Tai, Flick et al. 1996). Likewise, in our study, carprofen could have uncoupled oxidative phosphorylation in mitochondria of epithelial cells of the colonic mucosa of the dog, thereby compromising the functional integrity of intercellular tight junctions and consequently increasing electrical conductance.

Carprofen increased the in vitro permeability to mannitol of the colonic mucosa of the dog. This increase in permeability occurred over time and was most evident for the last mannitol flux period (180-240min). These findings are consistent with a previous study in which non-steroidal anti-inflammatory drugs increased permeability of the colon of people to radiolabelled ethylenediaminetetraacetate (^{51}CrEDTA), a molecule used as a marker of paracellular permeability (Jenkins, Trew et al. 1991). In the colonic mucosa of the dog, mannitol is passively transported along a gradient of concentration using both paracellular and transcellular pathways (Nejdfors, Wang et al. 1998). Because there is no active transport of mannitol in the colonic mucosa of the dog, increased permeability to mannitol is likely due to compromised functional integrity of intercellular tight junctions of the colonic mucosa. In the previously mentioned study by Somasundaram and co-workers, oral administration of indomethacin was associated with increased permeability to radiolabelled ethylenediaminetetraacetate of the intestinal mucosa of rats (Somasundaram, Sigthorsson et al. 2000). A similar increase in permeability was also observed with dinitrophenol, an agent that uncouples oxidative phosphorylation in the mitochondriae of epithelial cells of the intestinal mucosa without affecting the activity of cyclooxygenase enzymes. Parenteral administration of aspirin on the other hand did not increase permeability of the intestinal mucosa. These findings suggest that non-steroidal anti-inflammatory drugs have a direct local damaging effect on the intestinal mucosa (Somasundaram, Sigthorsson et al. 2000). This has been called the "topical" phase of non-steroidal anti-inflammatory drug induced injury to the intestinal mucosa (Somasundaram, Rafi et al. 1997). The "topical" phase occurs at the time of absorption of the drug by the epithelium of the intestinal mucosa and results in rapid and dose-dependant structural changes to mitochondria and uncoupling of oxidative phosphorylation (Somasundaram, Rafi et al. 1997). This affects the functional integrity of the intercellular tight junctions which results in increased permeability of

the intestinal mucosa. In the study by Tai and co-workers, compromise to the functional integrity of intercellular tight junctions due to increased intracellular concentration of calcium ions in a monolayer of human colonic cells was associated with increased permeability to mannitol (Tai, Flick et al. 1996). Likewise, in our study, carprofen could have uncoupled oxidative phosphorylation in mitochondria of epithelial cells of the colonic mucosa of the dog, thereby compromising the functional integrity of intercellular tight junctions and consequently increasing permeability to mannitol of the colonic mucosa.

Carprofen caused sloughing of cells from the surface epithelium and erosions of the colonic mucosa of the dog in vitro. These histologic findings are indicative of compromise of the integrity and loss of barrier function of the colonic mucosa and add support to the changes in electrical conductance and permeability to mannitol previously discussed.

Sloughing of cells from the surface epithelium was observed in our study to a significantly greater extent in the carprofen group where 92.1% of sections of colonic mucosa showed moderate or severe sloughing of cells from the surface epithelium compared to 44.4% of sections of colonic mucosa in the control group. Polentarutti and co-workers studied the relation between permeability to mannitol and histologic changes in the intestinal mucosa of rats during Ussing chamber experiments (Polentarutti, Peterson et al. 1999). They observed that permeability of the duodenal and jejunal mucosa to mannitol gradually increased throughout the duration of the experiment (180 minutes). This gradual increase in permeability to mannitol was attributed to gradual sloughing of cells from the surface epithelium of the intestinal mucosa and extensive repair process. Repair of the epithelium of the mucosa was observed histologically by reduced villi index and reduced nucleo-apical distance which indicate a reduction in the number of cells in the epithelium with the remaining cells flattening out to compensate for cell loss (Polentarutti, Peterson et al. 1999). Sloughing of cells from the surface epithelium is likely to

have contributed to the gradual increase in permeability to mannitol of the colonic mucosa of the dog in the carprofen group.

Erosions of the epithelium of the colonic mucosa were observed in our study to a significantly greater extent in the carprofen group where 98.5% of sections of colonic mucosa had erosions compared to 37.1% of sections of colonic mucosa from the control group. Erosions are areas of discontinuity of the epithelium of the mucosa. In the study by Polentarutti and co-workers, erosions of the epithelium of the intestinal mucosa were not evaluated or discussed separately from sloughing of cells from the surface epithelium. Rather, they were assigned a high morphologic score for tissue damage (Polentarutti, Peterson et al. 1999). Erosions of the epithelium of the colonic mucosa are likely to result in changes in permeability similar in nature but greater in magnitude to sloughing of cells from the surface epithelium as the indiscriminate passage of small and large molecules across the mucosa through areas devoid of epithelial cells is possible. Electrical conductance should also be affected as indiscriminate passage of ions through areas devoid of epithelial cells would preclude establishment of a concentration gradient necessary for normal transport of ions across the epithelium of the colonic mucosa.

Compromise of the integrity and loss of barrier function of the colonic mucosa indicated by increased electrical conductance, increased permeability to mannitol, sloughing of cells from the surface epithelium and erosions of the epithelium could be of concern in vivo. The epithelium of the colonic mucosa functions as a selectively permeable barrier between the lumen and the interstitium. When the integrity of the epithelium of the colonic mucosa is preserved, segregation of molecules to the luminal space or the interstitium is possible in conjunction with absorption of selected molecules from the lumen to the interstitium and secretion of yet other molecules from the interstitium to the lumen (Guilford, Center et al. 1996). Increased permeability of the intestinal mucosa constitutes the earliest and mildest stage of injury to the

intestinal mucosa caused by non-steroidal anti-inflammatory drugs. Increased permeability of the intestinal mucosa leads to delivery of luminal agents such as bile acids and bacteria to the intestinal mucosal immune system which may trigger an inflammatory reaction. Inhibition of prostaglandin synthesis by non-steroidal anti-inflammatory drugs facilitates progression of mucosal inflammation to mucosal ulcers (Thiefin and Beaugerie 2005). In people with Crohn's disease and other inflammatory bowel disease, increased permeability of the intestinal mucosa may underlie development and relapses of active disease (Kaufmann and Taubin 1987; Meddings 1997; Meddings 2000; Breslin, Nash et al. 2001; Otani, Yamaguchi et al. 2006). During times of increased intestinal permeability in people with Crohn's disease and other inflammatory bowel disease, abnormally high delivery of antigenic luminal agents to the intestinal mucosal immune system would trigger an inflammatory reaction and clinical signs of colitis (Meddings 2000). Support for this hypothesis comes from studies in animals in which inflammatory bowel disease can develop by increasing delivery of luminal agents to the intestinal mucosal immune system (Kiliaan, Saunders et al. 1998; Meddings, Jarand et al. 1999; Meddings and Swain 2000).

Electrical conductance was higher in the mucosa of the transverse colon compared to the proximal descending and the distal descending colon of the dog in the absence of carprofen. Carprofen annulled this difference in electrical conductance. Regional differences in electrical conductance of the colonic mucosa have been reported in rabbits, mice and people (Sellin and DeSoignie 1984; Sellin and DeSoignie 1987; Charney, Egnor et al. 2001). In rabbits, electrical conductance is higher in the proximal colon compared to the distal colon (Sellin and DeSoignie 1984). This is consistent with our findings in dogs. Regional differences in electrical conductance in rabbits are due to the presence of a sodium-chloride ion co-transport pathway in the proximal colon and not in the distal colon (Sellin and DeSoignie 1984). In people, electrical

conductance was lower in the proximal colon compared to the transverse and descending colon (Sellin and DeSoignie 1987). Comparison of these findings with our findings is complicated by major anatomical differences between the colon of people and the colon of dog. Regional differences in electrical conductance in people were attributed to an aboral gradient of increasing transport of sodium ions (Sellin and DeSoignie 1987). Regional differences in electrical conductance of the colonic mucosa of the dog may reflect differences in transport of ions across the epithelium of the colonic mucosa or variations in intercellular tight junctions. Hypothetically, greater secretion of chloride ions in the mucosa of the transverse colon compared to the descending colon could explain the regional differences we observed. Mayol and co-workers showed that depletion of adenosine tri-phosphate inhibits secretion of chloride ions in the distal colon of rats to a greater extent than in the proximal colon (Mayol, Alarma-Estrany et al. 2005). A similar situation could be present in the dog where gradual depletion of adenosine tri-phosphate in the Ussing chamber environment would lead to lower secretion of chloride ions in the mucosa of the descending colon which would result in lower electrical conductance compared to the transverse colon. Alternately, greater transport of sodium ions in the mucosa of the descending colon compared to the transverse colon could explain the regional differences in electrical conductance we observed. Systems for the transport of ions across the epithelium of the colonic mucosa have not been extensively studied in the dog and further studies are needed to investigate these hypotheses.

Regional differences in electrical conductance of the colonic mucosa of the dog could reflect variations in intercellular tight junctions. The seal formed by intercellular tight junctions is relative and despite their name, tight junctions are in fact the most permeable element of the epithelium of the mucosa. The impermeability of intercellular tight junctions to molecules varies considerably from one epithelium to another. Studies in mice have shown that there are

variations in the expression of the molecular constituents of tight junctions resulting in differences in paracellular transport of ions across the epithelium of the mucosa over different regions of the intestinal tract (Muresan, Paul et al. 2000; Fujita, Chiba et al. 2006). It is plausible that variations in intercellular tight junctions also exist between different regions of the colonic mucosa of the dog.

Permeability to mannitol gradually increased over the three flux periods in the mucosa of the transverse colon but not in the proximal descending or distal descending colon in the absence of carprofen. This result, together with regional differences in electrical conductance, suggests that the colonic mucosa of the transverse colon of the dog is distinct from that of the descending colon. Preservation of integrity and barrier function of tissues in the Ussing chamber is of major concern. The in vitro environment created in the Ussing chamber will eventually be unable to maintain integrity and barrier function of tissues. Polentarutti and co-workers showed that integrity and barrier function of the colonic and ileal mucosa of rats is better preserved in the Ussing chamber compared to the duodenal and jejunal mucosa (Polentarutti, Peterson et al. 1999). This finding was based on observation of a greater increase in permeability to mannitol over time as well as greater histologic evidence of mucosal damage in the duodenum and jejunum compared to the colon and ileum. The authors did not hypothesize regarding the cause of the differences observed (Polentarutti, Peterson et al. 1999). Such regional differences among regions of the colonic mucosa of the dog may exist. The mucosa of the transverse colon of the dog may be more susceptible to loss of integrity and loss of barrier function in the in vitro environment of the Ussing chamber.

Histologic examination of all sections of colonic mucosa from the Ussing chamber was performed to verify viability and preservation of structural integrity at the end of the experiment in the control group and to compare to the histologic findings of the sections of colonic mucosa

from the carprofen group. Polentarutti and co-workers observed sloughing of cells from the surface epithelium, sloughing of epithelial cells within the mucosal glands and edema in the colonic mucosa of rats following Ussing chamber experiments. Sloughing of cells from the surface epithelium, sloughing of epithelial cells within the mucosal glands and edema were present 60 minutes after the start of the experiment and progressively worsened throughout the duration of the experiment (180 minutes) (Polentarutti, Peterson et al. 1999). Sloughing of cells from the surface epithelium and sloughing of epithelial cells within the mucosal glands may represent loss of epithelial cells within the limits of repair of the epithelium and therefore does not indicate complete loss of tissue integrity or barrier function. Edema does not result in loss of continuity or function of the epithelium of the mucosa and therefore does not indicate complete loss of tissue integrity or barrier function. Another indication of preserved integrity and barrier function of the colonic mucosa in their study was that permeability to mannitol was stable until the end of the experiment (Polentarutti, Peterson et al. 1999). The epithelium was intact (without erosions) in most sections of colonic mucosa from the control group (63%). Erosions involving more than 10% of the epithelium were present in only 2% of the sections. Sloughing of epithelial cells within the mucosal glands was absent or mild in 83.3% of the sections. Edema was absent or mild in 63% of the sections. These mild histologic changes in combination with relatively stable permeability throughout the experiment suggest that integrity and barrier function were adequately preserved for the colonic mucosa from the control group. Thus, presence of severe damage to the epithelium of the colonic mucosa (sloughing of cells from the surface epithelium and erosions) in the sections from the carprofen group can reasonably be attributed to the effect of carprofen.

One section of colonic mucosa placed in formalin immediately after harvesting of the colon (not bathed in the Ussing chamber) from each dog in the control and the carprofen group

was examined to look for histologic evidence of preexisting colonic disease. While it is presumed that the findings in the section examined represent the entire colon it is taken from, we cannot determine whether any changes observed in one section represent a focal change limited to that section or diffuse disease. While isolated sections had some mild changes, there was no significant difference in the frequency of this finding between groups. Thus, under the constraints of the experimental design, we believe pre-existing disease was not a factor in the results.

The concentration of carprofen in the bathing solution for the carprofen group was 400µg/ml. The maximum plasma concentration after an oral dose of 25mg of carprofen in beagles reported by Clark and co-workers is 18.7µg/ml. This corresponds approximately to 2.2mg/kg or half the total recommended daily dose of carprofen (Fox and Johnston 1997) assuming that the dogs were of normal weight and size for the breed (Deavers, Huggins et al. 1972). Clark and co-workers showed that plasma concentration of carprofen is proportional to the dose administered orally (Clark, Chieffo et al. 2003). It is unknown whether plasma concentrations of 400µg/ml carprofen can be achieved in vivo and whether this linear relation would be maintained at such high plasma concentrations. If so, the colonic mucosa in our study was exposed to a concentration of carprofen approximately ten times that achieved with a full single daily dose administered orally in vivo. This magnitude of concentration is not outside the realm of a clinical overdose. Our results show that at a concentration of 400µg/ml, carprofen does compromise the in vitro integrity and barrier function and causes sloughing of cells and erosions of the colonic mucosa of the dog. The minimum concentration of carprofen resulting in damage to the colonic mucosa of the dog has not been determined. Whether the effect of a given plasma concentration of carprofen on the colonic mucosa of the dog in vivo is the same as the

effect of the corresponding concentration of carprofen in vitro is unknown. Whether repeated dosing of carprofen has a cumulative damaging effect on the colonic mucosa of the dog in vivo is also unknown.

CHAPTER 5. CONCLUSION

Carprofen increases in vitro electrical conductance and permeability to mannitol and causes sloughing of cells from the surface epithelium and erosions of the colonic mucosa of the dog. Together, these findings suggest compromise of the integrity and loss of barrier function of the colonic mucosa.

Cells of the epithelium of the colonic mucosa are held together by intercellular tight junctions. The tight junctions allow the epithelium of the mucosa to function as a selectively permeable barrier between the lumen and the interstitial space. The presence of intercellular tight junctions forces transport of molecules across the epithelium of the colonic mucosa through either the transcellular or the paracellular pathway. Increased electrical conductance of the colonic mucosa in this study may reflect changes in transcellular and paracellular transport of ions across the epithelium of the colonic mucosa, or compromise of the functional integrity of intercellular tight junctions. Because there is no active transport of mannitol in the colon of the dog, increased permeability to mannitol in this study is likely due to compromised functional integrity of intercellular tight junctions of the colonic mucosa.

We propose that increased electrical conductance, increased permeability to mannitol, sloughing of cells from the surface epithelium and erosions are due to a direct effect of carprofen on the colonic mucosa of the dog. The direct effect of non-steroidal anti-inflammatory drugs has previously been demonstrated. Non-steroidal anti-inflammatory drugs uncouple oxidative phosphorylation in the mitochondria of the epithelium of the intestinal mucosa which secondarily affects the functional integrity of intercellular tight junctions.

To clarify the mechanism underlying the effects of carprofen on the colonic mucosa of the dog, further studies are needed. Systems for the transport of ions across the epithelium of the colonic mucosa of the dog under normal conditions and in the presence of carprofen can be

studied by use of radiolabelled ions in an Ussing chamber system similar to the one used in this study. The effect of carprofen on intercellular tight junctions can be studied through permeability experiments such as this study with complementary information obtained from electron microscopic assessment of mitochondrial and intercellular tight junction morphology.

The information obtained in this study is of clinical importance as compromise of integrity and loss of barrier function of the colonic mucosa could be of concern in vivo. Increased permeability of the intestinal mucosa constitutes the earliest and mildest stage of injury to the intestinal mucosa caused by non-steroidal anti-inflammatory drugs. This may progress to mucosal ulcers. In people with Crohn's disease, as well as in animals with inflammatory bowel disease, increased permeability of the intestinal mucosa may underlie development and relapses of active disease.

Further information regarding the clinical consequences of the effects of carprofen on the colonic mucosa of the dog could be obtained by conducting experiments similar to this one with non-steroidal anti-inflammatory drugs other than carprofen and comparing their effects to those of carprofen. The results of our study could also be compared to results of experiments in which carprofen would be administered in vivo, prior to euthanasia rather than added to the bathing solution in the Ussing chamber. Our study does not evaluate the effects of varying plasma concentrations of carprofen on the colonic mucosa of dogs. Permeability and structure of the colonic mucosa of dogs with clinical signs of colonic disease (mostly hematochezia) associated with the administration of carprofen or other non-steroidal anti-inflammatory drugs could be studied with the Ussing chamber system, light microscopy and electron microscopy. Similar experiments could be conducted on the colonic mucosa of dogs with inflammatory bowel disease receiving carprofen or other non-steroidal anti-inflammatory drugs.

Electrical conductance and permeability vary between anatomical regions of the colonic mucosa in omnivorous species. In this study, electrical conductance was higher in the mucosa of the transverse colon compared to the mucosa of the descending colon of the dog in the absence of carprofen. Carprofen annulled this difference in electrical conductance. Regional differences in electrical conductance of the colonic mucosa of the dog may reflect differences in transport of ions across the epithelium of the colonic mucosa or variations in intercellular tight junctions. Permeability to mannitol gradually increased over time in the mucosa of the transverse colon but not in the mucosa of the descending colon in the absence of carprofen. The mucosa of the transverse colon of the dog may be more susceptible to loss of integrity and loss of barrier function in an in vitro environment such as the Ussing chamber.

Regional differences in electrical conductance and permeability to mannitol should be taken into account in designing future studies of the colonic mucosa of the dog.

To better explain the mechanism underlying regional difference in electrical conductance and permeability between the mucosa of the transverse and the descending colon, further studies are needed. Systems for transport of ions across the epithelium of the colonic mucosa in the transverse colon and in the descending colon of the dog can be studied by use of radiolabelled ions in the Ussing chamber system. Intercellular tight junctions in the epithelium of the mucosa of the transverse and the descending colon of the dog can be studied by use of electron microscopy, immunohistochemistry of tight junctions associated proteins and western blot analysis.

BIBLIOGRAPHY

Ahrens, F., G. Gabel, et al. (2003). "A23187-activated mast cells affect intestinal function in the pig proximal colon--role for prostaglandins." Inflamm Res **52 Suppl 1**: S15-6.

Alberts, B., D. Bray, et al. (1994.). Biologie moléculaire de la cellule. Paris, Flammarion.

Amasheh, S., T. Schmidt, et al. (2005). "Contribution of claudin-5 to barrier properties in tight junctions of epithelial cells." Cell Tissue Res **321**: 89-96.

Barrera, S., J. Lai, et al. (1996). "Regulation by prostaglandin E2 of interleukin release by T lymphocytes in mucosa." J Cell Physiol **166**(1): 130-7.

Binder, H. J., V. Rajendran, et al. (2005). "Bicarbonate secretion, a neglected aspect of colonic ion transport." J Clin Gastroenterol **39**: S53-S58.

Bjarnason, I., J. Hayllar, et al. (1993). "Side effects of nonsteroidal anti-inflammatory drugs on the small and large intestine in humans." Gastroenterology **104**(6): 1832-47.

Breslin, N. P., C. Nash, et al. (2001). "Intestinal permeability is increased in a proportion of spouses of patients with Crohn's disease." Am J Gastroenterol **96**(10): 2934-8.

Charney, A. N., R. W. Egnor, et al. (2001). "Effect of E. coli heat-stable enterotoxin on colonic transport in guanylyl cyclase C receptor-deficient mice." Am J Physiol **280**: G216-G221.

Clark, T. P., C. Chieffo, et al. (2003). "The steady-state pharmacokinetics and bioequivalence of carprofen administered orally and subcutaneously in dogs." J Vet Pharmacol Ther **26**(3): 187-92.

Day, J., B. King, et al. (2005). "A nonneuronal 5-hydroxytryptamine receptor 3 induces chloride secretion in the rat distal colonic mucosa." Am J Surg **190**(5): 736-738.

Deavers, S., R. A. Huggins, et al. (1972). "Absolute and relative organ weights of the growing beagle." Growth **36**(3): 195-208.

Dharmsathaphorn, K., K. G. Mandel, et al. (1985). "Vasoactive intestinal polypeptide-induced chloride secretion by a colonic epithelial cell line. Direct participation of a basolaterally localized Na+,K+,Cl- cotransport system." J Clin Invest **75**(2): 462-71.

Dharmsathaphorn, K. and S. J. Pandol (1986). "Mechanism of chloride secretion induced by carbachol in a colonic epithelial cell line." J Clin Invest **77**(2): 348-54.

Eberhart, C. E. and R. N. Dubois (1995). "Eicosanoids and the gastrointestinal tract." Gastroenterology **109**(1): 285-301.

Engelmann, B. E., N. Bindslev, et al. (2002). "Effects of cyclooxygenase and lipoxygenase inhibition on basal- and serotonin-induced ion transport in rat colon." Comp Biochem Physiol C Toxicol Pharmacol **132**(1): 37-52.

Evans, H. E. (1993). Miller's anatomy of the dog. Philadelphia, W.B. Saunders.

Evans, H. E. and A. deLaHunta (1996). Miller's Guide to the Dissection of the Dog. Philadelphia, W.B. Saunders.

Evans, J. M., A. D. McMahon, et al. (1997). "Non-steroidal anti-inflammatory drugs are associated with emergency admission to hospital for colitis due to inflammatory bowel disease." Gut **40**(5): 619-22.

Fawcett, D. W. (1994). Bloom and Fawcett, A Textbook of Histology. New York, Chapman & Hall.

Fox, S. M. and S. A. Johnston (1997). "Use of carprofen for the treatment of pain and inflammation in dogs." J Am Vet Med Assoc **210**(10): 1493-8.

Fujita, H., H. Chiba, et al. (2006). "Differential expression and subcellular localization of claudin-7, -8, -12, -13, and -15 along the mouse intestine." J Histochem Cytochem **54**(8): 933-44.

Gana, T. J., B. R. MacPherson, et al. (1988). "Gastric mucosal blood flow in misoprostol pretreated aspirin-induced ulceration." Ann Surg **207**(3): 327-34.

Gartner, L. P. and J. L. Hiatt (1997). Color Textbook of Histology. Philadelphia, W.B. Saunders.

Gawenis, L. R., H. Hut, et al. (2004). "Electroneutral sodium absorption and electrogenic anion secretion across murine small intestine ae regulated in parallel." Am J Physiol Gastrointest Liver Physiol **287**: G1140-G1149.

Guilford, W. G., S. A. Center, et al. (1996). Small and large intestine: Normal structure and function. Strombeck's Small Animal Gastroenterology. Philadelphia, W. B. Saunders**:** 318-350.

Hall, R. I., A. H. Petty, et al. (1983). "Enteritis and colitis associated with mefenamic acid." Br Med J (Clin Res Ed) **287**(6400): 1182.

Hardman, J. G., L. E. Limbird, et al. (2001). Autacoids;drug therapy of inflammation. Goodman & Gilman's The pharmacological basis of therapeutics. J. G. Hardman and L. E. Limbird. New York, McGraw-Hill**:** 643-733.

Hass, M. (1994). "The Na-K-Cl cotransporters." Am J physiol Cell Physiol **267:**: C869-C885.

Heitzmann, D., R. Warth, et al. (2000). "Regulation of the Na+2Cl-K+ cotransporter in isolated rat colon crypts." Eur J Physiol **439**: 378-384.

Hendel, J. and O. H. Nielsen (1997). "Expression of cyclooxygenase-2 mRNA in active inflammatory bowel disease." Am J Gastroenterol **92**(7): 1170-3.

Hongyu, L., D. N. Sheppard, et al. (2004). "Transepithelial electrical measurements with the Ussing chamber." J Cyst Fibrosis **3**: 123-126.

Jenkins, A. P., D. R. Trew, et al. (1991). "Do non-steroidal anti-inflammatory drugs increase colonic permeability?" Gut **32**(1): 66-9.

Josephs, M. D., G. Cheng, et al. (1999). "Products of cyclooxygenase-2 catalysis regulate postoperative bowel motility." J Surg Res **86**(1): 50-4.

Kaufmann, H. J. and H. L. Taubin (1987). "Nonsteroidal anti-inflammatory drugs activate quiescent inflammatory bowel disease." Ann Intern Med **107**(4): 513-6.

Kiliaan, A. J., P. R. Saunders, et al. (1998). "Stress stimulates transepithelial macromolecular uptake in rat jejunum." Am J Physiol **275**(5 Pt 1): G1037-44.

King, B. N., S. M. Haque, et al. (2004). "Effect of cyclooxygenase inhibition on serotonin-induced chloride secretion from rat distal colon." Surgery **136**(2): 240-5.

Kurahara, K., T. Matsumoto, et al. (2001). "Clinical and endoscopic features of nonsteroidal anti-inflammatory drug-induced colonic ulcerations." Am J Gastroenterol **96**(2): 473-80.

Lanas, A., J. Panes, et al. (2003). "Clinical implications of COX-1 and/or COX-2 inhibition for the distal gastrointestinal tract." Curr Pharm Des **9**(27): 2253-66.

Mall, M., M. Bleich, et al. (1998). "Cholinergic ion secretion in human colon requires coactivation by cAMP." Am J Physiol Gastrointest Liver Physiol **275**: G1274-G1281.

Mayol, J. M., P. Alarma-Estrany, et al. (2005). "Effect of luminal ATPase inhibitors on electrogenic ion transport in rat distal colon." Journal of Surgical Research **129**: 85-89.

Mayol, J. M., P. Alarma-Estrany, et al. (2005). "Effects of luminal ATPase inhibitors on electrogenic ion transport in rat distal colon." J Surg Res **129**(1): 85-9.

McCarn, K., B. Yursik, et al. (2002). "Peri-epithelial origin of prostanoids in the human colon." Journal of Cellular Physiology **194**: 176-185.

Meddings, J. (2000). "Barrier dysfunction and Crohn's disease." Ann N Y Acad Sci **915**: 333-8.

Meddings, J. B. (1997). "Review article: Intestinal permeability in Crohn's disease." Aliment Pharmacol Ther **11 Suppl 3**: 47-53; discussion 53-6.

Meddings, J. B., J. Jarand, et al. (1999). "Increased gastrointestinal permeability is an early lesion in the spontaneously diabetic BB rat." Am J Physiol **276**(4 Pt 1): G951-7.

Meddings, J. B. and M. G. Swain (2000). "Environmental stress-induced gastrointestinal permeability is mediated by endogenous glucocorticoids in the rat." Gastroenterology **119**(4): 1019-28.

Mlodzik-Danielewicz, N. and T. Tyrakowski (2005). "Effects of amiloride and bumetanide on hyperpolarization after movement across the distal colon epithelium." Pharmacol Rep **57**(4): 489-97.

Muresan, Z., D. L. Paul, et al. (2000). "Occludin 1B, a variant of the tight junction protein occludin." Mol Biol Cell **11**(2): 627-34.

Nedergaard, S., E. H. Larsen, et al. (1999). "Sodium recirculation and isotonic transport in toad small intestine." J Membr Biol **168**(3): 241-51.

Nejdfors, P., Q. Wang, et al. (1998). "Increased colonic permeability in patients with ulcerative colitis: an in vitro study." Scand J Gastroenterol **33**(7): 749-53.

Otani, T., K. Yamaguchi, et al. (2006). "Levels of NAD(+)-dependent 15-hydroxyprostaglandin dehydrogenase are reduced in inflammatory bowel disease: evidence for involvement of TNF-alpha." Am J Physiol Gastrointest Liver Physiol **290**(2): G361-8.

Palmer, L. G. (1992). "Epithelial Na channels: function and diversity." Annu Rev Physiol **54**: 51-66.

Pierrugues, R. and J. Fontes (1994). "[Hemorrhagic angiodysplasia of the right colon after ingestion of non-steroidal anti-inflammatory agents]." Gastroenterol Clin Biol **18**(11): 1041-2.

Polentarutti, B. I., A. L. Peterson, et al. (1999). "Evaluation of viability of excised rat intestinal segments in the Ussing chamber: investigation of morphology, electrical parameters, and permeability characteristics." Pharm Res **16**(3): 446-54.

Raab, Y., C. Sundberg, et al. (1995). "Mucosal synthesis and release of prostaglandin E2 from activated eosinophils and macrophages in ulcerative colitis." Am J Gastroenterol **90**(4): 614-20.

Rampton, D. S. and G. E. Sladen (1984). "Relationship between rectal mucosal prostaglandin production and water and electrolyte transport in ulcerative colitis." Digestion **30**(1): 13-22.

Rechkemmer, G., R. A. Frizzell, et al. (1996). "Active potassium transport across guinea-pig distal colon: action of secretagogues." J physiol **493.2**: 485-502.

Redfern, J. S. and M. Feldman (1989). "Role of endogenous prostaglandins in preventing gastrointestinal ulceration: induction of ulcers by antibodies to prostaglandins." Gastroenterology **96**(2 Pt 2 Suppl): 596-605.

Reimer, R., H. K. Heim, et al. (1992). "Effects of EP-receptor subtype specific agonists and other prostanoids on adenylate cyclase activity of duodenal epithelial cells." Prostaglandins **44**(5): 485-93.

Rubio, F., S. Seawall, et al. (1980). "Metabolism of carprofen, a nonsteroid anti-inflammatory agent, in rats, dogs, and humans." J Pharm Sci **69**(11): 1245-53.

Sahi, J., M. P. Wiggins, et al. (1996). "Calcium regulated chloride permeabilities in primary cultures of rabbit colonocytes." J Cell Physiol **168**(2): 276-83.

Schmitz, H., M. Fromm, et al. (1996). "Tumor necrosis factor-alpha induces Cl- and K+ secretion in human distal colon driven by prostaglandin E2." Am J Physiol **271**(4 Pt 1): G669-74.

Schultheiss, G., A. Siefjediers, et al. (2005). "Muscarinic receptor stimulation activates a Ca2+-dependant Cl- conductance in rat distal colon." J Membrane Biol **204**: 117-127.

Seibert, K. and J. L. Masferrer (1994). "Role of inducible cyclooxygenase (COX-2) in inflammation." Receptor **4**(1): 17-23.

Sellin, J. and R. DeSoignie (1984). "Rabbit proximal colon: a distinct transport epithelium." Am J Physiol **246**: G603-G610.

Sellin, J. H. (1999). "SCFAs: The enigma of weak electrolyte transport in the colon." News Physiol Sci **14**: 58-64.

Sellin, J. H. and R. DeSoignie (1987). "Ion transport in human colon in vitro." Gastroenterology **93**: 441-448.

Shull, G. E., M. L. Miller, et al. (2000). "Lessons from genetically engineered animal models VIII. Absorption and secretion of ions in the gastrointestinal tract." Am J Physiol Gastrointest Liver Physiol **278**: G185-G190.

Singer, II, D. W. Kawka, et al. (1998). "Cyclooxygenase 2 is induced in colonic epithelial cells in inflammatory bowel disease." Gastroenterology **115**(2): 297-306.

Singh, V. P., C. S. Patil, et al. (2004). "Aggravation of inflammatory bowel disease by cyclooxygenase-2 inhibitors in rats." Pharmacology **72**(2): 77-84.

Smith, P. R. and D. J. Benos (1991). "Epithelial Na+ channels." Annu Rev Physiol **53**: 509-530.

Soll, A. H. (1992). "Nonsteroidal anti-inflammatory drugs and ulcers." West J Med **157**(4): 465-8.

Somasundaram, S., S. Rafi, et al. (1997). "Mitochondrial damage: a possible mechanism of the "topical" phase of NSAID induced injury to the rat intestine." Gut **41**(3): 344-53.

Somasundaram, S., G. Sigthorsson, et al. (2000). "Uncoupling of intestinal mitochondrial oxidative phosphorylation and inhibition of cyclooxygenase are required for the development of NSAID-enteropathy in the rat." Aliment Pharmacol Ther **14**(5): 639-50.

Tai, Y. H., J. Flick, et al. (1996). "Regulation of tight junction resistance in T84 monolayers by elevation in intracellular Ca2+: a protein kinase C effect." J Membr Biol **149**(1): 71-9.

Thiefin, G. and L. Beaugerie (2005). "Toxic effects of nonsteroidal antiinflammatory drugs on the small bowel, colon, and rectum." Joint Bone Spine **72**(4): 286-94.

Vidyasagar, S., V. M. Rajendran, et al. (2004). "Three distinct mechanisms of HCO3- secretion in rat distal colon." Am J Physiol Cell Physiol **287**: 612-621.

Whittle, B. J. (1981). "Temporal relationship between cyclooxygenase inhibition, as measured by prostacyclin biosynthesis, and the gastrointestinal damage induced by indomethacin in the rat." Gastroenterology **80**(1): 94-8.

Wilson, J. E., N. V. Chandrasekharan, et al. (2004). "Determination of expression of cyclooxygenase-1 and -2 isozymes in canine tissues and their differential sensitivity to nonsteroidal anti-inflammatory drugs." Am J Vet Res **65**(6): 810-8.

APPENDIX I. CONDUCTANCE CONTROL

Conductance (mS/cm^2) for control group. Time is in minutes. Trans = transverse colon; Prox = proximal descending colon; Dist = distal descending colon

Dog	Time	Trans	Trans	Trans	Prox	Prox	Prox	Dist	Dist	Dist
1	30	22	25	16	12	11	12	21	7	14
1	45	19	22	14	12	11	12	17	9	13
1	60	21	21	13	11	1	12	15	9	13
1	75	21	20	13	11	11	12	15	10	14
1	90	21	20	13	11	12	11	14	10	12
1	105	23	21	13	10	12	11	15	10	11
1	120	22	21	13	10	12	11	14	10	12
1	135	24	23	15	10	12	11	15	11	13
1	150	25	23	17	11	12	11	14	11	14
1	165	26	24	18	11	12	11	14	12	16
1	180	28	27	20	11	13	12	15	12	18
1	195	32	30	24	12	14	11	15	13	20
1	210	37	35	27	13	15	11	15	13	25
1	225	42	38	33	13	15	12	15	14	31
1	240	42	43	35	14	15	12	15	15	37
2	30	10	13	14	.	11	12	15	11	.
2	45	13	13	12	.	11	12	11	10	.
2	60	15	15	11	.	10	14	12	10	.
2	75	14	14	11	.	10	13	10	10	.
2	90	18	15	11	.	11	8	11	10	.
2	105	20	16	12	.	10	17	10	9	.
2	120	32	16	12	.	11	17	10	10	.
2	135	32	17	13	.	12	19	11	11	.
2	150	32	18	13	.	14	19	12	11	.
2	165	35	19	14	.	13	20	13	11	.
2	180	40	20	14	.	14	20	14	11	.
2	195	40	21	15	.	15	21	14	12	.
2	210	47	23	15	.	17	21	20	12	.
2	225	56	23	16	.	17	23	21	12	.
2	240	56	24	16	.	18	23	24	12	.
3	30	13	14	14	12	13	12	7	7	9
3	45	11	12	12	12	13	10	7	7	8
3	60	10	11	12	11	11	9	7	7	8
3	75	10	10	11	11	11	8	7	7	7
3	90	10	10	10	12	10	8	7	8	7
3	105	10	10	11	10	11	7	7	9	7
3	120	10	10	10	10	11	7	7	10	7
3	135	11	10	11	10	12	7	7	12	8
3	150	10	9	11	11	13	7	7	16	9

3	165	1	9	11	11	15	7	8	17	9
3	180	12	10	12	11	16	8	8	19	10
3	195	14	10	13	12	17	8	10	19	10
3	210	15	11	14	12	19	9	11	19	12
3	225	16	12	15	12	18	10	12	19	12
3	240	16	13	16	12	19	11	14	18	12
4	30	9	13	13	11	10	13	21	14	14
4	45	9	13	13	10	10	10	20	11	12
4	60	8	12	12	10	9	11	19	11	10
4	75	8	12	12	9	9	10	18	10	9
4	90	8	10	12	8	9	12	19	9	8
4	105	8	9	12	9	9	11	20	9	8
4	120	8	9	12	9	9	9	19	9	9
4	135	9	9	13	9	8	10	19	9	9
4	150	9	10	13	9	9	9	19	10	9
4	165	10	10	13	9	9	10	19	10	9
4	180	12	11	14	10	10	10	20	12	9
4	195	13	12	15	11	11	10	20	13	10
4	210	15	13	15	12	11	9	21	14	11
4	225	17	14	15	14	12	9	23	14	11
4	240	17	15	16	17	13	10	24	16	12
5	30	32	.	.	11	11	9	8	11	9
5	45	29	.	.	11	11	8	7	11	10
5	60	14	.	.	8	10	9	8	11	9
5	75	12	.	.	10	10	9	8	10	9
5	90	12	.	.	11	10	9	8	10	8
5	105	12	.	.	11	9	10	7	10	8
5	120	12	.	.	11	10	10	7	10	8
5	135	11	.	.	12	10	10	7	9	8
5	150	11	.	.	13	10	11	7	9	8
5	165	11	.	.	13	11	12	10	9	8
5	180	10	.	.	14	12	13	7	9	8
5	195	10	.	.	14	13	13	7	9	9
5	210	10	.	.	16	16	15	8	9	10
5	225	10	.	.	17	17	16	8	9	10
5	240	10	.	.	19	19	17	8	10	12
6	30	18	10	15	16	24	13	10	16	17
6	45	18	9	14	14	23	12	9	16	16
6	60	16	8	13	13	22	11	9	14	15
6	75	15	8	12	13	21	12	8	14	15
6	90	14	8	11	12	21	12	7	13	14
6	105	14	7	10	11	19	11	7	12	13
6	120	13	7	9	10	17	10	7	11	12
6	135	14	7	9	8	16	9	7	10	12
6	150	14	7	8	8	14	8	7	9	11

6	165	14	8	8	8	14	8	7	9	11
6	180	13	8	9	8	13	7	7	8	10
6	195	13	8	8	8	13	8	7	8	10
6	210	13	8	9	8	13	9	7	8	10
6	225	13	8	10	8	12	8	6	8	10
6	240	13	9	11	9	6	9	6	10	11
7	30	11	9	8	10	11	8	8	9	.
7	45	9	8	8	10	11	8	9	9	.
7	60	9	7	7	10	0	8	9	8	.
7	75	10	7	7	9	10	8	10	8	.
7	90	11	6	7	9	10	9	11	8	.
7	105	12	6	7	9	11	9	12	8	.
7	120	13	6	8	9	11	9	12	9	.
7	135	12	6	9	10	12	9	13	11	.
7	150	13	7	11	11	12	9	13	13	.
7	165	14	8	13	12	13	9	17	15	.
7	180	15	9	16	13	15	10	17	17	.
7	195	17	10	19	20	16	10	18	18	.
7	210	18	11	24	22	18	10	19	19	.
7	225	18	13	26	23	17	11	20	20	.
7	240	18	14	28	23	18	12	20	21	.

APPENDIX II. CONDUCTANCE CARPROFEN

Conductance (mS/cm^2) for carprofen group. Time is in minutes. Trans = transverse colon; Prox = proximal descending colon; Dist = distal descending colon

Dog	Time	Trans	Trans	Trans	Prox	Prox	Prox	Dist	Dist	Dist
1	30	8	.	10	11	8	8	10	12	7
1	45	6	.	9	7	9	8	9	14	6
1	60	8	.	7	8	7	8	7	12	6
1	75	6	.	9	9	11	9	11	13	7
1	90	6	.	9	7	10	9	8	14	8
1	105	8	.	8	8	9	9	8	15	8
1	120	8	.	9	8	10	11	9	17	9
1	135	13	.	9	9	12	13	11	15	10
1	150	16	.	11	10	14	15	12	16	11
1	165	18	.	13	11	18	15	14	17	13
1	180	19	.	15	14	21	19	16	18	14
1	195	21	.	17	14	24	23	18	18	15
1	210	27	.	19	16	29	24	21	19	16
1	225	29	.	21	19	35	29	23	21	19
1	240	29	.	21	23	40	35	27	23	21
2	30	.	17	13	9	12	.	11	11	10
2	45	.	17	11	11	14	.	13	11	13
2	60	.	16	10	10	13	.	12	11	13
2	75	.	20	15	14	23	.	16	16	19
2	90	.	19	14	13	24	.	23	13	26
2	105	.	18	14	13	27	.	25	13	28
2	120	.	17	19	15	32	.	28	23	32
2	135	.	20	21	19	27	.	32	24	34
2	150	.	21	24	21	40	.	34	32	37
2	165	.	24	27	24	34	.	37	45	40
2	180	.	27	32	29	43	.	39	39	43
2	195	.	31	34	30	44	.	43	38	47
2	210	.	33	40	35	49	.	48	45	.
2	225	.	36	41	39	51	.	50	48	49
2	240	.	36	46	40	52	.	52	50	49
3	30	9	8	9	9	7	8	9	9	8
3	45	9	9	10	8	10	10	9	9	8
3	60	10	8	14	9	9	10	8	9	7
3	75	10	8	11	13	12	9	9	12	10
3	90	10	15	13	12	12	11	11	20	9
3	105	12	16	18	14	12	15	13	53	13
3	120	13	14	21	16	13	19	13	27	15
3	135	14	16	26	24	18	19	14	29	
3	150	18	17	32	35	21	21	20	31	18

3	165	21	29	53	15	24	27	17	30	19
3	180	24	27	318	159	27	32	18	33	23
3	195	27	29	106	159	27	35	19	30	45
3	210	29	32	106	0	35	35	20	31	32
3	225	32	32	159	0	40	40	24	34	35
3	240	32	29	318	32	45	40	25	38	40
4	30	10	9	15	11	9	13	11	13	13
4	45	10	10	13	11	9	13	14	13	13
4	60	9	9	11	12	9	12	13	12	13
4	75	9	9	12	12	9	12	12	11	13
4	90	10	9	13	11	9	11	12	12	14
4	105	11	9	13	11	9	13	13	13	15
4	120	14	10	15	11	10	14	14	14	14
4	135	16	12	17	13	11	18	16	15	16
4	150	19	15	19	35	15	15	18	18	17
4	165	20	19	21	21	18	18	20	21	19
4	180	20	20	23	23	20	19	21	23	21
4	195	21	21	27	32	20	21	27	25	24
4	210	23	21	27	35	21	23	29	25	24
4	225	23	24	29		23	24	32	27	24
4	240	23	24	29	37	24	24	32	28	24
5	30	12	12	11	11	13	13	11	10	13
5	45	14	13	12	9	10	13	10	11	11
5	60	12	12	11	8	10	13	9	10	11
5	75	12	12	13	9	10	16	9	10	11
5	90	14	13	12	10	11	16	10	16	11
5	105	14	16	13	10	11	19	10	11	12
5	120	16	17	15	12	13	22	11	12	14
5	135	18	20	18	14	15	24	12	14	15
5	150	19	21	21	17	18	27	13	15	16
5	165	21	23	23	20	19	32	14	17	17
5	180	23	24	24	21	22	37	14	18	19
5	195	23	32	27	21	24	45	16	19	20
5	210	29	32	32	23	32	53	17	19	23
5	225	35	45	45	30	29	63	23	21	30
5	240	35	45	53	32	53	45	27	23	33
6	30	10	8	9	8	12	8	9	9	12
6	45	10	8	8	7	11	7	8	8	11
6	60	11	10	9	7	10	7	8	8	11
6	75	12	12	11	7	10	7	9	8	12
6	90	13	11	10	7	9	8	9	9	13
6	105	15	17	11	8	10	9	10	10	14
6	120	16	19	12	9	11	10	12	11	14
6	135	16	20	14	9	11	11	13	13	16
6	150	16	21	17	10	12	12	13	15	16

6	165	18	30	15	11	13	13	14	14	17
6	180	20	33	18	12	14	14	15	15	17
6	195	23	33	20	12	17	16	17	16	20
6	210	24	36	24	14	-13	18	18		21
6	225	27	39	27	16	21	20	19	19	24
6	240	27	40	32	17	23	23	20	19	24
7	30	18	9	10	10	9	11	10	11	11
7	45	17	9	10	11	9	10	9	10	10
7	60	15	7	7	11	9	10	8	9	10
7	75	17	8	8	13	8	9	9	9	11
7	90	19	8	10	9	9	10	7	10	13
7	105	19	7	9	9	8	9	8	10	12
7	120	21	8	9	11	8	9	8	10	14
7	135	0	9	11	12	10	10	9	12	13
7	150	27	10	13	14	11	11	10	13	7
7	165	35	11	16	13	12	13	12	14	16
7	180	40	12	13	15	13	14	12	15	18
7	195	53	14	15	17	15	14	14	15	18
7	210	53	15	17	21	18	14	14	17	19
7	225	53	17	18	21	19	15	16	18	20
7	240	53	19	19	21	21	17	18	19	20

APPENDIX III. FLUX CONTROL

Mucosal to serosal flux (µmol/cm^2*h) of mannitol for control group. [a]2 = 60 to 120 minutes, 3 = 120 to 180 minutes, 4 = 180 to 240 minutes. trans = transverse colon; [b]prox = proximal descending colon; dist = distal descending colon

Dog	Chamber	Period[a]	Region[b]	Flux
1	1	2	trans	0.10
1	1	3	trans	0.12
1	1	4	trans	0.23
1	2	2	trans	0.13
1	2	3	trans	0.19
1	2	4	trans	0.27
1	3	2	prox	0.02
1	3	3	prox	0.05
1	3	4	prox	0.10
1	4	2	prox	0.15
1	4	3	prox	0.18
1	4	4	prox	0.31
1	5	2	prox	0.05
1	5	3	prox	0.33
1	5	4	prox	0.27
1	6	2	dist	0.13
1	6	3	dist	0.05
1	6	4	dist	0.32
1	7	2	dist	0.16
1	7	3	dist	0.15
1	7	4	dist	0.21
2	1	2	trans	0.15
2	1	3	trans	0.21
2	1	4	trans	0.24
2	2	2	trans	0.15
2	2	3	trans	0.26
2	2	4	trans	0.29
2	3	2	trans	0.08
2	3	3	trans	0.14
2	3	4	trans	0.17
2	4	2	prox	0.02
2	4	3	prox	0.23
2	4	4	prox	0.09
2	5	2	prox	0.09
2	5	3	prox	0.18
2	5	4	prox	0.15
2	6	2	prox	0.07
2	6	3	prox	0.12

2	6	4	prox	0.12
2	7	2	dist	0.11
2	7	3	dist	0.14
2	7	4	dist	0.17
2	8	2	dist	0.10
2	8	3	dist	0.13
2	8	4	dist	0.19
2	9	2	dist	0.03
2	9	3	dist	0.18
2	9	4	dist	0.08
3	1	2	trans	0.14
3	1	3	trans	0.24
3	1	4	trans	0.22
3	2	2	trans	0.20
3	2	3	trans	0.24
3	2	4	trans	0.24
3	3	2	trans	0.13
3	3	3	trans	0.15
3	3	4	trans	0.21
3	4	2	prox	0.15
3	4	3	prox	0.19
3	4	4	prox	0.14
3	5	2	prox	0.13
3	5	3	prox	0.35
3	5	4	prox	0.17
3	6	2	prox	0.09
3	6	3	prox	0.10
3	6	4	prox	0.11
3	7	2	dist	0.08
3	7	3	dist	0.08
3	7	4	dist	0.15
3	8	2	dist	0.12
3	8	3	dist	0.25
3	8	4	dist	0.29
3	9	2	dist	0.07
3	9	3	dist	0.16
3	9	4	dist	-0.01
4	1	2	trans	0.24
4	1	3	trans	0.26
4	1	4	trans	0.23
4	2	2	trans	0.16
4	2	3	trans	0.16
4	2	4	trans	0.27
4	3	2	trans	0.14
4	3	3	trans	0.16

4	3	4	trans	0.21
4	4	2	prox	0.14
4	4	3	prox	0.15
4	4	4	prox	0.24
4	5	2	prox	0.1
4	5	3	prox	0.12
4	5	4	prox	0.14
4	6	2	prox	0.18
4	6	3	prox	0.3
4	6	4	prox	0.38
4	7	2	dist	0.13
4	7	3	dist	0.09
4	7	4	dist	0.14
4	8	2	dist	0.13
4	8	3	dist	.13
4	8	4	dist	.23
4	9	2	dist	0.13
4	9	3	dist	0.12
4	9	4	dist	0.20
5	1	2	trans	.15
5	1	3	trans	.33
5	1	4	trans	.35
5	2	2	trans	.21
5	2	3	trans	.21
5	2	4	trans	.22
5	3	2	trans	.23
5	3	3	trans	.23
5	3	4	trans	.27
5	4	2	prox	.11
5	4	3	prox	.18
5	4	4	prox	.10
5	5	2	prox	.12
5	5	3	prox	.2
5	5	4	prox	.27
5	6	2	prox	.15
5	6	3	prox	.20
5	6	4	prox	.23
5	7	2	dist	.1
5	7	3	dist	.18
5	7	4	dist	.23
5	8	2	dist	.13
5	8	3	dist	.18
5	8	4	dist	.191
5	9	2	dist	.08
5	9	3	dist	.15

5	9	4	dist	.17
6	1	2	trans	.13
6	1	3	trans	.19
6	1	4	trans	.13
6	2	2	prox	.25
6	2	3	prox	.32
6	2	4	prox	.24
6	3	2	dist	.2
6	3	3	dist	.23
6	3	4	dist	.16
6	4	2	trans	.21
6	4	3	trans	.24
6	4	4	trans	.15
6	5	2	prox	.33
6	5	3	prox	.36
6	5	4	prox	.27
6	6	2	dist	.19
6	6	3	dist	.24
6	6	4	dist	.22
6	7	2	trans	.39
6	7	3	trans	.36
6	7	4	trans	.24
6	8	2	prox	.21
6	8	3	prox	.24
6	8	4	prox	.2
6	9	2	dist	.22
6	9	3	dist	.28
6	9	4	dist	.24
7	1	2	trans	.17
7	1	3	trans	.11
7	1	4	trans	.
7	2	2	prox	.18
7	2	3	prox	.17
7	2	4	prox	.
7	3	2	dist	.25
7	3	3	dist	.22
7	3	4	dist	.
7	4	2	trans	.23
7	4	3	trans	.21
7	4	4	trans	.
7	5	2	prox	.27
7	5	3	prox	-.26
7	5	4	prox	.
7	6	2	dist	.27
7	6	3	dist	.28

7	6	4	dist	.
7	7	2	trans	.27
7	7	3	trans	.19
7	7	4	trans	.
7	8	2	prox	.27
7	8	3	prox	.24
7	8	4	prox	.
7	9	2	dist	.32
7	9	3	dist	.38
7	9	4	dist	.

APPENDIX IV. FLUX CARPROFEN

Mucosal to serosal flux (µmol/cm^2*h) of mannitol for carprofen group. [a]2 = 60 to 120 minutes, 3 = 120 to 180 minutes, 4 = 180 to 240 minutes. trans = transverse colon; [b]prox = proximal descending colon; dist = distal descending colon

Dog	Chamber	Period [a]	Region [b]	Flux
1	1	2	trans	.
1	1	3	trans	0.13
1	1	4	trans	.
1	2	2	prox	0.06
1	2	3	prox	0.11
1	2	4	prox	.
1	3	2	dist	0.06
1	3	3	dist	0.14
1	3	4	dist	.
1	4	2	trans	0.07
1	4	3	trans	0.12
1	4	4	trans	.
1	5	2	prox	0.06
1	5	3	prox	0.12
1	5	4	prox	.
1	6	2	dist	0.07
1	6	3	dist	0.15
1	6	4	dist	.
1	7	2	prox	0.05
1	7	3	prox	0.12
1	7	4	prox	.
1	8	2	dist	0.08
1	8	3	dist	0.18
1	8	4	dist	.
2	1	2	trans	0.29
2	1	3	trans	0.62
2	1	4	trans	0.97
2	2	2	prox	0.20
2	2	3	prox	0.39
2	2	4	prox	0.81
2	3	2	dist	0.08
2	3	3	dist	0.44
2	3	4	dist	0.41
2	4	2	trans	0.21
2	4	3	trans	0.69
2	4	4	trans	1.07
2	5	2	prox	0.18
2	5	3	prox	0.47

2	5	4	prox	0.59
2	6	2	dist	0.33
2	6	3	dist	0.89
2	6	4	dist	1.27
2	7	2	trans	-0.07
2	7	3	trans	0.34
2	7	4	trans	0.69
2	8	2	prox	0.24
2	8	3	prox	0.57
2	8	4	prox	0.61
2	9	2	dist	0.10
2	9	3	dist	0.23
2	9	4	dist	0.43
3	1	2	trans	0.07
3	1	3	trans	0.14
3	1	4	trans	0.23
3	2	2	prox	0.07
3	2	3	prox	0.16
3	2	4	prox	0.30
3	3	2	dist	0.06
3	3	3	dist	0.14
3	3	4	dist	0.24
3	4	2	trans	0.08
3	4	3	trans	0.21
3	4	4	trans	0.40
3	5	2	prox	0.11
3	5	3	prox	0.36
3	5	4	prox	0.55
3	6	2	dist	0.07
3	6	3	dist	0.24
3	6	4	dist	0.31
3	7	2	trans	0.08
3	7	3	trans	0.25
3	7	4	trans	0.39
3	8	2	prox	0.06
3	8	3	prox	0.20
3	8	4	prox	0.27
3	9	2	dist	0.09
3	9	3	dist	0.32
3	9	4	dist	0.38
4	1	2	trans	0.18
4	1	3	trans	0.38
4	1	4	trans	0.66
4	2	2	prox	0.10
4	2	3	prox	0.19

4	2	4	prox	0.37
4	3	2	dist	0.12
4	3	3	dist	0.18
4	3	4	dist	0.25
4	4	2	trans	0.08
4	4	3	trans	0.16
4	4	4	trans	0.24
4	5	2	prox	0.12
4	5	3	prox	0.20
4	5	4	prox	0.32
4	6	2	dist	0.12
4	6	3	dist	0.19
4	6	4	dist	0.40
4	7	2	trans	0.13
4	7	3	trans	0.27
4	7	4	trans	0.45
4	8	2	prox	0.09
4	8	3	prox	0.14
4	8	4	prox	0.26
4	9	2	dist	0.11
4	9	3	dist	0.17
4	9	4	dist	0.43
5	1	2	trans	0.13
5	1	3	trans	0.20
5	1	4	trans	0.46
5	2	2	prox	0.09
5	2	3	prox	0.19
5	2	4	prox	0.44
5	3	2	dist	0.07
5	3	3	dist	0.13
5	3	4	dist	0.26
5	4	2	trans	0.12
5	4	3	trans	0.22
5	4	4	trans	0.45
5	5	2	prox	0.08
5	5	3	prox	0.15
5	5	4	prox	0.29
5	6	2	dist	0.07
5	6	3	dist	0.13
5	6	4	dist	0.22
5	7	2	trans	.08
5	7	3	trans	.12
5	7	4	trans	.21
5	8	2	prox	0.14
5	8	3	prox	0.25

5	8	4	prox	0.54
5	9	2	dist	0.00
5	9	3	dist	0.23
5	9	4	dist	0.29
6	1	2	trans	0.13
6	1	3	trans	0.15
6	1	4	trans	0.30
6	2	2	prox	0.10
6	2	3	prox	0.13
6	2	4	prox	0.26
6	3	2	dist	0.08
6	3	3	dist	0.14
6	3	4	dist	0.28
6	4	2	trans	0.10
6	4	3	trans	0.12
6	4	4	trans	0.30
6	5	2	prox	0.09
6	5	3	prox	0.15
6	5	4	prox	0.23
6	6	2	dist	0.13
6	6	3	dist	0.19
6	6	4	dist	0.34
6	7	2	trans	0.09
6	7	3	trans	0.11
6	7	4	trans	0.36
6	8	2	prox	0.07
6	8	3	prox	0.14
6	8	4	prox	0.28
6	9	2	dist	0.09
6	9	3	dist	0.15
6	9	4	dist	0.32
7	1	2	trans	0.05
7	1	3	trans	0.06
7	1	4	trans	0.07
7	2	2	prox	0.07
7	2	3	prox	0.11
7	2	4	prox	0.26
7	3	2	dist	0.05
7	3	3	dist	0.10
7	3	4	dist	0.20
7	4	2	trans	0.07
7	4	3	trans	0.12
7	4	4	trans	0.28
7	5	2	prox	0.09
7	5	3	prox	0.16

7	5	4	prox	0.24
7	6	2	dist	0.09
7	6	3	dist	0.13
7	6	4	dist	0.27
7	7	2	trans	0.10
7	7	3	trans	0.18
7	7	4	trans	0.29
7	8	2	prox	0.08
7	8	3	prox	0.19
7	8	4	prox	0.30
7	9	2	dist	0.09
7	9	3	dist	0.14
7	9	4	dist	0.24

APPENDIX V. HISTOLOGIC EXAMINATION CONTROL

Findings for histologic examination of sections of colonic mucosa for control group: Inflammation, edema, sloughing of cells from the surface epithelium, erosion and sloughing of epithelial cells within the mucosal glands. [a]A = absent; + = mild; ++ = moderate; +++ = severe. [b]A = absent; <10% = erosion involving less than 10% of the surface epithelium; ≥10% = erosions involving 10% of the surface epithelium or more.

Sample	Inflammation [a]	Edema [a]	Sloughing of surface epithelium [a]	Erosions [b]	Sloughing within mucosal glands [a]	Comments
CONTROL 1						
Pre-experiment	A	A	A	<10%	A	
DD	A	+++	A	<10%	A	
TRANS	A	+	++	<10%	+++	
TRANS	A	+	++	A	+++	Muscularis
PD	A	+	+	A	A	
PD	A	++	+	A	+	
DD	A	++	+	<10%	A	
DD	A	++	++	A	+	
TRANS	A	+++	+	A	A	
PD	A	++	A	A	A	
CONTROL 2						
Pre-experiment	A	A	A	A	A	
PD	A	++	+	<10%	A	
TRANS	A	A	+	A	+	Muscularis
PD	A	+	++	<10%	++	
TRANS	A	A	A	A	A	Small sample
PD	A	++	+	A	A	
DD	A	++	+	A	A	
DD	A	A	++	A	++	
TRANS	A	+	+	A	A	Lymph nodule
DD	A	+	+	A	A	artefactual cut of epithelium
CONTROL 3						
Pre-experiment	A	A	A	A	A	Parasite
DD	A	+	++	<10%	A	Bacteria within lamina propria
TRANS	A	A	+	A	A	

TRANS	A	+	+	<10%	+	Intralesional parasites
PD	A	+	+	<10%	A	
TRANS	A	+	+	A	+	
PD	A	A	A	A	+++	Muscularis; small sample
DD	A	+	A	<10%	A	lymph nodule
DD	A	+	+	A	A	
PD	A	+++	++	A	+	Superficial bacteria
CONTROL 4						
Pre-experiment	A	A	A	A	A	Lymph nodule
PD	A	++	+	<10%	+	Lymph nodule
TRANS	A	+	+	<10%	A	
DD	A	+	A	A	A	
TRANS	A	++	++	<10%	+	Lymph nodule
TRANS	A	+	+	<10%	A	
DD	A	+	A	A	A	
PD	A	+	+++	<10%	A	
DD	A	+	+	A	A	Lymph nodules
PD	A	+	+	A	+	
CONTROL 5						
Pre-experiment	A	A	+	A	A	
TRANS	A	+++	++	A	+	
PD	A	++	+++	A	+	
DD	A	+	++	<10%	A	
PD	A	+++	++	A	+	Artefactual loss of epithelium
TRANS	A	++	+++	A	A	
DD	A	++	+++	A	+	Muscularis
DD	A	++	++	A	+	
PD	A	+++	++	A	A	
TRANS	A	++	+++	A	+	
CONTROL 6						
Pre-experiment	A	A	A	A	A	Lymph nodule
TRANS	A	+	+	A	++	
TRANS	A	+	++	A	+	Lymph nodule
PD	A	A	++	<10%	++	
DD	A	A	+++	<10%	+	
PD	A	+	+	A	+	

DD	A	A	+++	**≥10%**	++	
DD	A	A	++	<10%	+	Superficial bacteria
TRANS	A	+	+++	<10%	++	
PD	A	+	+	A	+	

APPENDIX VI. HISTOLOGIC EXAMINATION CARPROFEN

Findings for histologic examination of sections of colonic mucosa for carprofen group: Inflammation, edema, sloughing of cells from the surface epithelium, erosion and sloughing of epithelial cells within the mucosal glands. [a]A = absent; + = mild; ++ = moderate; +++ = severe. [b]A = absent; <10% = erosion involving less than 10% of the surface epithelium; ≥10% = erosions involving 10% of the surface epithelium or more.

Sample	Inflammation	Edema	Sloughing of surface epithelium	erosions	Sloughing within mucosal glands	Comments
CARPROFEN 1						
Pre-experiment	A	A	A	A	A	
PD	A	+	+++	**≥10%**	A	
DD	A	+	++	**≥10%**	A	Lymph nodule
PD	A	+	+++	<10%	+	
TRANS	A	+	++	**≥10%**	A	
PD	A	+	++	**≥10%**	+	
TRANS	A	+	+++	<10%	A	
TRANS	A	+	+++	**≥10%**	+	
DD	A	A	+++	**≥10%**	+	
DD	+	A	+++	**≥10%**	+++	Focal neutrophilic inflammation
CARPROFEN 2						
Pre-experiment	A	A	++	<10%	A	Lymph nodule
PD	A	++	++	<10%	A	
TRANS	A	+	+++	<10%	A	Lymph nodule; focal crypt abcess
DD	A	+	++	**≥10%**	+	
DD	A	++	+++	**≥10%**	+	Lymph nodule
PD	A	++	+++	**≥10%**	A	
TRANS	A	+	++	**≥10%**	+	
DD	A	A	+++	<10%	A	
PD	A	++	+++	<10%	A	
TRANS	A	++	+++	**≥10%**	+	
CARPROFEN 3						
Pre-experiment	A	A	A	A	A	
PD	A	+	++	<10%	A	
DD	A	A	+++	<10%	A	Lymph nodule
TRANS	A	+	+++	<10%	+	

TRANS	A	A	+++	<10%	A	
PD	A	+	+++	**≥10%**	A	
DD	A	+	+++	<10%	A	Lymph nodule
TRANS	A	+	+++	**≥10%**	A	
DD	A	A	++	<10%	A	Lymph nodule
PD	A	+	+++	**≥10%**	A	
CARPROFEN 4						
Pre-experiment	A	A	++	**≥10%**	A	
PD	A	+	+++	**≥10%**	+	
DD	A	+	+++	A	A	
TRANS	A	A	+++	<10%	A	
TRANS	A	+	+++	**≥10%**	A	
PD	A	+	+++	<10%	A	
DD	A	A	+++	<10%	A	Superficial bacteria
TRANS	A	+	+++	<10%	A	
DD	A	+	+++	<10%	A	
PD	A	+	+	**≥10%**	+	
CARPROFEN 5						
Pre-experiment	A	A	+	<10%	A	Lymph nodule
TRANS	A	++	++	**≥10%**	A	
DD	A	A	++	**≥10%**	+	Lymph nodule
DD	A	+	+++	**≥10%**	A	
PD	A	++	+++	**≥10%**	A	
DD	A	A	+	**≥10%**	A	
PD	A	+	+++	**≥10%**	A	
TRANS	A	+	+++	**≥10%**	+	
TRANS	A	+	+++	**≥10%**	+	Lymph nodule; Plant foreign body with focal neutrophilic inflammation
PD	A	A	+++	**≥10%**	+	
CARPROFEN 6						
Pre-experiment	A	A	A	A	A	
DD	A	+	++	<10%	+	
TRANS	A	+	+++	<10%	++	Muscularis
PD	A	+	++	<10%	A	Lymph nodules
DD	A	+	+	<10%	A	Lymph nodule
TRANS	A	+	++	**≥10%**	A	Muscularis
PD	A	+	+++	**≥10%**	A	

DD	A	A	++	<10%	A	
PD	A	++	+++	<10%	A	
TRANS	A	++	+	**≥10%**	A	
CARPROFEN 7						
Pre-experiment	+	A	A	A	A	Focal neutrophilic inflammation
TRANS	A	+	++	**≥10%**	+	
PD	A	A	++	**≥10%**	A	
DD	A	+	++	**≥10%**	A	
PD	A	A	+++	**≥10%**	+	
TRANS	A	++	+	**≥10%**	++	
DD	A	+	+++	<10%	A	
DD	A	A	+++	<10%	+	
PD	A	A	+++	<10%	++	
TRANS	A	+	+++	<10%	+	

VITA

Catherine Briere was born in Shawinigan, province of Quebec, Canada. She graduated from College Jean-de Brebeuf in Montreal, province of Quebec, Canada, in 1992. From 1992 to 1995 she completed a three years professional degree in equine techniques. After completing a minor degree in biology at the University of Montreal, province of Quebec, Canada, she entered the Faculty of Veterinary Medicine of the University of Montreal in 1998. She graduated with honors in June 2002.

From June 2002 to June 2003 she completed a rotating internship in small animal medicine and surgery at Washington State University in Pullman, Washington. The following year, from July 2003 to May 2004 she completed an internship in small animal surgery at Affiliated Veterinary Specialists in Orange Park and Jacksonville, Florida. In July 2005, she started a Residency in Companion Animal Surgery at Louisiana State University, in Baton Rouge, Louisiana. In August 2005 she was admitted to the graduate school at Louisiana State University. She will be awarded the degree of Master of Science in veterinary medical sciences in May 2007 and will complete her residency training in July 2007.