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Assessment of an adapter modified Ussing chamber for evaluation of endoscopically obtained duodenal and colonic biopsy samples from cats and dogs

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Dedicated to Florian

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List of Abbreviations

ARD	antibiotic responsive diarrhea
Ag/AgCl	silver/silver chloride
ATP	adenosine tri phosphate
ATPase	adenosine tri phosphatase
°C	degree Celsius
cAMP	cyclic adenosine monophosphate
CFTR	cystic fibrosis transmembrane conductance regulator
cGMP	cyclic guanosine monophosphate
CIBDAI	canine inflammatory bowel disease activity index
DVC-3	trade name of the preamplifier used
DVC-1000	trade name of the amplifier used
EKC	voltage electrode
EKV	current electrode
ENaC	epithelial sodium channel
EP	prostaglandin type E receptor
FRD	food responsive diarrhea
f	female, intact
fs	female, spayed
g	gram
G	conductance
G_s	stimulating G-protein
GLUT	glucose transporter
H_1	histamine receptor 1
I	current
IBD	idiopathic inflammatory bowel disease
IFNγ	interferon gamma
I_{sc}	short circuit current
KCC1	chloride/potassium co-transporter
KCl	potassium chloride
KCNA	voltage gated potassium channel
µA	microampere
µM	micromolar (µM = micro moles of solute/liter of solution)
µg	microgram
mA	milliampere
mc	male, castrated
mg	milligram
min	minute
ml	milliliter
mM	millimolar (mM = milli moles of solute/liter of solution)
mm^2	square millimeter
mV	millivolt
n	number
Ω	Ohm
p	p-value
PBS	phosphate buffered saline
PD	potential difference
PGE	prostaglandin E

PGE_2	prostaglandin E_2
pH	-log 10 concentration of H^+ ions activity in solution
R	resistance
SD	standard deviation
SGLT	sodium-linked glucose transporter
SGLT-1	sodium-linked glucose transporter 1
TNFα	tumor necrosis factor alpha
Tris	2-amino-2-hydroxymethyl-1,3-propanediol
V	volt
WSAVA	World Small Animal Veterinarian Association
5-HT	5-hydroxytryptamine

List of Materials and Methods

Amplifier DVC-1000	World Precision Instruments, Inc., Sarasota, FL, USA
Dow Corning® high vacuum grease	Dow Corning Corporation, Midland, MI, USA
D-Glucose	Sigma-Aldrich Chemicals, St Louis, MO, USA
D-Sorbitol	Sigma-Aldrich Chemicals, St Louis, MO, USA
Electrodes EK1	World Precision Instruments, Inc., Sarasota, FL, USA
GraphPad Prism 5	GraphPad Software, Inc., La Jolla, CA, USA
Histamine	Sigma-Aldrich Chemicals, St Louis, MO, USA
iWorx LabScribe2	iWorx Inc., Dover, NH, USA
Olympus GIF-160 Gastroscope, 2.8 mm working channel	Olympus America, Inc., Center Valley, PA, USA
Olympus FB-36 K-1	Olympus America, Inc., Center Valley, PA, USA
Olympus SZ 30 Stereomicroscope	Olympus America, Inc., Melville, NY, USA
O_2/CO_2	Praxair, Houston, TX, USA
Ouabain octahydrate	Sigma-Aldrich Chemicals, St Louis, MO, USA
Phloridzin dihydrate	Sigma-Aldrich Chemicals, St Louis, MO, USA
Preamplifier DVC-3	World Precision Instruments, Inc., Sarasota, FL, USA
Prostaglandin E_2	Sigma-Aldrich Chemicals, St Louis, MO, USA
Recirculating Chiller, Model 13270-116	VWR International, West Chester, PA, USA

Serotonin hydrochloride	Sigma-Aldrich Chemicals, St Louis, MO, USA
Sodium chloride	Sigma-Aldrich Chemicals, St Louis, MO, USA
Sodium hydroxide 50%	Sigma-Aldrich Chemicals, St Louis, MO, USA
Sodium phosphate, dibasic	Sigma-Aldrich Chemicals, St Louis, MO, USA
Sodium phosphate, monobasic	Sigma-Aldrich Chemicals, St Louis, MO, USA
Standard biopsy forceps (Olympus FB-36K-1, diameter 2.8)	Olympus America, Inc., Center Valley, PA, USA
Supporting discs	eMachine Shop, Mahwah, NJ, USA
Ussing System CHM8	World Precision Instruments, Inc., Sarasota, FL, USA
Vacu/Trol Regulated Vacuum Pump, 115V	Spectrum Laboratories, Inc., Rancho Dominguez, CA, USA

Table 1: Meyler's Buffer
This table lists the ingredients of Meyler's Buffer (DE JONGE et al., 2004). The buffer solution was adjusted to pH 7.4 with sodium hydroxide. All chemicals were purchased from Sigma-Aldrich Chemicals, St Louis, MO, USA.

HEPES	10 mM
Na_2HPO_4	0.3 mM
NaH_2PO_4	0.4 mM
$MgCl_2$	1.0 mM
$CaCl_2$	1.3 mM
KCl	4.7 mM
NaCl	105 mM
$NaHCO_3$	20.2 mM

1. Introduction

Chronic enteropathies are common in both cats and dogs. The diagnosis of the underlying cause can be challenging due to the non-specific clinical presentation. For correct diagnosis, diseases with similar clinical signs, such as idiopathic inflammatory bowel disease (IBD), food allergies, presence of pathogenic bacteria, parasitic infestations, and others must be distinguished, which requires a systematic work-up of affected patients. Several guidelines for staging patients with chronic enteropathies have been developed in the past. (ALLENSPACH et al., 2007; JERGENS et al., 2003; JERGENS et al., 2010). Recently, the World Small Animal Veterinary Association (WSAVA) Gastrointestinal Standardization Group has published endoscopic and histopathologic criteria for classification of IBD in dogs and cats (WASHABAU et al., 2010). However, approximately 50% of the patients with chronic gastrointestinal diseases and suspected IBD show no histopathological changes of mucosal biopsies (CRAVEN et al., 2004).
In humans, several gastrointestinal disorders can be further characterized by assessment of tissues using Ussing chambers (DE JONGE et al., 2004; BIJLSMA et al., 2004). Studies using Ussing chambers allow for measurements of transepithelial resistance and for assessment of secretory and absorptive processes within the intestinal mucosa. The Ussing chamber has proven its value in studies of membranes of various tissue origins and in a wide variety of species. Thus, studies involving assessment of the intestinal mucosa using an Ussing chamber might be a useful tool to identify and classify functional changes of the gastrointestinal mucosa in both dogs and cats. The device, which is commercially available, requires samples that are 3 cm^2 in size, which can only be collected during abdominal exploratory surgery. The invention of specially designed adapter discs enabled investigation of endoscopically obtained biopsies in humans (LARSEN et al., 2001). This adapter modified Ussing Chamber provides a unique possibility to use endoscopically collected biopsies rather than surgically obtained intestinal samples and may thus also be beneficial in canine and feline patients.

1.1 Hypothesis and Objectives

The hypothesis of the study described here was that the adapter modified Ussing chamber can be successfully used to evaluate mucosal transport physiology in endoscopically collected duodenal and colonic biopsies from cats and dogs and can be used to observe altered intestinal transport in dogs with chronic enteropathies. In order to prove or disprove this hypothesis, the objectives of this study were:

1) to evaluate the feasibility of an adapter modified Ussing chamber for assessment of intestinal mucosal transport physiology in endoscopically collected biopsies from canine and feline duodenum and colon,
2) to evaluate mucosal transport physiology in the duodenum of healthy dogs,
3) to evaluate mucosal transport physiology in the duodenum of dogs with inflammatory bowel disease, and
4) to compare mucosal transport physiology between healthy dogs and dogs with inflammatory bowel disease.

2. Literature Review

2.1 Epithelial Function

The intestinal tract is characterized by a large functional diversity, which is apparent both macroscopically and microscopically. The functional diversity results from a large number of cell types, transporter molecules, and a variation of structure and number of the tight junctions.

Selective transport processes can take place through a transcellular or an intercellular pathway. In general, the transcellular pathway is energy-dependent and can occur against an electrochemical gradient, while the passive paracellular transport depends on an electrochemical gradient (BINDSLEV et al., 1974).

Two characteristics of the epithelial membrane can be determined experimentally: permeability and permselectivity. While permeability simply reflects the transepithelial resistance, permselectivity describes the qualitative tissue property of a preference of absorption of some electrolytes over others (POWELL, 1981).

2.1.1 Permeability

According to Kirchhoff's law ($Resistance_{total} = R_1 \times R_2/(R_1 + R_2)$), the total membrane resistance is composed of the resistance of the transcellular pathway (resistance of the apical cell barrier, the resistance of the basolateral cell barrier, and resistance of the cytosol) in parallel connection to the paracellular resistance (resistance of the tight junction and resistance of the lateral intercellular space). The resistance of the intestine is mainly determined by tight junctions (FRIZZELL and SCHULTZ, 1972). Figure 1 displays the various components of resistance.

According to the overall measured resistance, epithelia can be classified as leaky, moderately tight, or tight.

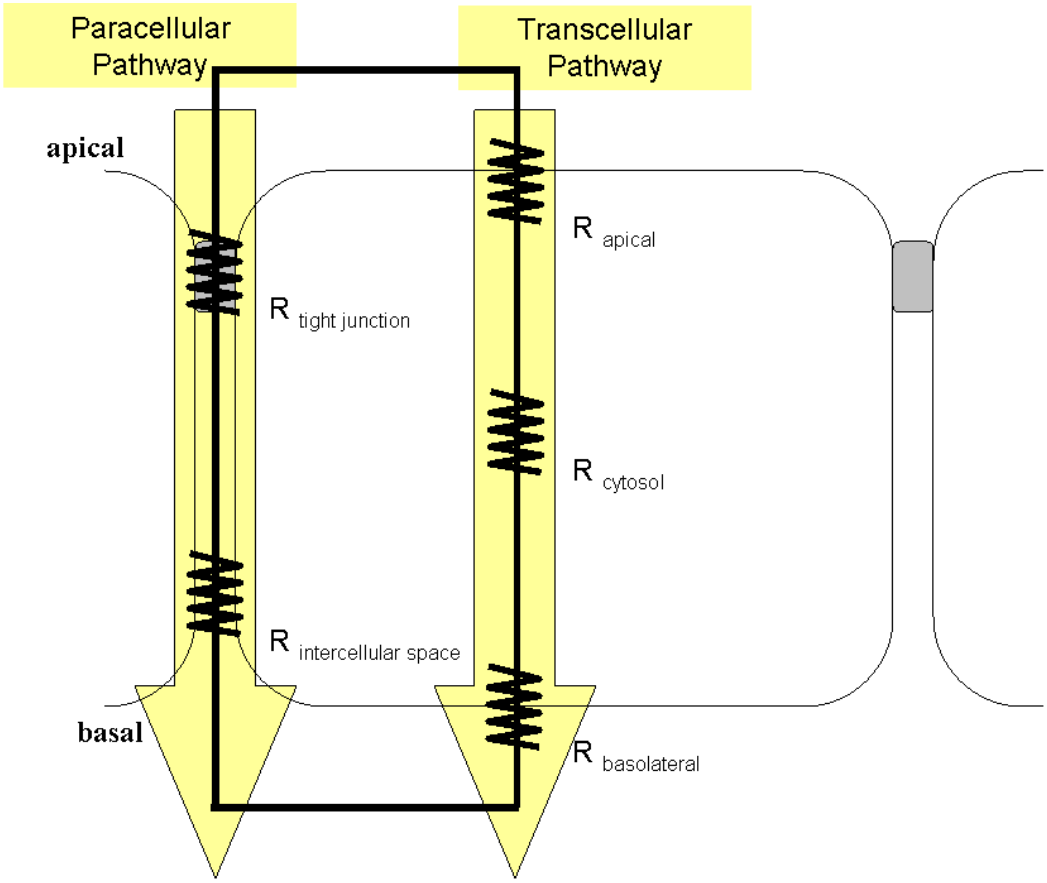

Figure 1: Illustration of epithelial resistances
This figure shows the different components of resistance that affect the overall cellular resistance of a biological membrane. The resistances of the apical membrane, the cytosol, and the basolateral membrane are in series. Parallel to this transcellular resistance is the paracellular resistance that is made up of the resistance of paracellular tight junctions and the resistance of the lateral intercellular space.

2.1.1.1 Leaky Epithelia

Based on the definition proposed by Powell, tissues with a resistance of <1000 Ω•cm^2 are considered to be leaky (POWELL, 1981). However, different investigators have differing opinions about the specific resistance values that classify membrane permeability. Another way of defining a leaky epithelium is an epithelium with a transcellular/paracellular resistance ratio that is greater than 1, or an epithelium with a paracellular conductance that is greater than 50% of the total tissue conductance. Those ratios in conductance can be used to classify epithelia, but cut-off values may vary (GITTER et al., 2000a). According to Frizzell, leaky epithelia can be defined by the paracellular pathway making up >85% of the overall conductance (FRIZZELL et al., 1972).
The epithelia of the gall bladder, small intestine, and the proximal colon are three examples of leaky epithelia. In these epithelia, the majority of mass transport occurs though the paracellular passive route. Also, paracellular transport is the main determinant of total resistance.

2.1.1.2 Moderately Tight Epithelia

Some authors refer to epithelia as medium resistance epithelia if the tissue resistance is greater than 200-300 Ω•cm^2, but less than 1000 Ω•cm^2. Examples of moderately tight epithelia in the gastrointestinal tract are the epithelia of the distal colon or the gastric antrum (CLAUSS et al., 1985).

2.1.1.3 Tight Epithelia

Tight epithelia are characterized by a low level of passive, paracellular transport. The barrier function in tight epithelia is controlled by the apically and basolaterally located transporter systems. In this type of epithelia, transcellular resistance is the predominant component of the total resistance. The total resistance of tight epithelia is typically >1000 Ω•cm^2. The epithelia of the gastric fundus and the urinary bladder are considered to be tight epithelia (POWELL, 1981).

2.1.2 Permselectivity

Permselectivity is determined by a combination of transepithelial transporter functions and the number and type of pumps, carriers, and channels that are integrated into the membrane. Every tissue has its characteristic permselectivity, which is generally associated with its function.

The permselectivity of the paracellular transport is strongly influenced by the osmotic gradient (DOBSON et al., 1976). The strength of the electrochemical gradient may vary and therefore the ability of several electrolytes or other solutes to cross the zona occludens also varies. The ability of solutes to cross the membrane barrier via the paracellular pathway increases as the resistance of the tissue decreases.

The following sections outline paracellular and transcellular transport and further detail the underlying mechanisms behind permeability and permselectivity.

2.1.3 Paracellular Transport

Cell to cell adhesion is governed by several structures including the zonulae occludentes, zonulae adhaerentes, desmosomes, and gap junctions. Tight junctions not only determine the total conductance of the epithelia, but also control the fine-tuning of paracellular substrate absorption (BINDSLEV et al., 1974; SCHNEEBERGER and LYNCH, 2004). Three major properties enable the zonulae occludentes to serve its purpose: First, tight junctions separate the apical and basolateral membrane, preventing integrated membrane proteins from changing their localization. Secondly, the charge and size selective barrier prevents solubles from freely passing the epithelial barrier. Thus, tight junctions simultaneously serve as a barrier, gateway, and channel. Thirdly, tight junctions interact with transmembrane proteins, such as those comprising the actin cytoskeleton, thus stabilizing membrane integrity not only in a functional but also in a mechanical fashion (WILL et al., 2008). In order to achieve function, tight junctions consist of three different subunits: junctional adhesion molecules, claudins, and occludins (GONZALEZ-MARISCAL et al., 2003; VAN ITALLIE and ANDERSON, 2004; WILL et al., 2008). Desmosomes and gap junctions complete the morphological alliances of the lateral intercellular space (ANDERSON and ITALIE, 1995).

2.1.4 Transcellular Transport

Certain molecules can cross the intestinal membrane via a non-selective pathway. Water, oxygen, carbon dioxide, small (4.4-16.5 Ångström) polar particles like ethanol and urea, and certain lipophilic substances, such as steroid hormones, can cross biological membranes by diffusion (SIFLINGER-BIRNBOIM et al., 1987). This net transport of molecules from a region of higher concentration to a region of lower concentration is based on random molecular motion and occurs until equal concentrations are reached on both sides of the cell.

As it is impossible for hydrophilic substances to directly cross the phospholipid bilayer of the intestinal epithelia, integrated membrane proteins provide selective permeability and protection from hydrophobic interactions. Integral proteins control not only the charge, but also the size of passing molecules. This whole process is passive and cannot be saturated. Both the chemical and electrical gradient plays a major role in this movement.

Facilitated diffusion describes the spontaneous movement of molecules or ions across a biological membrane through specific transmembrane proteins. The passive carrier proteins that provide facilitated diffusion recognize a specific substrate (VOET et al., 2002). The substrate binds with a specific binding affinity. The carrier then captures the substrate within its molecular structure, causing an internal translocation of the former. This translocation carries the substrate across the membrane, where it is then released. All these steps are reversible (KING, 1996). The speed of this transport may be faster than unfacilitated diffusion.

Primary active transport carriers work in a similar fashion regarding substrate binding, as well as conformational changes. The crucial difference is that the active carrier mediated process occurs against a concentration gradient (PARDEE, 1968). The energy needed for this process comes from chemical energy, such as the coupled hydrolysis of adenosine tri phosphate (ATP).

Secondary active transporters utilize an electrochemical gradient, established by a primary active carrier (VOET et al., 2002). The best known example might be the secondary transport due to the activity of the basolaterally located sodium-potassium pump (Na^+/K^+ ATPase) (LINGREL and KUNTZWEILER, 1994). As the ATP dependent Na^+/K^+- transporter (primary active) pumps potassium into the cell, sodium is forced to leave the cytosol, generating a strong concentration gradient. This strong urge for sodium to enter the cell along its ionic concentration gradient, drags other carrier mediated substances (e.g., glucose) into the cytosol (i.e., secondary active transport).

2.1.5 Absorption

The fluid, nutrients, and electrolytes generated and secreted during digestion need to be absorbed, which results in the assimilation of food components and electrolytes into the animal's circulation.

The single most important process that takes place in the small intestine enabling absorption is the establishment of an electrochemical gradient of sodium across the epithelial boundary (KIMMICH, 1973). The importance of this system is exemplified by the fact that a single small intestinal enterocyte from a rat contains 150,000 sodium pumps, transporting 4.5 billion sodium ions/minute that are ultimately responsible for absorption of water, amino acids, and carbohydrates (HARMS and WRIGHT, 1980).

By the time the ingesta reach the large intestine, approximately 80% of its fluid has been absorbed (DEBONGNIE and PHILLIPS, 1978). The net movement of water

occurs by osmosis: the absorption of water is tightly coupled and therefore dependent on the absorption of solutes, particularly sodium. Even though net absorption is the result of small intestinal transport, secretion, predominantly of chloride and bicarbonate, also occurs. The mechanisms are very similar to the secretory process in the large intestine described in section 2.1.6.

2.1.5.1 Absorption of Sodium and Chloride

Electroneutral sodium chloride (NaCl) transport occurs due to luminal potassium/proton (Na^+/H^+) exchange, as well as chloride/bicarbonate (Cl^-/HCO_3^-) exchange. Basolaterally located Na^+/K^+ ATPases generate the driving force for activating those secondary active transporters by lowering the cytosolic Na^+ concentration. Electrogenic Na^+ absorption happens mainly via epithelial Na^+ channels (ENaC) located at the apical enterocyte surface (DAWSON, 1991; KOEFOED-JOHNSEN and USSING, 1958). As the electric potential of the cell is negative compared to the intestinal lumen, a large electrical driving force for sodium absorption is established by this electrochemical gradient. The channel gated sodium uptake is accompanied by passive chloride movements through paracellular and cystic fibrosis transport receptor (CFTR) pathways. CFTR channels strongly influence the activity of the Na^+/H^+, as well as Cl^-/HCO_3^- electroneutral transporter systems (BERDIEV et al., 2009; POULSEN et al., 1994).
Absorbed sodium is rapidly exported from the cell via basolaterally located Na^+/K^+ ATPases. When a lot of sodium is entering the apical surface of the cell, a lot of sodium also is excreted on the basolateral side, creating a high osmolarity in the intercellular space between the individual enterocytes. Water, as well as sodium, then diffuses into the capillary blood within the villus. Figure 2 shows the process of Na^+ absorption in enterocytes. Significant species-specific differences exist regarding the localization and contribution of the electroneutral and electrogenic transport processes. (BINDER et al., 1987; POTTER and BURLINGAME, 1986). However, limited information is available on this process in the canine and feline intestine.

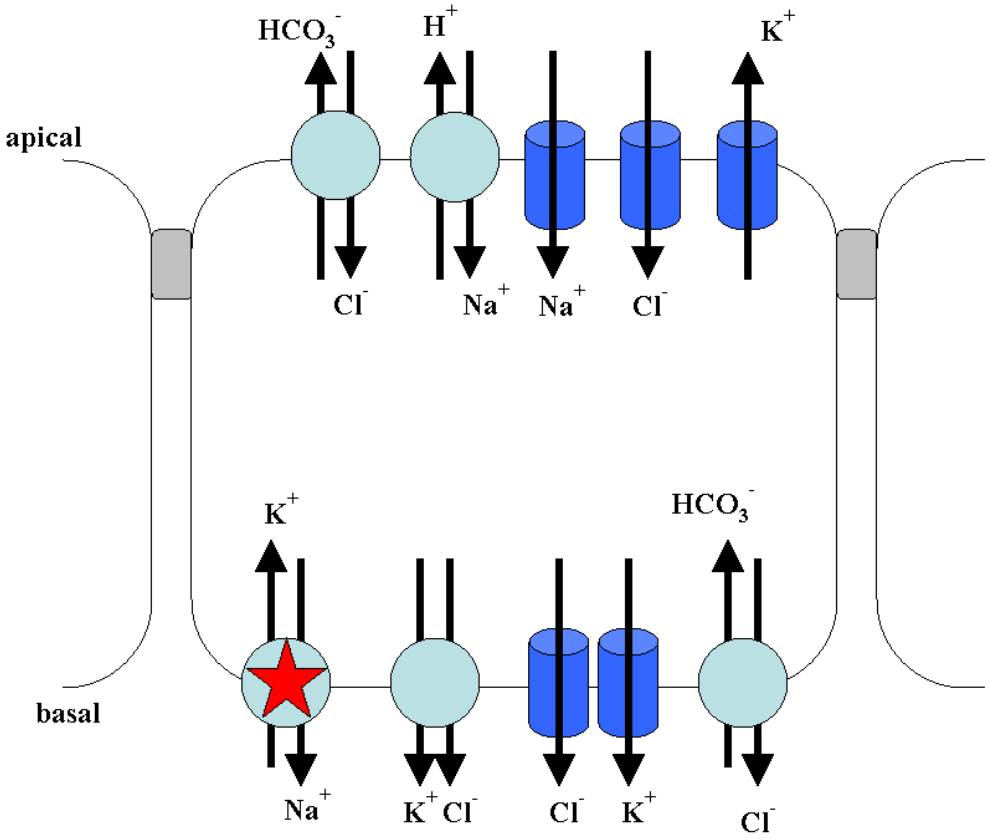

Figure 2: Model for electrolyte absorption of the enterocyte
This figure shows the transporters and channels responsible for intestinal sodium absorption. Transporters are represented by the light blue colored circles, channels by dark blue colored cylinders. The red star identifies an energy-dependent transporter (i.e., ATP dependent). While electro neutral electrolyte movement is achieved by the apical Cl^-/HCO_3^- exchanger, as well as the Na^+/H^+ exchanger, electrogenic Na^+ transport occurs due to sodium transport via the apically located epithelial sodium transporter ENaC.

2.1.5.2 Absorption of Potassium

Active potassium (K^+) absorption is mediated by two different types of apical potassium-proton-pumps. Because they consist of different subunits, they show differences in function and can be distinguished by their sensitivity to ouabain inhibition (DORGE et al., 1998). While the ouabain-sensitive type is predominantly present in crypt cells, the ouabain-insensitive type is present in enterocytes of the villi (BINDER et al., 1999). Cytosolic potassium is discharged by basolateral potassium channels and the chloride/potassium co-transporter KCC1 (SCHOUMACHER et al., 1987). Intracellular Ca^+ concentrations, as well as cAMP concentrations stimulate the potassium channels independently, maintaining the membrane potential necessary for physiologic cell function (MALL et al., 1998b).

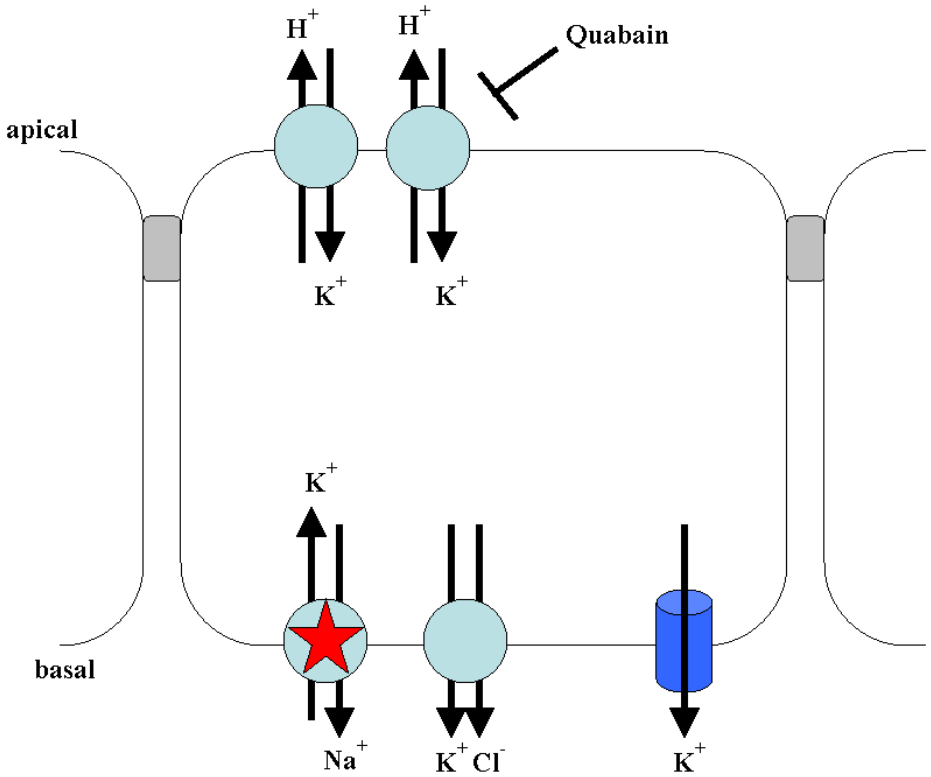

Figure 3: Mechanism of potassium absorption on a cellular level

This figure shows the mechanism of potassium uptake and discharge in mammalian colonic enterocytes. Dark blue cylinders represent channels, light blue circles represent transporters. A red star symbolizes an active transport mechanism. Luminal potassium is taken up by two different potassium transporters. These transporters are functionally equivalent but differ morphologically, and can be distinguished by ouabain sensitivity. Basolateral discharge of potassium occurs through potassium channels and KCC1 transporters.

2.1.5.3 Absorption of Glucose

Two types of glucose transporters carry glucose, which is a polar molecule, across the lipophilic membrane; the sodium-linked glucose transporter (SGLT) and the facilitative glucose transporter (GLUT) (HEDIGER and RHOADS, 1994; WRIGHT, 1993; WRIGHT et al., 2007). The transporter carrying glucose and galactose into the enterocyte is a sodium dependent hexose transporter called sodium-glucose transporter 1 (SGLT-1) (MILLER and CRANE, 1961). As the name indicates, this transporter moves both glucose and sodium into the cell and, in fact, will not transport either molecule alone. The transport of glucose by the SGLT-1 involves a series of conformational changes (HEDIGER and RHOADS, 1994; WRIGHT, 1993; WRIGHT et al., 2007):

1) The binding site of the transporter faces luminally, and is capable of binding two sodium ions, but not glucose.
2) Sodium is bound by the transporter, inducing a conformational change that opens the glucose binding pocket.
3) Glucose is bound, and the transporter re-orientates itself within the membrane. The pocket holding glucose and sodium is moved to the inner surface of the cell.
4) Sodium and glucose dissociate into the cytoplasm and the unloaded transporter re-orientates itself back to its original, outwards facing position.

After glucose has reached the cytosol it exits the enterocyte through a facilitative glucose transporter, the glucose transporter 2 (GLUT-2) located in the basolateral membrane (WOOD and TRAYHURN, 2003). After the monosaccharides are released, the force of the concentration gradient drags the substrate into the capillaries located within the villus.

The glucose/sodium coupled uptake is accompanied by transcellular water transport (LOO et al., 1996). The SGLT-1 mediated sodium-glucose transport has been shown to be electrogenic and dependent on a potential difference. Therefore, its activity can be monitored under voltage-clamping conditions (UMBACH et al., 1990).

Kellet and Brot-Laroche hypothesized about the additional occurrence of apically located GLUT-2 transporters promoted by luminal glucose (KELLETT and BROT-LAROCHE, 2005). Glucose activates a protein kinase C, a mitogen activated protein kinase, as well as a Ca^{+} dependent pathway resulting in the insertion of GLUT-2 transporters within minutes (PFANNKUCHE and GÄBEL, 2009).

Luminal glucose also induces a serotonin-mediated release of sodium and chloride in enterocytes, but since this mechanism requires an intact enteric nervous system, this pathway is irrelevant for in vitro studies such as studies in Ussing chambers as described here (SEE and BASS, 1993).

2.1.5.4 Phloridzin

Phloridzin is a specific competitive inhibitor of the SGLT-1 transporter as it binds to the transporter but is not being transferred (FERRARIS and DIAMOND, 1986; RESTREPO and KIMMICH, 1986). Originally isolated from apple trees, this substance serves as a growth and development regulator. Numerous studies have demonstrated the inhibitory effect of phloridzin on intestinal glucose uptake. Thus, this substance is widely used to study SGLT-1 transporter function (SANFORD, 1967). Concentration of $5x10^{-5}$ M and $2x10^{-3}$ M phloridzin have been shown to inhibit intestinal glucose uptake by 64% and 96%, respectively (NEWEY et al., 1963).

2.1.6 Secretion

Secretion can be defined as the release of fluid, enzymes, or electrolytes from a cell.
Along the entire length of the intestine, the three major electrolytes that determine the secretory status of the intestine are bicarbonate, potassium, and chloride. The active secretion of chloride leads to bicarbonate secretion, as chloride is recycled into the cell through an active apical antiport with bicarbonate (HOGAN et al., 1997; ISENBERG et al., 1993). As the chloride secretion is accompanied by the paracellular transport of sodium, an osmotic force for water secretion is generated. Therefore, excessive chloride secretion can lead to diarrhea.

2.1.6.1 Secretion of Chloride

Figure 4 displays the mechanism of chloride secretion by enterocytes.

1) Chloride enters the crypt epithelial cell via electroneutral cotransport with sodium and potassium. The basolaterally located sodium-potassium-chloride cotransporter ($1Na^+/1K^+/2Cl^-$ transporter) accumulates the desired chloride in the cytosol, as the large sodium gradient is the major force for this transporter.
2) The sodium gradient is established by the basolaterally located Na^+/K^+ ATPase. Several potassium channels are also located in the basolateral membrane.
3) While the voltage gated potassium channels of subtypes KCNA1 and KCNA2 have been shown to be present in the cell membranes of canine colonic mucosa, others are expressed in canine smooth muscle cells, where they contribute to gastrointestinal motility (HART et al., 1993; HEITZMANN and WARTH, 2008). The large quantities of voltage gated potassium channels prevent the cells from depolarizing and enable potassium to leave the cell to its determined amount (DONG et al., 2006).
4) Chloride accumulates in the cell and is released on the luminal side of the intestinal membrane through Ca^+ dependent chloride channels, or the cAMP-dependent CFTR (BARRETT and KEELY, 2000). The CFTR channel, which is regulated by the intracellular cAMP concentration, is essential for chloride secretion (MALL et al., 1998a).

In general, two pathways of chloride secretion can be distinguished and even some evidence of cross talk between those mechanisms has been reported (KACHINTORN et al., 1993; VAJANAPHANICH et al., 1994). Increased levels of cyclic nucleotides, such as cAMP and cGMP induce a persistent increase in chloride secretion, whereas calcium mediated stimuli cause a short term increase in chloride secretion (BARRETT, 1997).
The reason for the difference between the two pathways of secretion is that the calcium dependant pathway is subject to a negative feedback mechanism.
The calcium mediated chloride secretion results from intracellular Ca^{2+} action, that does not only activate the apical Cl^- channels, but also induces a negative feedback due to the release of muscarinic agents, followed by the mobilization of inositol 3,4,5,6 tetrakisphosphate, that ultimately blocks the apical chloride channels.
The second mechanism of Cl^- secretion is the cAMP/cGMP mediated pathway.
Stimulation of the cAMP pathway enhances Cl^- conductance by opening CFTR channels. This strong driving force results in massive Cl^- secretion leading to a depolarization of the luminal membrane. Especially in the small intestinal epithelium, where the leaky tight junctions do not definitively differentiate between the apical and basal membrane, this apical hyperpolarization can contribute to activation of basolaterally located voltage-dependent potassium channels, supporting

chloride secretion (HEITZMANN and WARTH, 2008).
The mechanisms described above were all reported in *in vitro* experiments using the human colonic epithelial cell line, T_{84}. However, the authors of these publications hypothesize that these findings can also be applied to *in vivo* conditions (BARRETT and KEELY, 2000; KEELY and BARRETT, 2000).
The negatively charged chloride forces the positively charged sodium to cross paracellular tight junctions, leading to accumulation of both sodium and chloride in the intestinal crypts. This accumulation of sodium chloride in the crypt is responsible for a strong osmotic gradient, dragging water into the lumen.

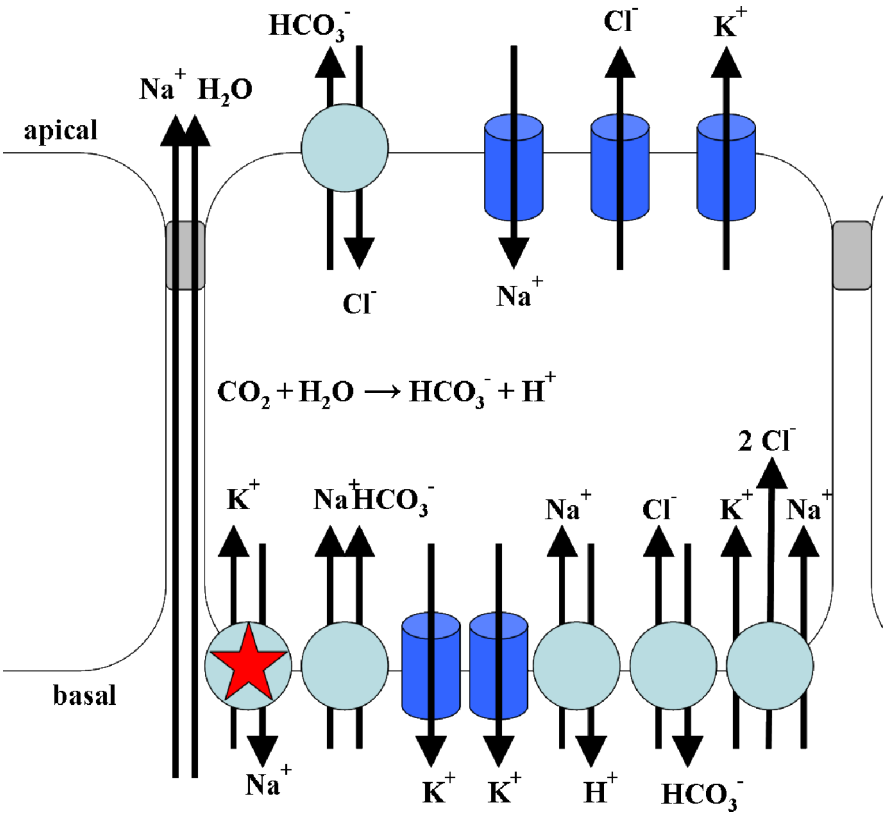

Figure 4: Principles of chloride secretion
This figure shows the physiological processes relevant for chloride secretion in enterocytes. The apical cell surface is located at the top of this diagram, while the basal cell membrane is displayed on the bottom. The tight junctions linking the enterocytes are represented by gray rectangles. Dark blue colored cylinders represent cellular channels, light blue circles symbolize transporters. Transporters marked with a red star represent active transporters that use energy from the hydrolysis of ATP. Arrows indicate the direction of electrolyte transport.
Chloride enters the enterocyte basolaterally through the $1Na^+/1K^+/2Cl^-$ co-transporter. Potassium is constantly excreted due to basolaterally located potassium channels. Sodium leaves the cytosol by the primary active Na^+/K^+ ATPase. Chloride remains in the cell, accumulating until it is released by CFTRs, or calcium modulated chloride channels. The two different secretory pathways result in a different duration of calcium secretion, as detailed in the text.

2.1.6.2 Secretion of Bicarbonate

Under physiologic conditions, the duodenum actively secretes large amounts of bicarbonate (HCO_3^-) into the intestinal lumen to buffer the acidic chyme (FURUKAWA et al., 2005). As mentioned above, bicarbonate transport into the cell is mediated by a basolaterally located sodium/bicarbonate co-transporter (JACOB et al., 2000). Additionally, bicarbonate is produced intracellularly from carbon dioxide in a reaction catalyzed by carbonic anhydrase.

The apical secretion of bicarbonate is mediated by the CFTR, as well as three different anion/HCO_3^- exchangers (SEIDLER et al., 1997). While CFTR is mainly expressed in enterocytes of the crypt region, the anion exchangers are predominantly expressed in epithelial cells of the intestinal villi (SIMPSON et al., 2007).

Activation of bicarbonate transport occurs through several pathways: prostaglandin E_2 activates cAMP, but cGMP and Ca^+ mediated pathways also do exist (TUO et al., 2006). Activation of the cGMP kinase II appears to be the most powerful stimulating factor of HCO_3^- release (PFEIFER et al., 1996). Duodenal electrogenic HCO_3^- secretion is proportionally greater than electroneutral HCO_3^- secretion (FLEMSTRÖM et al., 1982) and is the secretory pathway activated by most secragogues (ALLEN et al., 1993; FLEMSTRÖM et al., 1982; GUBA et al., 1996).

2.1.6.3 Secretion of Potassium

Potassium (K^+) secretion is induced by luminal accumulation of ATP followed by activation of the $P2Y_2$ receptor, but luminal cAMP and Ca^+ activated channels also exist (KERSTAN et al., 1998; KUNZELMANN et al., 2001). In fact, costimulation of the Ca^+ and cAMP pathway lead to a potentiated secretion (BANKS et al., 2004). The basolateral extrusion of K^+ repolarizes the cell, strengthening the electrochemical driving force for chloride secretion. Together with luminal chloride channels this leads to electroneutral KCl secretion (HEITZMANN and WARTH, 2008) (Figure 3). Potassium secretion is usually not detectable by short circuit current (I_{sc}) measurements, as the total amount of K^+ efflux is masked by the dominating chloride efflux (MALL et al., 1998b).

Although the apical potassium permeability and the secretory capacity of K^+ in normal human colonic epithelium are relatively small, recent studies suggest that enhanced activity and/or expression of high conductance apical K^+ channels may play an important role in mediating increased colonic K^+ secretion in several diseases. (PERRY and SANDLE, 2009).

2.1.6.4 Histamine

Histamine is a biogenic immune mediator accumulating in the cytoplasm of mast cells. Among other effects, histamine is known to cause fluid secretion in the canine intestine (LEE and SILVERBERG, 1976).

The histamine H_1 receptor of enterocytes is located on the basolateral membrane (WASSERMAN et al., 1988). After histamine binding to this receptor the amount of free cytosolic calcium in the cell increases, which induces a rapid and transient increase in chloride secretion (WASSERMAN et al., 1988). This pathway leaves intracellular levels of cAMP and cGMP unchanged but triggers the response of secretagougues whose response is mediated by cyclic nucleotides, such as prostaglandin (WASSERMAN et al., 1988).

Aside from the direct effect on free cytosolic calcium, histamine also influences homeostasis of chloride by an indirect mechanism. Because histamine does not cause an increase in 3,4,5,6 tetrakisphosphate and does also not activate any other inhibitory messengers of the calcium-mediated pathway, it indirectly contributes to unregulated chloride secretion (KACHINTORN et al., 1993).

It has also been demonstrated, that histamine inhibits prostaglandin-induced bicarbonate secretion via stimulation of the H_2 receptor (HOGAN et al., 1995). This pathway relies on H_2 receptors located on enteric nerve endings, but can also be induced by endogenously stored histamine deposits (BARRETT, 1997). Activation of the H_2 receptors on enteric nerve endings also leads to depolarization of the submucosal plexus, resulting in prolonged chloride secretion in the intestine (COOKE et al., 1993).

Ussing chamber experiments in canine proximal colonic mucosa demonstrate that histamine causes a marked increase in short circuit current, which is accompanied by significant Cl^- fluxes (RANGACHARI and PRIOR, 1994).

2.1.6.5 Serotonin

Serotonin, also known as 5-hydroxytryptamine (5-HT), is stored in secretory granules of enterochromaffin cells. Serotonin is believed to be the primary humoral mediator of diarrhea in human patients with carcinoid syndrome (MODLIN et al., 2005). While multiple serotonin receptor subtypes (5-HT_X) exist in the body, the receptors responsible for serotonin's intestinal function are 5-HT_2, 5-HT_3, and 5-HT_4.

The 5-HT_3 receptors are known to be responsible for nausea, vomiting, and diarrhea in patients with irritable bowel syndrome of humans. In contrast, 5-HT_4 receptors activate protein kinase A, which leads to excitatory responses and increased gastric motility. 5-HT_2 receptors located on enterocytes mainly influence absorptive and secretory function. However, pathways and interactions of these receptors are very complex and vary depending on the animal species, as well as the intestinal location (DE PONTI, 2004). The following general pathways of molecular mechanisms have been described:

Serotonin binds to 5-HT_2 receptors on the surface of epithelial cells, resulting in activation of G proteins, subsequent activation of protein kinase C, and finally an increase in free cytoplasmic calcium as well as a decreased synthesis of prostaglandins (BROWN, 1996; SIRIWARDENA et al., 1991). The final effect is the secretion of bicarbonate and the inhibition of sodium chloride absorption. In addition to the calcium–dependent secretory pathway in healthy animals there is also a submucosal neuron and interneuron mediated pathway. In this pathway serotonin binds to the 5-HT_3 and 5-HT_4 receptors, located on enterochromaffin cells of the epithelial cell layer, inducing acetylcholine release, as well as the vasopeptide IP

cascade, resulting in ion secretion (FRIELING et al., 1991; SIRIWARDENA et al., 1991).
Serotonin applied to the serosal side of intestinal membranes is known to increase net chloride and bicarbonate secretion. This is detectable as a rapid and transient rise in the short circuit current (DONOWITZ et al., 1980; KELLUM et al., 1994). However, these effects depend on the intestinal segment affected: while the mid-jejunum and ileum of rabbit intestine showed markedly altered water and electrolyte movement, the proximal jejunum and colon did not show such changes (DONOWITZ et al., 1977).
Serotonin applied to the serosal side of the intestinal mucosa is known to rapidly and transiently enhance I_{sc}, which is usually manifested as an increased net flux of Cl^- or HCO_3^- (BROWN, 1996; KELLUM et al., 1994; RANGACHARI et al., 1986). An application of serotonin at concentrations below 300 nM resulted in a monophasic increase of the short circuit current, while application of serotonin at concentrations higher than 300 nM was shown to induce a biphasic increase in I_{sc}. An effect of serotonin on the intercellular space or conductance of rabbit ileum could not be observed, when evaluated by Ussing chamber experiments and electron microscopic examination (DONOWITZ et al., 1977; DONOWITZ et al., 1980) .

2.1.6.6 Prostaglandin E_2

The response of the intestinal epithelium to prostaglandin (PGE) was first discovered in the early 70`s (KIMBERG et al., 1971) and it has been shown that prostaglandins aid in the recovery of membrane barrier function after mucosal injury. (BLIKSLAGER et al., 1999; GOOKIN et al., 2003; MOESER et al., 2006). Both cAMP and Ca^+ were shown to be responsible for mediating the effects of prostaglandin in the recovery of transmucosal resistance (BLIKSLAGER et al., 1999). However, PGE-mediated inhibition of the Na^+/H^+ exchanger also plays a major role (BLIKSLAGER et al., 2001; MOESER et al., 2006). The rapid recovery of tissue integrity, observed as an increase in tissue epithelial resistance accompanied by a significant increase of transmucosal potential difference has been demonstrated in porcine ileal and colonic samples after prostaglandin administration (BLIKSLAGER et al., 2001; MOESER et al., 2007).
The chloride secretion stimulating effect of prostaglandin was demonstrated by Reddix et al. (REDDIX et al., 1998). Stimulating effects of prostaglandin E_2 (PGE_2) on bicarbonate and sodium secretion have also been described. These effects can be visualized by a significant increase of a transmucosal potential difference after PGE_2 (BEUBLER et al., 1986; HANSEN and JAFFE, 1993; KNUTSON et al., 1995; MUNCK et al., 1988).
At least eleven different prostaglandin type E receptors (EP) interact with prostaglandin E_2. Depending on the prostaglandin concentration and the receptor involved, either stimulating or inhibiting G proteins are activated, resulting in different levels of cAMP and Ca^+ concentrations within the cell (BLIKSLAGER et al., 2000; DING et al., 1997; ICHIKAWA et al., 1996; MOSA et al., 2008, WEYMER et al., 1985). The intracellular Ca^+ mediated pathway of PGE leads to opening of basolateral K^+ channels, while increasing the intracellular cAMP leads to opening of apical Cl^- channels (type 2 Cl^- channels, expressed in the interepithelial tight junction), thus resulting in Cl^- secretion, (RECHKEMMER et al., 1996; MOESER et al., 2007; NIELSEN et al., 1998).
EP_1 and EP_3 receptors are located in the muscular layer of the stomach and duodenum, respectively, while EP_4 receptors are expressed in the mucosal layer of

both organs (DING et al., 1997). The activation of EP_1 leads to increases of free cytosolic calcium, stimulating protein kinase C and therefore smooth muscle contractions. Protein kinase C may also stimulate CTFR receptors directly, or maybe increasing their sensitivity to protein kinase A. The EP_4 receptor subtype of the mucosal layer stimulates cAMP production, resulting in enhanced chloride secretion by opening apically located CFTR channels (MOSA et al., 2008). However, the distribution and function of these EP receptors may be species-specific (GRASA et al., 2006). For example, in mice duodenal EP_3 and EP_4 receptors stimulate intracellular Ca^{2+} and cAMP concentrations, resulting in HCO_3^- secretion (TAKEUCHI et al., 2005).

2.1.6.7 Forskolin

The diterpene forskolin was originally extracted from the root of the Indian plant *coleus forskolhii* (SEAMON and DALY, 1986). Forskolin directly activates adenylyl cyclases, resulting in increased intracellular cAMP levels (TANG and HURLEY, 1998). cAMP in turn, activates prostaglandin kinase A, which in turn stimulates apical potassium and CFTR channels. Hence forskolin induces cellular potassium and chloride loss (PERRY and SANDLE, 2009). The cAMP mediated effect of forskolin is significantly supported by PGE_2 induced expression of stimulating G-proteins (G_s) as these support intracellular cAMP concentrations and hence trigger the forskolin action (INSEL and OSTROM, 2003).

Forskolin has also been proven to stimulate bicarbonate secretion by activating CFTR channels and promoting the activity of nonselective cation channels (SIEMER and GOGELEIN, 1993; TUO et al., 2009). Activation of the CFTR receptor is demonstrated by a significant increase of intestinal I_{sc} in less than five minutes after forskolin exposure (ZHANG et al., 2007).

Forskolin does not only interact with adenylyl cyclase, but also has a direct effect on glucose transporters and ion channels (ONO et al., 1995; SHANAHAN et al., 1987). As forskolin increases intracellular cyclic AMP levels, it was shown to increase Na^+/glucose cotransport two- to eightfold within minutes of administration (WRIGHT et al., 1997).

2.1.1.8 Ouabain

Ouabain, also known as g-strophantin, is a cardiac glycoside that originated from the African plant *strophantus gracus*. The name ouabain stems from the Somali word "waabaayo", which means arrow poison. The deadly effect of ouabain is due to an irreversible inhibition of the Na^+/K^+ ATPase (PRESSLEY, 1996). The Na^+/K^+ ATPase, located on the basolateral membrane of the enterocyte, is another target of indirectly regulating chloride secretion.

In 1968, scientists observed the effect of ouabain inhibiting glucose transfer in the small intestine of rats (NEWEY et al., 1968). Henriques confirmed these findings by recording the short circuit current of rabbit ileum being abolished after ouabain exposure (HENRIQUES, 1977).

However, Wasserman et al. discovered that the response to histamine cannot be abolished by ouabain. Incubation times of up to one hour with high concentrations of ouabain (1 mM) resulted in only minor degrees of inhibition of this pathway (WASSERMAN et al., 1988).

A study by Read et al. demonstrated that incubation with ouabain does not result in the expected decrease of absorption of sodium and chloride in samples of canine ileum. As these scientists perfused the ileum of 18 dogs with ouabain, no significant

changes in sodium and chloride transport, unidirectional flux of ions, or transmucosal potential difference could be observed. However, the Na^+/K^+ ATPase activity was reduced by 50%. This group concluded, that the Na^+/K^+ ATPase is not the limiting step in the transport of sodium and chloride, although it might be an important factor (READ et al., 1979).

Another possible explanation for these observations might be a basolaterally located ouabain-insensitive Na^+/K^+ ATPase. Although its activity in native tissue is about ten times lower than ouabain-sensitive Na^+/K^+ ATPase, it may be responsible for the observations mentioned above (MORETTI et al., 1991; PROVERBIO et al., 1991).

In addition, to its action on the Na^+/K^+ ATPase, ouabain completely inhibits the bicarbonate stimulatory effects of prostaglandin, as indicated by a decreased I_{sc} and decreased potential difference (PD). In tissue that has not been exposed to serotonin, ouabain decreases apical HCO_3^- efflux as well as I_{sc} for approximately 80 minutes (YAO et al., 1993).

2.2 Chronic Enteropathies in Cats and Dogs

Clinical signs of chronic enteropathies are non-specific in nature. Vomiting, diarrhea, weight loss, anorexia, and other clinical signs are commonly seen in small animal patients with chronic enteropathies. However, these clinical signs can also occur with a variety of other disorders. The following section discusses some of the common chronic intestinal disorders in small animals with special regard to the resultant alteration in cellular transport.

2.2.1 Idiopathic Inflammatory Bowel Disease

Idiopathic inflammatory bowel disease (IBD) is one of the most common chronic enteropathies in companion animals. IBD is characterized by an idiopathic inflammation of the small intestine with histological evidence of infiltration of the intestinal mucosa with inflammatory cells. The infiltrate may be comprised of lymphocytes, eosinophils, neutrophils, plasma cells, macrophages, or any combination of these (GERMAN et al., 2001; JERGENS, 1999). IBD can be classified as lymphoplasmacytic, eosinophilic, neutrophilic, granulomatous, or mixed, depending on the predominant population of inflammatory cells. In humans, the mucosal inflammation is unevenly distributed. In severe cases the invasion of inflammatory cells is accompanied by epithelial necrosis, erosion, hyperplasia, and fibrosis (CASAMIAN-SORROSAL et al., 2010; JACOBS et al., 1990). Peripheral eosinophilia may be present in cases of eosinophilic gastroenteritis (QUIGLEY and Henry, 1981). The following breeds appear to be predisposed to IBD: Siamese cats, Shar Peis, Soft Coated Wheaten Terriers, and German Shepherd dogs (JERGENS et al., 1992). However, often a specific breed develops a specific syndrome, such as ulcerative colitis in Boxer dogs, protein-losing enteropathy in Soft Coated Wheaton Terriers, or the immune proliferative enteropathy of Basenji's. (BREITSCHWERDT et al., 1984; HALL et al., 1994; HOSTUTLER et al., 2004; LITTMAN et al., 2000).

As the clinical signs of IBD are relatively nonspecific, this disease might be misdiagnosed. According to current guidelines of the WSAVA International Standardization Group, several criteria should be fulfilled in order to reach a diagnosis of IBD. These include the chronic occurrence of gastrointestinal symptoms, histopathologically confirmed mucosal inflammation, inadequate response to dietary, antibiotic, and antiparasitic treatment, clinical response to anti-inflammatory treatment, and the failure to identify other causes of gastrointestinal inflammation (WASHABAU et al., 2010).

Although, by definition the cause of IBD is idiopathic, several factors may contribute to this inflammatory complex. Genetic, environmental, as well as microbial influences may lead to an altered interaction between the intestinal microbiota and the immune system of the animal (CHICHLOWSKI and HALE, 2008). During intestinal inflammation, antigenic stimulation leads to accumulation of inflammatory mediators in the lamina propria, and in some cases also within the epithelium (PERDUE and MCKAY, 1994). Such inflammatory reaction affects the physiological functions of the epithelium (MCKAY and PERDUE, 1993). Mast cell degranulation may also be a contributing factor to IBD.

In human patients, coeliac disease, ulcerative colitis, and Crohn's disease are associated with the infiltration of the intestinal mucosa with mast cells. Also, degranulated mast cells have been found in proximity to intestinal nerve fibers in human IBD patients (BARRETT and METCALFE, 1984; DVORAK et al., 1980; STROBEL et al., 1983). Infiltration of mast cells also seems to be important in the pathogenesis of canine IBD (KLEINSCHMIDT et al., 2007). As described above

(section 2.1.6.4.), histamine release from mast cells can cause a marked alteration of intestinal function. Additionally, release of intracellular cAMP from neutrophils may also play a significant role in the pathogenesis of IBD. Neutrophils secrete a precursor of adenosine, called 5'AMP, that can be converted to adenosine by a 5' nucleotidase expressed on the apical membrane of enterocytes. This mechanism may be pertinent to the pathogenesis of diarrhea seen in patients with ulcerative colitis and other inflammatory bowel diseases in people, where neutrophils are known to migrate into the intestinal lumen forming crypt abscesses (GEWIRTZ et al., 2002). Inflammatory bowel disease, ulcerative colitis, and irritable bowel syndrome in humans are associated with an intracellular increase in 5-HT, an increase in enterochromaffin cells, increased serotonin release, as well as serotonin reuptake (COATES et al., 2004; MAGRO et al., 2002). These mediators are thought to induce their effects on epithelial cells via an elevation in cAMP, although the wide spectrum of prostanoid receptors, many of which are present in intestinal epithelial cells, make it difficult to interpret these data (DING et al., 1997).

In general, inflammatory mediators are well known to enhance electrolyte secretion while reducing NaCl absorption (TRAYNOR et al., 1993). More than 25 neurotransmitters have been identified that can modulate intestinal ion and fluid transport (HANSEN and SKADHAUGE, 1995).

Cytokine-secreting macrophages, which are also commonly associated with the inflammation, release tumor necrosis factor α (TNFα), which induces apoptosis (SATEGNA-GUIDETTI et al., 1993). This TNFα induced disruption of the epithelial barrier can be detected by alterations in mucosal conductance (GITTER et al., 2000b).

Several studies involving colonic perfusion have characterized absorptive and secretory processes leading to diarrhea in human patients with IBD (KREYDIYYEH et al., 2006; RASK-MADSEN and HJELT, 1977). These studies demonstrated a total dependence of the electrical potential difference and short circuit current on sodium transport (RASK-MADSEN and HJELT, 1977). The intestinal electroneutral Na^+/H^+ exchanger is known to be negatively affected not only by enteropathogenic bacteria, but also by colonic inflammation and inflammatory mediators such as nitric oxide, TNFα, and interferon γ (IFNγ) (HODGES et al., 2008). During acute intestinal inflammation, electrogenic bicarbonate secretion most likely increases due to increased concentrations of intracellular cAMP (ZHANG et al., 2007).

As chloride secretion is the primary driving force for water movement, its secretion might be an adaptive response of the host, to flush out toxins and proinflammatory cytokines from the intestinal lumen. Diarrhea can lead to HCO_3^- secretion, resulting in significant bicarbonate loss, which might lead to metabolic acidosis. Overall, IBD leads to significant morbidity and long-term mortality in dogs and cats. Also, the outcome of this disease is not predictable based on the site, type of primary inflammatory cell, and severity of inflammatory infiltration (CRAVEN et al., 2004).

2.2.2 Food Allergy

Adverse reactions to food can be classified as food allergy, which is mediated by the immune system and food intolerance, which does not have an immunological basis (ANDERSON, 1984). Food antigens can cause allergic reactions in the intestinal tract, resulting in impaired membrane permeability, accompanied by the secretion of fluid and mucus. These reactions are induced by the release of histamine and other mast cell mediators (KING and MILLER, 1984; LAKE et al., 1980; PERDUE et al., 1992). Mast cell degranulation leads to diarrhea, malabsorption, epithelial damage, villous atrophy, crypt hyperplasia, and increased mucous secretion (BRAVERMAN et al., 1985).

On a cellular basis, exposure of the enteric nervous system to histamine results in slow excitatory synapses accompanied by the release of serotonin (WOOD, 2006). 5-HT_1, 5-HT_2, and 5-HT_3 receptor antagonists have been proven to reduce antigen induced intestinal hypersensitivity in animals with food allergies (CROWE and PERDUE, 1992; FRIELING et al., 2000).

In humans, food allergy can be diagnosed by Ussing chamber experiments where defined amounts of antigens can be used to induce histamine release, which in turn leads to changes in I_{sc} (BIJLSMA et al., 2004). In humans, the interpretation of Ussing chamber studies appears to be both sensitive and specific for the diagnosis and characterization of this disorder (BIJLSMA et al., 2004).

2.2.2.1 Intestinal Anaphylaxis

Intestinal anaphylaxis is a special form of food allergy that is rarely seen in companion animals. However, the pathophysiology of anaphylaxis provides a deeper insight into the role of inflammatory transmitters.

During anaphylaxis the intestine rapidly reduces water, sodium, potassium, and chloride absorption. This process is mediated by histamine (PERDUE et al., 1984). Again, it was through experiments using Ussing chambers that the cellular mechanisms behind these findings were identified. Intestinal anaphylaxis leads to increases in short circuit currents and it has been hypothesized that these changes are associated with an enhanced net secretion of the ions mentioned above (PERDUE and GALL, 1986). The antigen mediated IgE reaction in the intestinal mucosa leads to Cl^- secretion, which is also reflected by an immediate increase of the short circuit current (PERDUE and GALL, 1986). Hypersensitivity reactions of the intestinal mucosa increase not only ion secretion, but also membrane permeability due to mast cell activation (PERDUE et al., 1992). The fast response of the intestine to allergens suggests that these substances undergo a paracellular uptake.

2.2.3 Antibiotic Responsive Diarrhea

Some canine and feline patients with clinical signs of chronic diarrhea respond to antibiotic therapy. A dysbalance of the intestinal microbiota is suspected to be responsible for this syndrome, but little is known about the pathogenesis. Antibiotics may be used to successfully treat patients with this condition because they provide selective pressure on the intestinal microbiota, support beneficial immunmodulatory effects to the host, or restore mucosal permeability in affected animals (HALL and BATT, 1990; PATEL et al., 1999; SUCHODOLSKI et al., 2009; SULLIVAN et al., 2001). The most commonly used antimicrobials are oxytetracycline, metronidazole, and tylosin (HOSTUTLER et al., 2004). Antibiotic responsive diarrhea (ARD) typically affects middle-aged, large breed dogs, most commonly German Shepherds (WESTERMARCK et al., 2005). The disease occurs specifically in dogs, and to a

lesser extent in the cat. Since studies using Ussing chambers have only rarely been performed in small animals, no such studies on animals with ARD have been published to date.

2.2.4 Summary

In summary, chronic intestinal diseases (IBD, food allergies, antibiotic responsive diarrhea) are accompanied by decreased epithelial integrity and altered ion flux. Both contribute to modified fluid homeostasis and diarrhea. Inflammation and inflammatory mediators lead to changes in the expression and function of key epithelial transporters, including Na^+/K^+ ATPase, the $Na^+/K^+/2Cl^-$ cotransporter 1, sodium hydrogen exchangers, as well as the ENaC (KIELA and GHISHAN, 2009). Cellular and molecular changes resulting in increased anion secretion accompanied with the shift from electro neutral to electrogenic electrolyte transport demonstrate the complexicity between inflammatory mediators and altered epithelial transport.

2.3 Ussing Chamber

As stated above, demonstrating histopathologic evidence of inflammation can play an important role in diagnosing chronic enteropathies. Unfortunately, there is poor correlation between the histopathological finding of inflammation on one side and the degree of clinical symptoms on the other (GARCIA-SANCHO et al., 2007; SCHREINER et al., 2008). As substantial interobserver variation between pathologists looking at the same histological sections of the intestine has been demonstrated, more objective criteria are warranted (WILLARD et al., 2002). Additionally, chronic enteropathies are distinguished and diagnosed many times by the patients' response to treatment, which often requires several weeks (GERMAN et al., 2003; GUILFORD et al., 2001; WESTERMARCK et al., 2005).

Ussing chamber studies have served not only as a valuable tool in characterizing membrane electrolyte transport but also in diagnosing inflammatory intestinal diseases in humans (DE JONGE et al., 2004; BIJLSMA et al., 2004).

The principle of Ussing chamber studies was introduced by the Danish scientists Ussing and Zerahn in 1951 (USSING and ZERAHN, 1951). Original studies investigated the physiological transport mechanisms in frog skin. Those observations led to the understanding of active NaCl transport, the theory of active and passive transport in general, as well as the Koenfoed-Johnsen-Ussing theory of the two-membrane-model (KOEFOED-JOHNSEN and USSING, 1958; USSING, 1994). Experiments indicating that the apical membrane is permeable to Na^+, and the basolateral membrane is permeable to K^+, have formed the basis of our present understanding of Na^+/K^+ ATPase.

Active transport reflects the capacity of an epithelium to transport substrates, such as nutrients or ions against a gradient (electrical or concentration). The current, after clamping the transepithelial voltage to zero, is referred as the short circuit current (I_{sc}). Since the Ussing chamber set up eliminates all forces leading to passive transport (hydrostatic, electrochemical, as well as the osmotic gradient), the I_{sc} reflects the sum of electrogenic ion movements by active transport. More precisely, the I_{sc} summarizes the currents of Na^+, Cl^-, HCO_3^-, and K^+. As the I_{sc} reflects all electrogenic secretory responses in the cell, chloride is still the predominant substance transported across the membrane barrier (UNMACK et al., 2001). The Ussing chamber is considered to be the gold standard for measurements of membrane transport processes involving ion movement in vitro (RAY et al., 2002).

Current and voltage measurements in Ussing chamber experiments have demonstrated that electrogenic Cl^- secretion can be the reason for enterotoxin-mediated as well as inflammatory-mediated secretory diarrhea. Furthermore, Ussing chamber experiments clearly indicated that some types of diarrhea are due to an activation of the apically located cystic fibrosis transmembrane conductance regulator, accompanied with the basolateral influx of Cl^- provided by the $Na^+/K^+/2Cl^-$ cotransporter as well as the Cl^-/HCO_3^- exchanger. (CLARKE, 2009; HOGAN et al., 1997; SEIDLER et al., 1997; WALKER et al., 2009). Another example of the impact of the Ussing chamber studies is the discovery of intestinal Na^+/glucose coupled uptake (WRIGHT, 1993; WRIGHT et al., 2007). The Ussing chamber revealed the characteristics and relative importance of this transport process in the intestinal epithelium, which was eventually identified at the molecular level (QUIGLEY and GOTTERER, 1969; SKOU, 1957; USSING and ZERAHN, 1951). Many studies, in virtually every type of epithelial tissue have been accomplished using Ussing chambers.

Apart from its role in these major scientific discoveries, the Ussing chamber is of use

in the investigation of many diseases. To mention just a few uses of bioelectrical Ussing chamber experiments, intestinal current measurements allowed the direct detection of cystic fibrosis induced changes in humans (VEEZE et al., 1991). Furthermore, with the help of Ussing chamber studies, researchers are able to discriminate between CFTR mediated Cl^- secretion and the secretion of Cl^- through alternative channels (DE JONGE et al., 2004). The CFTR receptor is responsible for electrogenic chloride secretion and is the main focus of many Ussing chamber experiments. Also, specific diseases, such as diverticulosis, were investigated with modified Ussing air suction chambers (OSBAK et al., 2007).

In general, there are two types of Ussing chambers: the continuously perfused chamber, and the circulating chamber. The continuously perfused chamber is a custom manufactured apparatus, enabling minimized hydrostatic pressure preventing epithelial damage. Special valves control the flow rate of the buffer bathing the membrane of interest. The disadvantage of this version is not only a lack of availability, but also the challenging modification of the buffers in this closed system. In contrast, the circulating chamber applies a hydrostatic pressure with the U-shaped glass cylinder serving as a buffer reservoir and fluid can be modified through open access from the top aperture. Further details on the principles of the circulating Ussing chamber are described in section 3.1.

The patch clamp technique and microelectrode studies have been developed using the principles of the Ussing chamber. Several methods have been developed that widen the applicability of Ussing chamber studies. Some of these include micro chambers, mounting plates, and the attachment of tissue samples with glue. These methods allowed studies in cultured cell lines, a variety of epithelia, the cornea, and other membranes (STOCKMANN et al., 1999; VEEZE et al., 1991; WALLON et al., 2005).

In summary, the Ussing chamber has been established as an investigative tool for the study of basic physiology as well as the pathophysiology of a variety of diseases. It is expected to continue to provide a useful method for integrating our knowledge of transporter function of the intestinal epithelium with clinical observations (KALTOFT et al., 2010; LUCAS, 2005).

3. Materials and Methods

3.1 Design of the Adapter Modified Ussing Chamber

As mentioned above, the principle of electrophysiological transepithelial measurements was introduced by Ussing and Zerahn in 1951 (USSING and ZERAHN, 1951). The Ussing chamber itself consists of a hollow cylinder made of Lucite (Ussing system CH8; World Precision Instruments, Inc., Sarasota, FL, USA). The hollow cylinder can be dissembled into two half-cylinders, which enables the investigator to place the membrane of interest in between these two cylinders, into the center of the Ussing chamber apparatus. The Ussing chamber cylinder has several ports.

In our setup, each half of the hollow cylinder was connected to a glass reservoir, filled with 10 ml Meyler´s buffer (Table 1). Thus, the inserted tissue was constantly surrounded by a physiologic buffer solution. The buffer reservoirs were heated to 37˚C by a recirculating chiller (VWR International, West Chester, PA, USA), and oxigenated with a 95%/5% mixture of O_2 and CO_2 (Praxair, Houston, TX, USA). This setup provided not only controlled environmental conditions but also ensured quick and steady mixing of the experimental buffer solution. Additional ports of the fluid reservoirs enabled modification of the buffers as the experiments were conducted.

Each half of the Lucite Ussing chamber was equipped with one voltage electrode, as well as one current generating electrode (EK1; World Precision Instruments, Inc., Sarasota, FL, USA). While both voltage electrodes were connected to the chamber close to the intestinal membrane (approximately 3 mm distance from the biopsy itself), the current generating electrodes were placed 20 mm apart, at the distal ends of each half of the Ussing chamber. Three % 3 M KCl agarose bridges ensured an electrical circuit between the buffer solution in the Ussing Chamber and the Silver/Silver-Chloride (Ag/AgCl) electrodes. The 3% 3 M KCl agarose bridges were prepared immediately before each experiment. The Ag/AgCl electrodes were connected to the preamplifier (DVC-3; World Precision Instruments, Inc., Sarasota, FL, USA) and operated by the amplifier DVC-1000 (World Precision Instruments, Inc, Sarasota, FL, USA). iWorx LabScribe2 computer software (iWorx, Inc., Dover, NH, USA) was used to record the electrical parameters.

The Ussing chamber used for this study was a modification of Ussing and Zerahn`s original invention (Ussing System CH8, World Precision Instruments, Inc., Sarasota, FL, USA; Figures 5 and 6). The discs that enabled fixation of the tissue by air suction were custom made (eMachineShop, Mahwah, NJ, USA; see details in section 3.2).

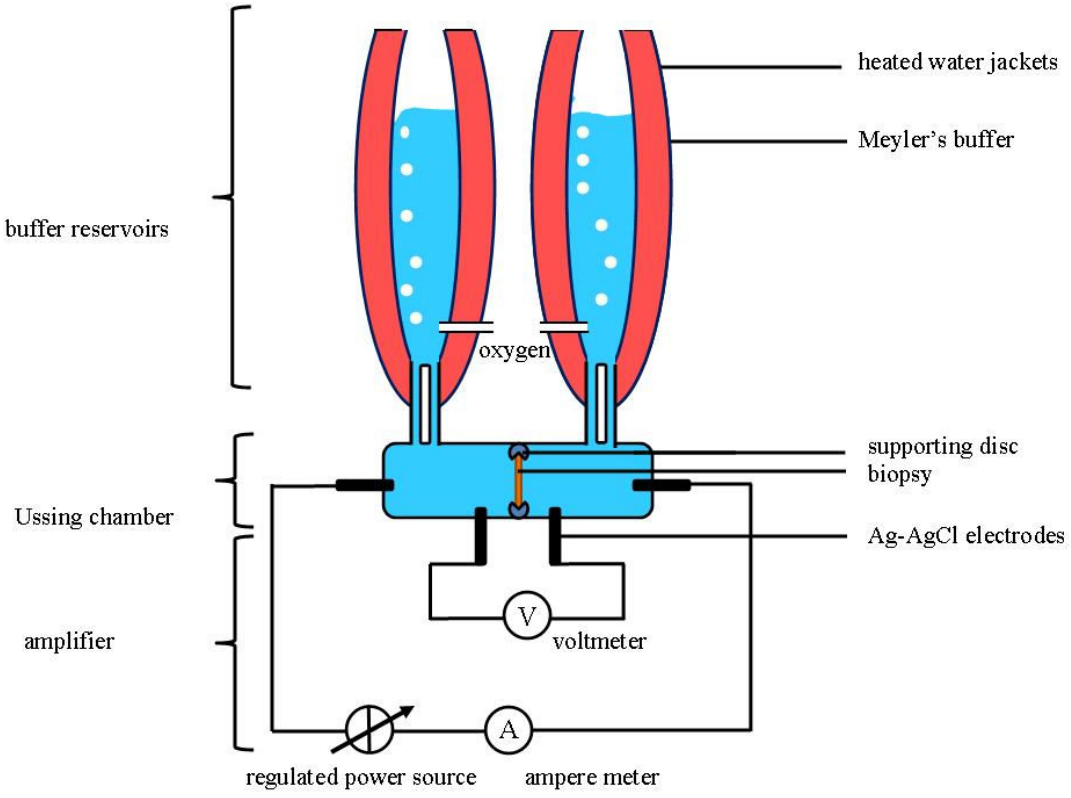

Figure 5: Sketch of the adapter modified Ussing chamber
The principle measurements of the Ussing chamber are based on two functional units: the physiological system, and the electrical circuit. The buffer reservoirs of 10 ml each of gassed and heated buffer enabled short-term physiological function of the intestinal mucosa. The connected amplifier was used to perform the electrical measurements.

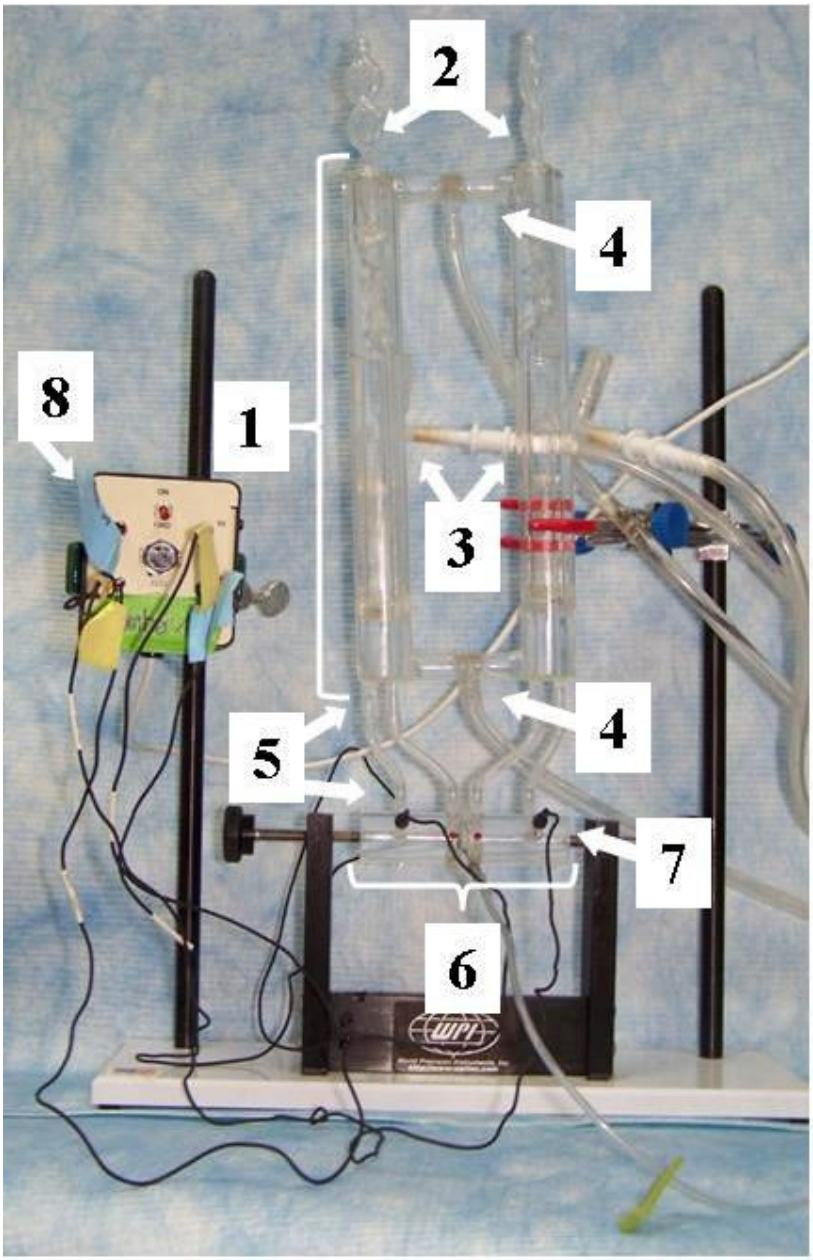

Figure 6: Picture of the adapter modified Ussing chamber
This photograph displays the original chamber set up in the laboratory. The tall buffer reservoirs (1) were filled with physiologic solution from the top twisted opening (2). The white connectors (3) provide oxygen to the buffer reservoir. The tubing at the top and bottom of the reservoir (4) is used to maintain the temperature of the water jackets. Four slender tubes (5) provide in- and outlets of the circulating buffer to the Ussing chamber itself. The small, hollow Ussing chamber (6) in the center of the picture is held in place by the black, wooden stator and the two lateral screws. Four black electrodes (7) and their cables connect the Ussing chamber with the preamplifier (8). While the preamplifier is set right next to the Ussing chamber, the amplifier itself is not displayed in this photograph.

3.2 Design of the Biopsy Discs

The aperture of the Ussing chamber dictates the size of the analyzed tissue sample and for the original Ussing chamber required a tissue sample of at least 3 cm^2. In order to overcome the size-related disadvantage of the conventional Ussing chamber, a team of Danish scientists designed a special disc (Figure 7) (LARSEN et al., 2001). This custom made disc, mounted between the Ussing chamber halves, enables the investigation of membranes covering a size of only 5 mm^2, which is the approximate size of endoscopically collected gastric or intestinal biopsies.

Before each experiment, the adapter disc was prepared by greasing (Dow Corning® high vacuum grease, Dow Corning Corporation, Midland, MI, USA) the edges of two hemi-discs and sliding them together to form a final sandwich-disc. A thin wire, twisted through two drill holes prevented the disc from sliding or twisting apart. Air suction was applied through a cannula passed through a side hole into the space between the two halves of the disc. The cannula was attached to a tubing system connected to a suction aperture (Vacu/Trol Regulated Vacuum Pump, 115V; Spectrum Laboratories, Inc., Rancho Dominguez, CA, USA). The suction allowed for sealing the disc and fixating the intestinal tissue sample in the center aperture of the assembled disc.

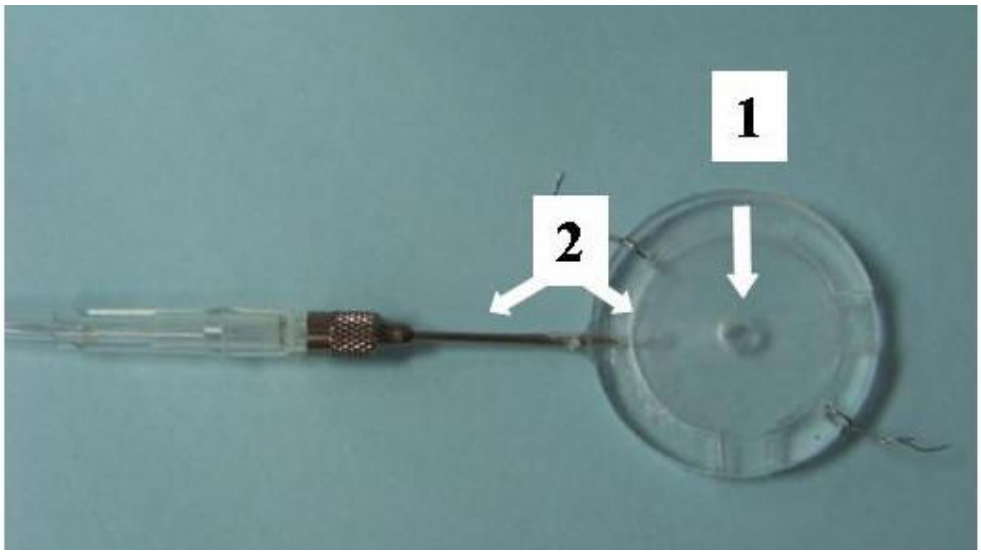

Figure 7: Adapter disc

The central aperture (1) of 5 mm^2 of the supporting disc allows for investigations of barrier and transport function across endoscopically collected intestinal biopsies. The biopsies were placed in the center hole, seated in this central opening, and held in place during the entire experiment by a vacuum created by air suction. The vacuum surrounds the intestinal sample from all sides, as a hollow space is formed in between the wired disc halves. Air suction is applied through the metal cannula (2) shown on the left side.

3.3 Sampling and Mounting of Biopsies into the Discs

Various clinicians at the Small Animal Clinic at Texas A&M University collected either six duodenal or colonic biopsies from each animal enrolled into the study.

An Olympus GIF-160 gastroscope with a 2.8 mm working channel was used to collect the biopsy samples (Olympus America, Inc., Center Valley, PA, USA). Biopsies were taken with standard biopsy forceps (Olympus FB-36 K-1, diameter 2.8; Olympus America, Inc., Center Valley, PA, USA). The sampled tissues did not contain serosa, but rather the entire thickness of the mucosa, as well as some submucosa. Tissue specimens were immediately placed into and then transported in a beaker filled with Meyler's buffer at room temperature.

While four biopsies were analyzed simultaneously in four separate Ussing chambers, two biopsies remained unused and served as replacements in case of difficulties during the transfer of a biopsy into the Ussing chamber. In some instances the two remaining biopsies served as control samples for histological evaluation for tissue damage, if they were not needed as a replacement specimen during the experiment.

With the aid of a stereomicroscope (SZ 30; Olympus America, Inc., Melville, NY, USA), each biopsy sample was oriented and mounted into a supporting disc. Under continuous suction of 400 mmHg, the discs were mounted between a set of Ussing chamber half-cylinders. The entire procedure from collecting the biopsy to mounting the biopsy in the final Ussing chamber lasted a total of 10-15 minutes at most.

The following terms were adopted (Figure 8):

1) The term "mucosal side" referred to the epithelial surface of the biopsy.

2) "Mucosal solution" was the solution in the Ussing chamber, which was in contact with the intestinal enterocytes on the mucosal side of the tissue biopsy.

3) The "serosal side" of the biopsy was the surface that faced the submucosa and muscularis layers of the intestinal mucosa.

4) "Serosal solution" was the buffer reservoir of the Ussing chamber-half that was in contact with the serosal side of the intestinal biopsy.

5) "Baseline" refers to the current or voltage values that were measured immediately before a stimulus was applied. Thus, the baseline value is a relative value that serves for the purpose of comparison, rather than a characteristic biopsy-related attribute.

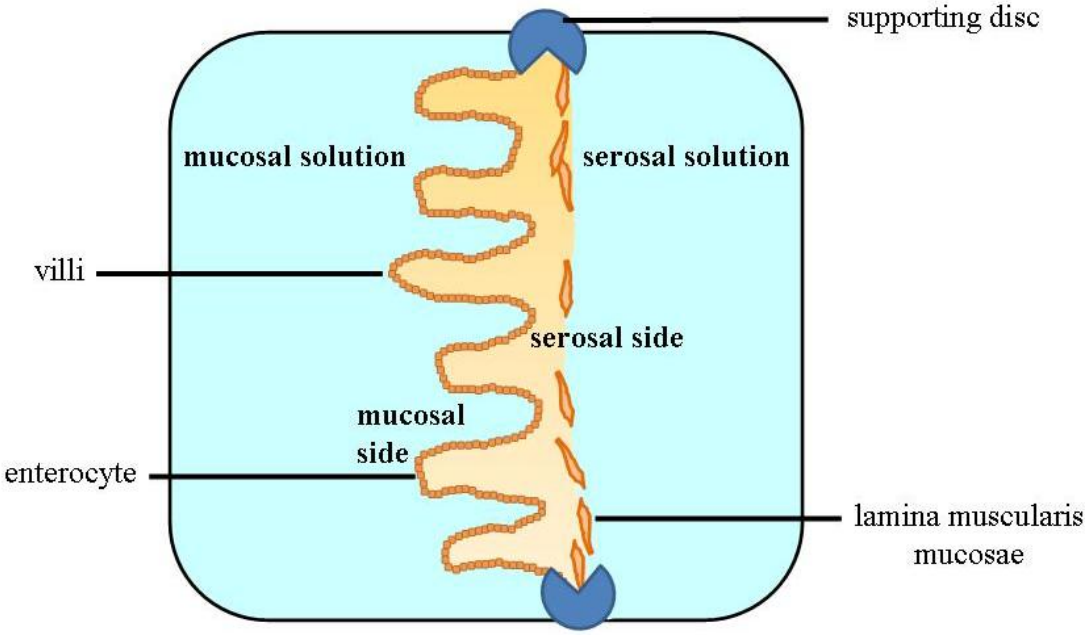

Figure 8: Illustration of a biopsy sample loaded into the Ussing chamber
The intestinal biopsy was placed in the center of the supporting disc, covering the transversal lumen of the Ussing chamber completely and dividing the solution into two compartments. While the enterocytes face the mucosal solution, the serosal side of the biopsy, containing fragments of the lamina muscularis mucosae as well as the submucosa, is facing the serosal solution.

3.4 Electrical Measurements

The electrical circuit of the Ussing chamber was established by four Ag/AgCl electrodes (EK1; World Precision Instruments, Inc., Sarasota, FL, USA). The two voltage electrodes (EKV) were placed approximately 3 mm from each side of the intestinal tissue. The electrodes that apply and measure current (EKC) were placed as far apart as possible from each other (approximately 20 mm away from each side of the biopsy) to achieve the most steady measurements. Electrical measurements were taken using a preamplifier (DVC-3, World Precision Instruments, Inc., Sarasota, FL, USA) and an amplifier (DVC-1000, World Precision Instruments, Inc., Sarasota, FL, USA).

In order to determine the system resistance, the unloaded Ussing chamber was filled with buffer solution before each experiment. This was followed by the measurement of the resistance and junction potential, which was then subtracted from the experimental measurements by default function. The system resistance represents a measure of the summary of fluid resistance, electrode resistance, and the resistance of the agarose bridges. After collection, the intestinal biopsy samples were transferred into the Ussing chamber and an equilibration time of 5-10 minutes was allowed. Then, the tissue was short-circuited by clamping the voltage to zero. During this period of voltage clamping, the zero voltage, as well as the tissue's generating current (named "short circuit current"; I_{sc}) were measured. This setting cycled every ten seconds (s) with the current clamp mode, a condition where the tissue was clamped to 18 μA. Current pulses were applied for 0.5 s each. During this period voltages (ΔV) were measured to calculate resistance of the tissue sample using Ohm's law ($R = \Delta V/\Delta I$). The voltage and the current were constantly being recorded during both voltage and current clamping, using the iWorx LabScribe2 software (iWorx, Inc., Dover, NH, USA).

The arrangement described above allowed for the measurement of the short circuit current (I_{sc}) and the calculation of the conductance (G):

Short circuit current (I_{sc})

While the voltage was maintained at zero, the current (in this case the short circuit current; I_{sc}) across the intestinal biopsy reflected the intensity of active ion transport. By comparing the intensity of the current in the resting epithelium to the current of the tissue that was exposed to a chemical substance (ΔI_{sc}), the specific effect of the chemical substance on transporter function could be assessed. This information reflected the active cellular response to specific substances. The time from the onset of I_{sc} until maximum I_{sc} values were reached was calculated (Δt).

Conductance (G)

The conductance was calculated as the reciprocal value of the resistance. Resistance, in turn, was calculated by dividing the change in voltage (ΔV) by the change of current (ΔI) during current clamping. The literature traditionally reports conductance, rather than resistance. In the current work, conductance is referred to as 1/resistance.

I_{sc} was recorded in microamperes (μA), the voltage was measured in millivolts (mV), and the resistance (R) was calculated according to Ohm's law. Resistance values slightly undulated. Therefore, the resistance was calculated at two time points close to each other and the mean value of the measurements from these time points was used for further calculations.

3.5 Experimental Design

Immediately before each experiment, the Ussing chamber was filled with heated and oxygenated Meyler's buffer and the unloaded supporting discs were inserted. The system was evaluated for possible leaks, as well as for accumulation of air bubbles. The system was adjusted for fluid resistance directly before each experiment by using the "fluid resistance compensation" feature of the DVC-1000 amplifier.

While the system was operating in standby mode, endoscopically collected biopsy specimens were transferred into the supporting discs.

Immediately before inserting the loaded discs into the chamber, fluid resistance was remeasured, eliminating any electrode drift and fluid junction potential that might have occurred in the meantime.

After mounting the loaded discs, each half of the water reservoir was concurrently filled with 10 ml Meyler's buffer solution. The serosal buffer solution was modified by adding 10 mM glucose, which was aimed at providing nutritional support to the intestinal cells. To maintain osmotic balance between the two sides of the chamber 10 mM sorbitol was added to the mucosal buffer.

After 5-10 min of equilibration time, the voltage applied to the tissue was short-circuited by clamping the voltage to zero. Voltage clamping was discontinued every 10 s and current clamping was applied for 0.5 s.

Absorptive activity was studied in each biopsy, using 40 mM glucose, followed by application of 500 μM phloridzin, with both of these substances being added to the mucosal bathing solution.

Secretory activity was subsequently studied, in response to application of 200 μM histamine, 200 μM 5-hydroxytryptamine (5-HT), 4 μM prostaglandin E_2 (PGE_2), or 5 μM forskolin, added to the serosal buffer.

If the intestinal biopsy showed a response to the initial glucose stimulus, another 40 mM glucose was applied to the mucosal buffer at the end of the entire experiment to evaluate biopsy viability. Finally, the experiment was concluded by addition of 600 μM ouabain to the serosal side of the buffer.

An overview of the experimental design is shown in Figure 9.

Time points:

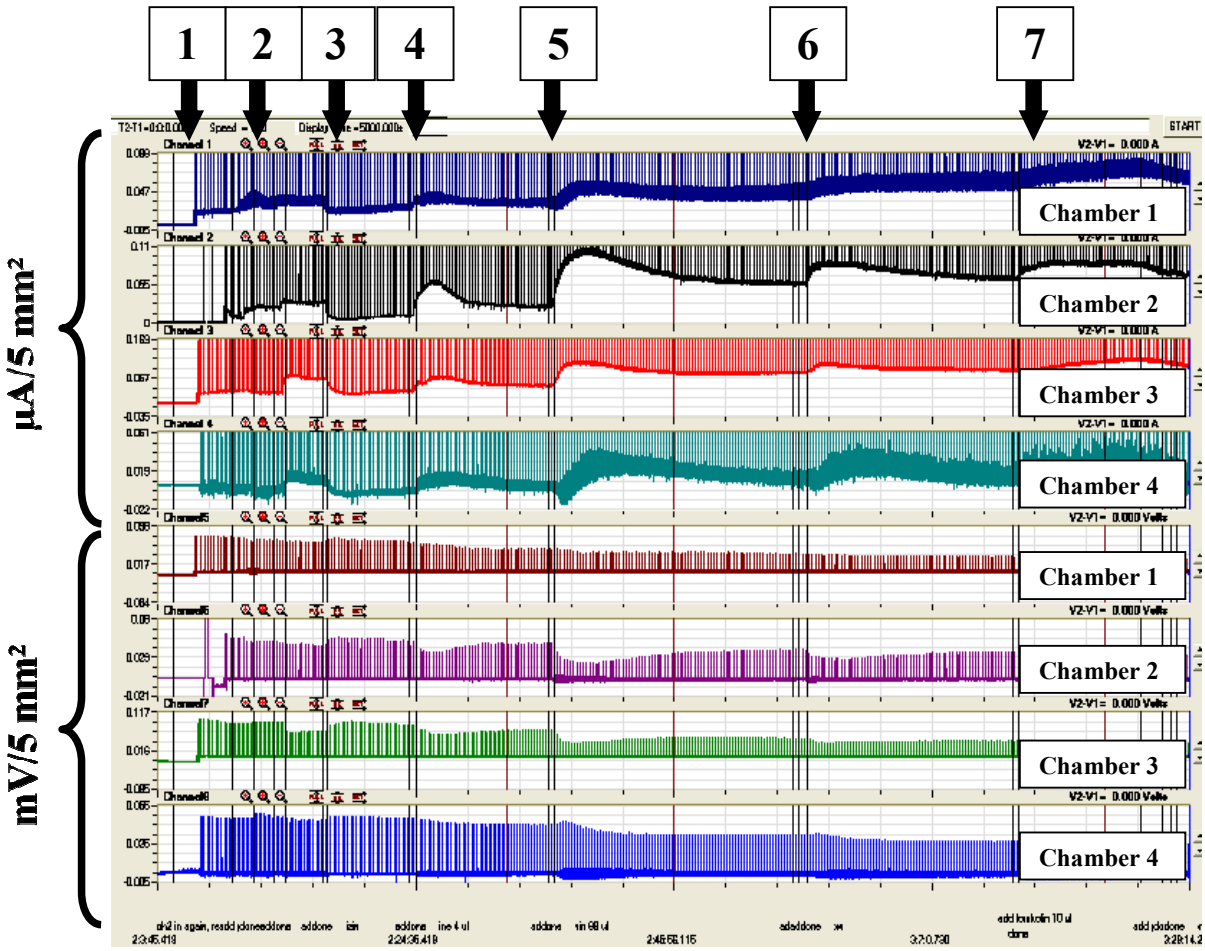

Figure 9: Overview of the experimental design

This figure shows the data of the raw measurements, as recorded by iWorx LabScribe2. While the top four graphs (blue, black, red, and green) show the current, recorded in µA/5 mm^2, the bottom four graphs (maroon, purple, green, and blue) show the voltage, recorded in mV/5 mm^2. In this experiment, all four Ussing chambers were loaded with canine colonic biopsies. Reactions of the biopsy in Ussing chamber number one can be observed by evaluating the first graph (current: blue) and the fifth graph (voltage: maroon). The biopsy of the Ussing chamber number two can be evaluated by looking at the second graph (current: black) as well as the sixth graph (voltage: purple). The current of the third sample is displayed in the third graph (current: red), while the voltage of this tissue is shown in channel seven (voltage: bright green). Measurements for the fourth intestinal sample can be seen in the fourth (current: dark green) and the eighth graph (voltage: blue). While the baseline voltage is continuously clamped to zero, the voltage during the time of current clamping (pulses) clearly adjusts to the changing conditions. The time points noted above the graph correspond to the following actions:

Time point 1: The arrow indicates the time point when the voltage-clamping was employed. Short current clamping pulses were applied to evaluate the baseline tissue resistance. The voltage of the Ussing chamber can be seen to adjust immediately

under these conditions.
Time point 2: Application of 40 mM glucose to the mucosal buffer. The glucose induced a rise of current, which could be abolished by the administration of 500 µM phloridzin (time point 3).
Time points 4, 5, 6, and 7: Application of 200 µM histamine, 200 µM serotonin, 4 µM prostaglandin E_2, and 5 µM forskolin. All of these substances induced a characteristic change of the short circuit current (ΔI_{sc}).

3.6 Study Population and Ethics

To investigate the feasibility of the adapter modified Ussing chamber, 24 duodenal biopsies were collected from five cats and 75 duodenal biopsies from 14 dogs during their routine diagnostic endoscopy at Texas A&M University. Also, a total of 22 and 21 colonic biopsies were collected from four cats and four dogs, respectively. Most of the animals showed a variety of clinical signs of gastrointestinal disease, such as vomiting, diarrhea, and/or anorexia and received various medications. Animals were hospitalized for at least 8 hours. Two healthy dogs underwent anesthesia for reasons unrelated to gastrointestinal disease, one for a spay and one for prophylactic dental care. For those two dogs, no history of gastrointestinal signs had been reported and no medication, other than medication for flea and heartworm prevention had been administered. Tables 2-7 provide details about the individual animals enrolled into this study.
Data of canine patients from which duodenal biopsies had been collected were analyzed as a whole, but were also grouped according to the histopathological standards for the diagnosis of gastrointestinal inflammation of endoscopic biopsy samples published by the World Small Animal Veterinary Association Gastrointestinal Standardization Group (DAY et al., 2008). The evaluation of the samples was performed by a board certified pathologist in a blinded manner:
Group 1 was made up of two healthy dogs with no history of signs of gastrointestinal disease (WSAVA score: <2.7; number of biopsies evaluated n=8).
Group 2 was made up of four dogs with clinical signs of gastrointestinal disease, but with unremarkable histopathological biopsy specimens (WSAVA score <2.7; number of biopsies evaluated n=15).
Group 3 consisted of seven dogs that showed clinical signs of gastrointestinal diseases and also showed moderate to severe histopathological lesions (WSAVA score >3.0, number of biopsies evaluated n=32).
Animals where the exact age was not known were assumed to be born on January 1st of their reported year of birth.
The study was approved by the Clinical Research Review Committee of the Texas A&M Veterinary Medical Center. All owners gave informed consent for their dog's or cat's participation in the study.

Table 2: Clinical data from enrolled cats from which duodenal biopsies had been collected
The table provides detailed information about the five cats from which duodenal biopsies had been collected.
fs = female spayed; mc = male castrated

	breed	**sex**	**age (month)**	**body weight (kg)**	**clinical signs**	**clinical diagnosis**	**WSAVA grading score**	**histopathologic diagnosis**
cat 1	Siamese	mc	143	6.2	chronic diarrhea, weight loss	mild IBD	5.3	lymphoplasmacytic inflammation
cat 2	American Domestic Shorthair	mc	9	4.3	chronic diarrhea	chronic large bowel diarrhea	3.2	lymphoplasmacytic inflammation
cat 3	American Domestic Shorthair	fs	135	5.9	chronic vomiting	no definite diagnosis	2.5	normal tissue
cat 4	American Domestic Shorthair	mc	29	5.7	chronic diarrhea	no definite diagnosis	3.7	lymphoplasmacytic inflammation
cat 5	American Domestic Shorthair	mc	53	3.6	chronic vomiting	chronic vomiting unknown etiology	3.2	lymphoplasmacytic inflammation
median			53	5.7			3.2	
range			9-143	3.6-6.2			2.5-5.3	

Table 3: Clinical data from enrolled cats from which colonic biopsies had been collected
This table summarizes the clinical data from four cats from which a colonic biopsy had been collected.
fs = female spayed; mc = male castrated

	breed	sex	age (month)	body weight (kg)	clinical signs	clinical diagnosis	WSAVA grading score	histopathologic diagnosis
cat 6	American Domestic Shorthair	fs	135	4.0	vomiting, chronic diarrhea	diffuse gastrointestinal disease	3.7	lymphoplasmacytic inflammation
cat 7	American Domestic Longhair	mc	118	5.2	chronic vomiting	esophageal stricture, gastritis	1.7	normal tissue
cat 8	American Domestic Shorthair	mc	105	3.8	weight loss, vomiting	chronic vomiting, weight loss	2.0	normal tissue
cat 9	Main Coon	mc	142	9.8	chronic diarrhea	enteritis	2.6	normal tissue
median			126.5	4.6			2.3	
range			105-142	3.8-9.8			1.7-3.7	

Table 4: Clinical data from dogs of group 1 from which duodenal biopsies had been collected
This table provides information about healthy dogs that were enrolled in this study.
f = female intact, fs = female spayed

group 1	breed	sex	age (month)	body weight (kg)	clinical signs	clinical diagnosis	WSAVA grading score	histopathologic diagnosis
dog 1	Labrador Retriever	f	67	43.5	none	none	2.7	normal tissue
dog 2	Weimaraner	fs	7	25.5	none	none	2.3	normal tissue
median			37	34.5			2.5	
range			7-67	25.5-43.5			2.3-2.7	

Table 5: Clinical data from dogs of group 2 from whom duodenal biopsies had been collected
This table provides clinical data from dogs exhibiting clinical signs of gastrointestinal disease, but no histopathological abnormalities from which duodenal biopsies were collected.
fs = female spayed; mc = male castrated; f = female intact

group 2	breed	sex	age (month)	body weight (kg)	clinical signs	clinical diagnosis	WSAVA grading score	histopathologic diagnosis
dog 3	Wheaton Terrier	fs	143	20	vomiting, diarrhea	protein losing enteropathy	2.0	normal tissue
dog 4	mixed breed	mc	61	34	weight loss, loose stool	no definite diagnosis	2.25	normal tissue
dog 5	Pit Bull Terrier	f	119	20.4	chronic vomiting	no definite diagnosis	2.4	normal tissue
dog 6	Miniature Schnauzer	f	149	3.5	chronic vomiting	gastric tumor	2.7	normal tissue
median			118	19.5			2.3	
range			61-149	3.5-34			2.0-2.7	

Table 6: Clinical data from dogs of group 3 from which duodenal biopsies had been collected
This table provides information from eight dogs exhibiting clinical signs of gastrointestinal disease from which duodenal biopsies were collected that showed histopathologic abnormalities. fs = female spayed; mc = male castrated

group 3	breed	sex	age (month)	body weight (kg)	clinical signs	clinical diagnosis	WSAVA grading score	histopathologic diagnosis
dog 7	Dachshund	fs	68	3.5	vomiting, chronic diarrhea	protein losing enteropathy	3.7	lymphoplasmacytic inflammation
dog 8	mixed breed	fs	51	4.5	loss of appetite, soft stool	intestinal histoplasmosis	6.5	lymphoplasmacytic inflammation, crypt necrosis
dog 9	German Shepherd	mc	24	21.59	chronic diarrhea	no definite diagnosis	3.8	lymphoplasmacytic inflammation
dog 10	English Springer Spaniel	mc	120	26.5	chronic intermittent vomiting, inappetence	gastric mucosal polyps, possible IBD	3	lymphoplasmacytic inflammation
dog 11	mixed breed	fs	36	21.4	intermittent, progressive vomiting, anorexia	protein losing enteropathy, suspected lymphangiectasia	3.3	lymphoplasmacytic inflammation
dog 12	Rat Terrier	fs	85	6.5	respiratory distress, hypoalbuminemia	protein-losing enteropathy; IBD	4.2	lymphoplasmacytic inflammation
dog 13	Boxer	mc	116	33.2	loss of appetite, weight loss	possible lymphoma, IBD, lymphangiectasia	4.3	lymphoplasmacytic inflammation
dog 14	Golden Retriever	fs	79	26.6	chronic diarrhea	no definite diagnosis	4.7	lymphoplasmacytic inflammation
median			72.4	18.0			4.0	
range			24-120	3.5-33.2			3.0-6.5	

Table 7: Clinical data from enrolled dogs from which colonic biopsies had been collected
This table provides clinical data from dogs from which colonic biopsies had been collected. fs = female spayed

	breed	**sex**	**age (month)**	**body weight (kg)**	**clinical signs**	**clinical diagnosis**	**WSAVA grading score**	**histopathologic diagnosis**
dog 15	German Shepherd	fs	102	33.5	loose bloody stool	colonic adenocarcinoma	2.8	crypt hyperplasia
dog 16	Miniature Schnauzer	fs	27	7.76	chronic diarrhea	behavioral disorder	1.0	normal tissue
dog 17	mixed breed	fs	105	27.5	hematochezia	rectal polyps	0.8	normal tissue
dog 17	mixed breed	fs	111	28.1	recheck colonoscopy	no definite diagnosis	2.8	crypt hyperplasia/ lymphoplasmacytic inflammatory
median			86.3	24.2			median: 1.9	
range			27-111	7.8-33.5			range: 0.8-2.8	

3.7 Data Analysis

Conductance measurements were used from all biopsies that had been successfully transferred into the Ussing chamber. In contrast, current measurements were only used for analysis if biopsies responded to a specific stimulus. Results (ΔI_{sc}, G) were expressed as medians (ranges), or means ±standard deviation (SD), when appropriate. Net changes of I_{sc} were calculated as the difference between the baseline current and the peak response. In order to compare electrophysiological parameters within the same experiment, data were evaluated for normal distribution by use of D´Agostino normality test, and compared using a paired Students t-test or Wilcoxon matched pairs test when appropriate. Data were analyzed by use of a commercial software program (GraphPad Prism 5; GraphPad Software Inc., La Jolla, CA, USA). Significance was set at $p < 0.05$ for all statistical analyses.

4. Results

4.1 Feline Duodenal Biopsies

Five cats were enrolled into this study and a total of 24 biopsies were collected from these cats. Of these biopsies, seven biopsies were evaluated separately in order to serve as histopathological controls, while 17 specimens underwent investigation in the Ussing chamber. Of the 17 samples transferred into the Ussing chamber, 14 were evaluated by the study protocol as described in materials and methods (section 3.5). In two cases, technical problems of the amplifier or electrodes precluded further measurements. In one additional biopsy, the sample was lost into the Ussing chamber solution. There were four biopsies (from two different cats) that did not respond to any substance. The total duration of the experiments varied between 40.3 and 220.2 minutes (median: 82.3 minutes). Results from the experiments with these feline duodenal biopsies including information how many samples per animal responded to the compound applied are summarized in Table 8.

4.1.1 Baseline Observations

The conductance was calculated for 14 feline duodenal biopsies (Figure 10). The mean (±SD) conductance at the beginning of the experiment was 61.7 ±28.7 mS/cm^2 (resistance: 20.3 ±10.8 Ω•cm^2). Values did not change significantly during the experiments (p-value: 0.1040). At the end of all experiments, the median conductance was 71.9 ±36.1 mS/cm^2 (resistance 18.5 ±11.8 Ω•cm^2).

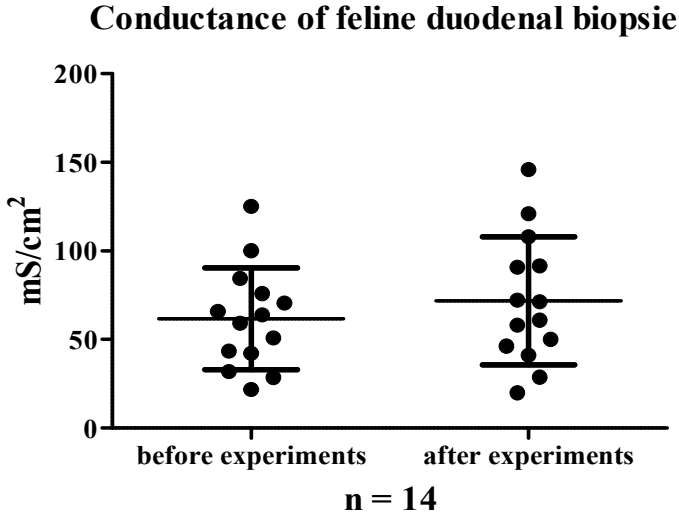

Figure 10: Conductance of feline duodenal biopsies

This scatter dot plot displays the conductance of feline duodenal biopsies before the start of the experiments and after the conclusion of the experiments. Lines represent mean ±SD. The y-axis of the graph shows the current in mS/cm^2. The mean conductance increased slightly from 61.7 (before the beginning of the experiments) to 71.9 mS/cm^2 (after the end of the experiments). However, this change did not reach statistical significance (p-value: 0.1040).

4.1.2 Absorptive Function

4.1.2.1 Response to Glucose

Of 17 biopsies tested, two feline duodenal specimens responded to stimulation with 40 mM glucose (Figure 11). Thus, the response rate of feline duodenal biopsies to glucose was poor (11.8%). However, immediately after transferring glucose into the mucosal bathing solution, the current measured for the two responding biopsies rose by 24 µA/cm² and 32 µA/cm², respectively. The specimen with the highest value took 111.4 s to reach its maximum value, while the other biopsy required only 68.4 s to reach maximum effect. The short circuit current remained on a plateau until it was abolished by phloridzin. Both biopsies were collected from the same cat.

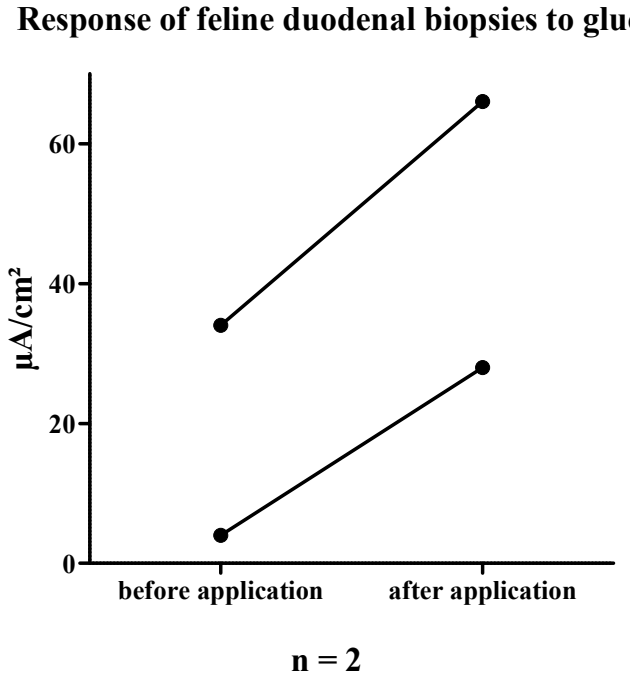

Figure 11: Response of feline duodenal biopsies to glucose

This figure shows the response of two feline duodenal biopsy samples to 40 mM glucose in the mucosal buffer. The y-axis of this figure represents the short circuit current (µA/cm²), while the x-axis plots the individual samples before and after exposure to glucose. Exposure to glucose increased the short circuit current by 24, µA/cm² and 32 µA/cm². The time until the maximum current values were reached (Δt) was 68.4 and 111.4 s, respectively.

4.1.2.2 Response to Phloridzin

At least one duodenal biopsy of each cat did respond to 500 µM phloridzin. Overall, twelve of 17 exposed biopsies responded (Figure 12). The median current reduction was 19 µA/cm² with values varying between 8 and 66 µA/cm². No correlation between time and the level of the ΔI_{sc} could be observed. The duration of time until the maximum current was reached ranged from 4.0 s (ΔI_{sc}: 20 µA/cm²) to 103.7 s (ΔI_{sc}: 66 µA/cm²) and the median time for this ΔI_{sc} was 39.1 s. An example of the raw current measurements is displayed in Figure 13.

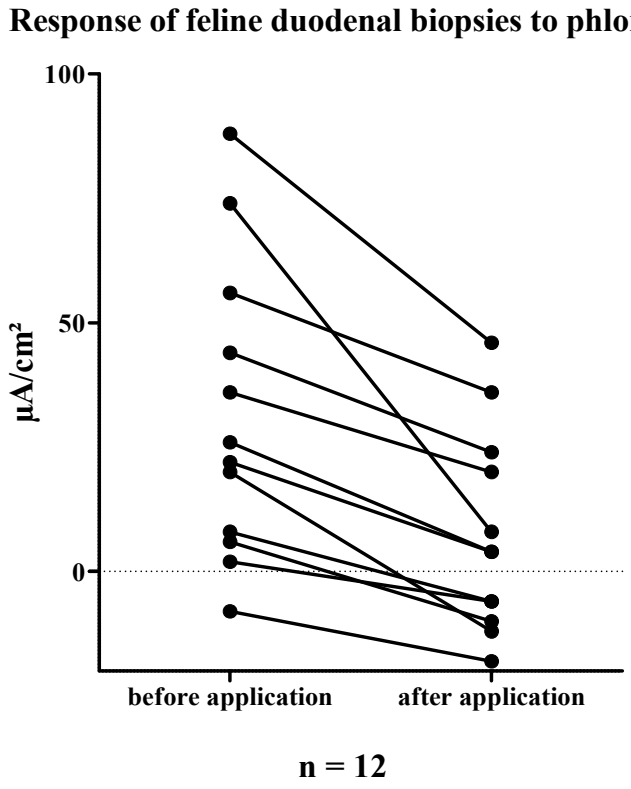

Figure 12: Response of feline duodenal biopsies to phloridzin

This figure shows the response of twelve feline colonic biopsy samples to 500 µM phloridzin in the mucosal buffer. Twelve of 17 exposed biopsies (70.6%) responded to the applied substance. Exposure to phloridzin decreased the median short circuit current by 19 µA/cm² (range: 8-66 µA/cm^2). The median duration until maximum current value decreases were reached (Δ t) was 39.1 s (range: 4.0-103.7 s).

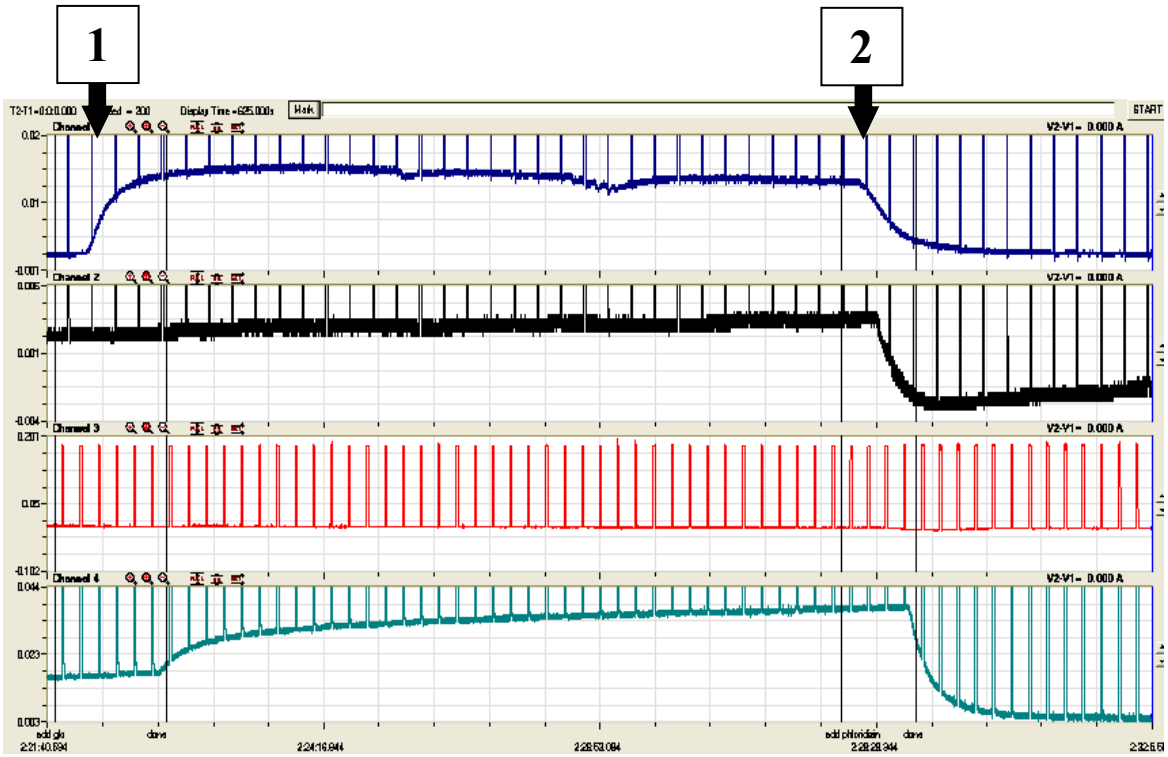

Figure 13: Raw recordings from an Ussing chamber experiment with four feline duodenal biopsies that were exposed to 40 mM glucose and 500 µM phloridzin

The graphs represent the raw current measurements of four feline duodenal biopsies during the experiment. Each Ussing chamber was loaded with one biopsy and results are displayed for each chamber as a separate channel, each shown in a different color. The arrows above the graphs indicate the time points for the application of glucose (1) and phloridzin (2), respectively. While the first and the fourth biopsy (blue and green graph) clearly responded to both chemicals, the second biopsy (black graph) is only responding to phloridzin. One biopsy (red graph) does not respond to either glucose or phloridzin and the current remains stable at baseline values.

4.1.3 Secretory Function

4.1.3.1 Response to Histamine

Of the 17 duodenal biopsies, eight specimens clearly responded to 200 µM histamine, dissolved in the bathing solution of the serosal side (Figure 14). In five experiments (ΔI_{sc} ranging from 4 to 8 µA/cm^2), a short rise of the current appeared and after a slow, but steady fall, the baseline level of the current was reached again after 56.8-771.5 s. In comparison, the current of three other biopsies (ΔI_{sc} ranging from 26 to 30 µA/cm^2) resulted in a biphasic graph (Figure 15). After an immediate sharp rise of the current, the current values kept increasing slowly until reaching the highest measurable points after 44.7-54.0 s. I_{sc} then slowly dropped until baseline values were reached again after 187.1-203.0 s. The three biopsies with this response had all been collected from the same cat. The median ΔI_{sc} of all samples was 7 µA/cm² (range: 4-30 µA/cm²). Median reaction time until maximum I_{sc} was reached was 46.7 s, with values ranging from 2.7 s to 54.6 s.

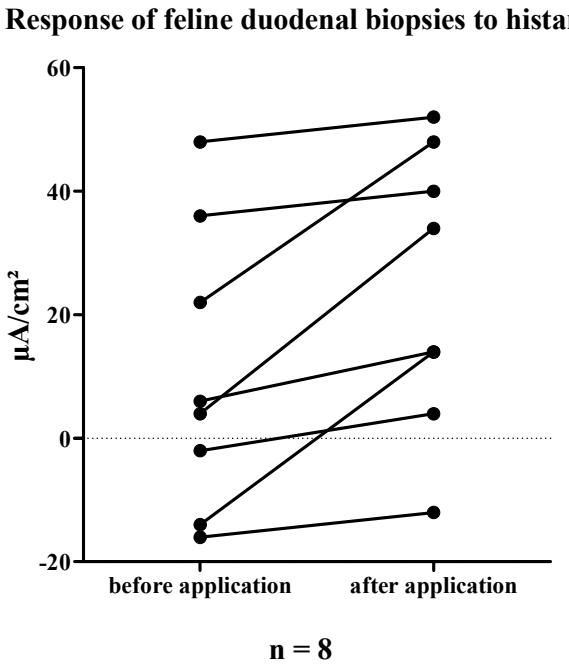

Figure 14: **Response of feline duodenal biopsies to histamine**
This figure shows the response of eight feline duodenal biopsy samples to 200 µM histamine in the serosal buffer. Exposure to histamine increased the median short circuit current by 7 µA/cm² (range: 4-30 µA/cm^2). Median reaction time until maximum current was reached was 46.7 s (range 2.7-54.6 s).

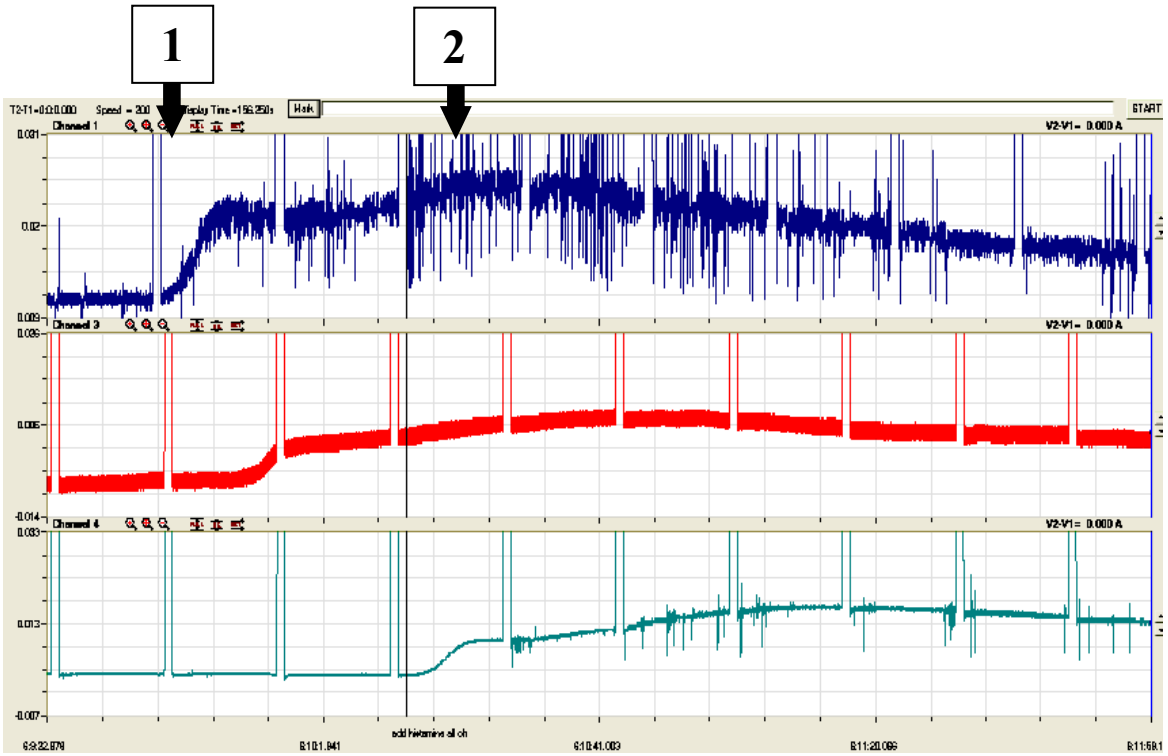

Figure 15: Raw current data collected during exposure of three feline duodenal biopsies to 200 µM histamine

The data recorded by the iWorx software displays the short circuit current of three feline intestinal samples. After exposure to histamine (arrow 1), the currents increased immediately in all three biopsies. Then, the currents remained on a plateau for a few seconds, before they rose again and reached maximum values (arrow 2). After a few minutes of a continuous decrease the current values reached baseline values.

4.1.3.2 Response to Serotonin

Sixteen feline duodenal biopsies were exposed to 200 µM serotonin. Overall, eight samples responded to this substance (Figure 16). The median change in short circuit current was 19 µA/cm², ranging from 6 to 32 µA/cm^2. The median time until the current reached the highest values was 83.9 s (range: 16.7-217.9 s). No correlation between the level of ΔI_{sc} and the time needed for that change to occur could be observed. While it took 45.0 s to reach the highest amplitude of 32 µA/cm^2, the smallest increase (6 µA/cm²) was reached after 100.7 s. In only two cases, the current dropped back to the original baseline values (after 13.3 and 18.4 minutes, respectively). The short circuit current of two other biopsies remained at maximum values for up to 18.5 minutes. The current of four biopsies steadily dropped as time went by, but did not reach baseline values even after 16-18 minutes. In those cases, the experiment was continued with the application of the next substance after 16-18 minutes regardless of the current values.

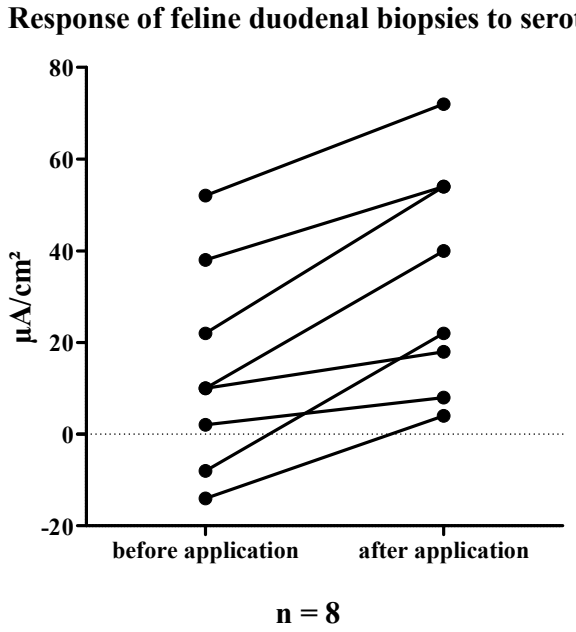

Figure 16: Response of feline duodenal biopsies to serotonin

This figure shows the response of eight feline duodenal biopsy samples to 200 µM serotonin in the serosal buffer. Fifty % of the exposed feline small intestinal biopsies responded to the applied substance. Exposure to serotonin increased the median short circuit current by 19 µA/cm² (range: 6-32 µA/cm^2). Those changes occurred after a median time of 83.9 s (range: 16.7-217.9 s).

4.1.3.3 Response to Prostaglandin E_2

Fifteen feline duodenal biopsies were exposed to 4 µM prostaglandin, but none of them showed any response.

4.1.3.4 Response to Forskolin

Of 14 feline duodenal biopsies exposed to 5 µM forskolin to the serosal side of the bathing solution, only one responded (Figure 17). The peak current change of 16 µA/cm² was reached within 89.7 s and remained increased for at least 15 minutes.

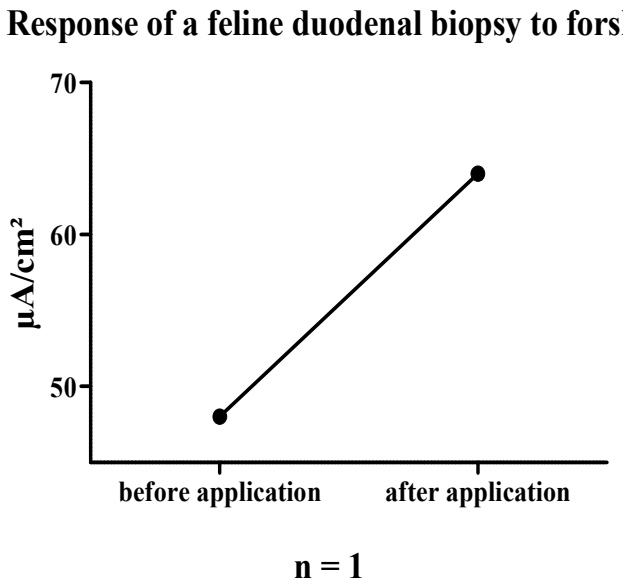

Figure 17: Response of a feline duodenal biopsy to forskolin

This figure shows the response of a feline duodenal biopsy sample to 5 µM forskolin in the serosal buffer. Exposure to forskolin increased the short circuit current by 48 µA/cm² within 89.7 s. The current remained steady at that level for more than 15 minutes.

4.1.4 Evaluation of Sample Viability

4.1.4.1 Response to Glucose after Completion of all Experiments

Only one of 14 feline duodenal biopsies exposed to 40 mM glucose at the end of all experiments responded. A ΔI_{sc} of 10 µA/cm ^{2} was reached after 96.0 s (Figure 18). Interestingly, this biopsy did not respond to glucose added at the beginning of the experiment, but did respond to phloridzin. Other biopsies only showed a tendency to an increase in ΔI_{sc} (i.e., ΔI_{sc} increased over a period of 5-10 minutes by about 4 µA/cm^2).

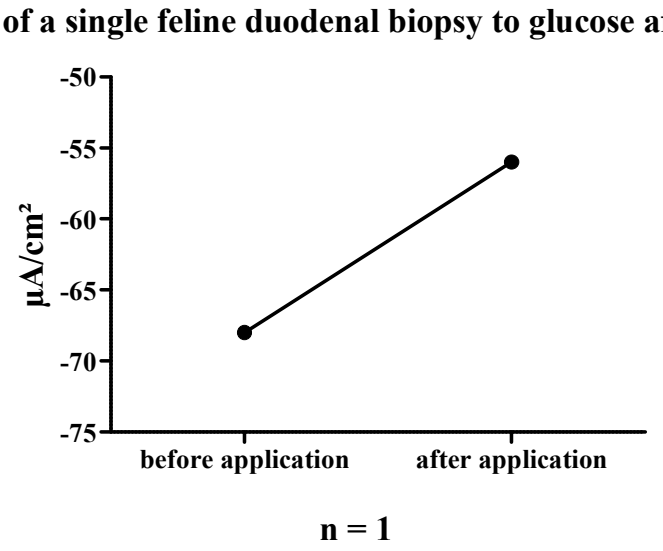

Figure 18: Response of a single feline duodenal biopsy to glucose after experiments
This figure shows the response of a single feline duodenal biopsy sample exposed to 40 mM glucose, after being in the Ussing chamber for more than 100 minutes. Exposure to glucose increased the short circuit current from -64 µA/cm^2 to -54 µA/cm^2 within 96.0 s. None of the other 14 biopsies evaluated showed a response to glucose at that time.

4.1.4.2 Response to Ouabain

Fourteen feline duodenal biopsies were exposed to 600 µM ouabain, which was added to the serosal bathing solution at the end of the experiment. Eleven specimens showed a reduction of the current (Figure 19). The median drop of the current in these eleven biopsies was 14 µA/cm² (range: 4-30 µA/cm²). The current of some specimens dropped quickly (within 5.4 minutes), while others took as long as 17.2 minutes (median: 9.1 minutes). Some biopsies were evaluated for 40 minutes, while the current constantly decreased. Changes of up to 66 µA/cm^2 could be observed. However, it remains unclear, whether the observed changes were due to the chemical, or were caused by something else. Thus, only obvious and immediate changes were considered to be relevant.

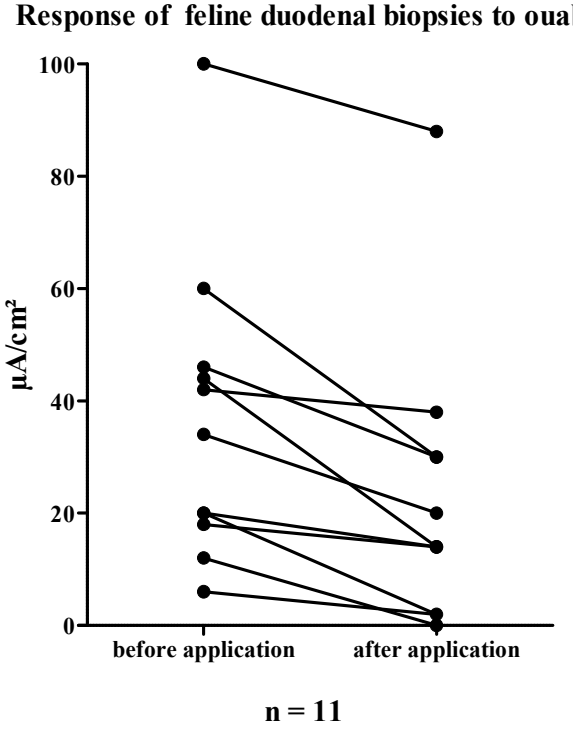

Figure 19: Response of feline duodenal biopsies to ouabain

This figure shows the response of eleven feline duodenal biopsy samples to 600 µM ouabain in the serosal buffer. Eleven of 14 exposed biopsies (78.6%) responded to the applied stimulus. Exposure to ouabain decreased the median short circuit current by 14 µA/cm² (range: 4-30 µA/cm^2). The median time required for this change was 9.1 minutes, ranging from 5.4 to 17.2 minutes.

Table 8: **Short circuit current measurements after exposure of feline duodenal biopsies to various compounds**
This table summarizes the response of the feline duodenal biopsies after exposure to various compounds. While most biopsies responded to phloridzin and ouabain, no changes could be observed in any tissue specimens that were exposed to prostaglandin. The reaction time varied widely between individual samples and a wide variability in response rate among samples from the same patient could be observed.

stimulus	n / N	%	median ΔI_{sc} (µA/cm²)	range (µA/cm²)	time (Δt) median (range)	number of cats, for which x biopsies showed a response				
						x=0	x=1	x=2	x=3	x=4
glucose 40 mM	2/17	11.8 %	28	24-32	89.9 (68.4-111.4)	4	0	1	0	0
phloridzin 500 µM	12/17	70.6 %	19	8-66	39.1 (4.0-103.7)	0	1	2	1	1
histamine 200 µM	8/17	47.1 %	7	4-30	46.7 (2.7-54.6)	1	1	2	1	0
serotonin 200 µM	8/16	50.0 %	19	6-32	83.9 (16.7-217.9)	0	3	1	1	0
PGE 4 µM	0/15	0 %	0	N/A	0 (N/A)	5	0	0	0	0
forskolin 5 µM	1/14	7.1 %	16	N/A	89.7 (N/A)	3	1	0	0	0
glucose 40 mM	1/14	7.1 %	12	N/A	96.0 (N/A)	3	1	0	0	0
ouabain 600 µM	11/14	78.6 %	14	4-30	9.4 min (5.4-17.2)	0	0	1	3	0
n = number of responding biopsies, N = number of exposed biopsies, N/A = not available										

4.2 Feline Colonic Biopsies

Twenty four colonic biopsies were collected from four cats. While 16 biopsies were transferred into the Ussing chamber, eight specimens served as backup samples. The experiments were completed with fifteen biopsies, technical problems hindered the completion of the protocol in one experiment. Median time for all experiments was 108.1 minutes with a range of 100.1-152.3 minutes. The total number of non-responding biopsies was five. A short summary of all results including information how many samples per animal responded to the compound applied is given at the end of this section (Table 9).

4.2.1 Baseline Observations

Conductance was calculated for 15 feline colonic biopsies (Figure 20). The mean (±SD) conductance at the start of the experiment was 101.6 ±57.4 mS/cm^2 (resistance: 17.1 ±20.3 Ω•cm^2). Values changed significantly during the period of the experiments (p-value: 0.0006). At the end of the experiments, the median conductance was 186.7 ±153.5 mS/cm^2 (resistance: 12.3 ±15.3 Ω•cm^2).

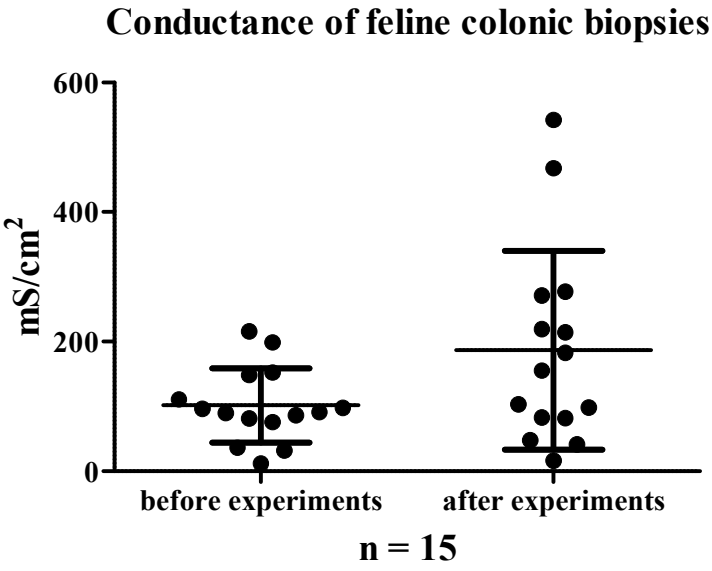

Figure 20: Conductance of feline colonic biopsies

This scatter dot plot shows the conductance values of feline colonic biopsies at the beginning and at the end of all experiments. Lines represent mean ±SD. Mean conductance rose significantly during the experiments (p-value: 0.0006).

4.2.2 Absorptive Function

4.2.2.1 Response to Glucose

Sixteen feline colonic biopsies were exposed to 40 mM glucose, but no response could be observed for any of the biopsies.

4.2.2.2 Response to Phloridzin

Sixteen feline colonic biopsies were exposed to 500 µM phloridzin, but no response could be observed for any of the biopsies.

4.2.3 Secretory Function

4.2.3.1 Response to Histamine

Sixteen feline colonic biopsies were exposed to 200 µM histamine. Five of the specimens showed a response to the stimulus as indicated by a rise of their short circuit current. (Figure 21) The median change of the current was 10 µA/cm² (range: 6-20 µA/cm^2). While the median reaction time (Δt) was 70.5 s, values ranged from 49.2-213.2 s. After reaching their maximum values, the current dropped steadily towards baseline values observed before exposure. Four biopsies reached the pre-exposure values after 225.0 to 354.5 s. However, the current of one biopsy did not reach baseline values within 12.4 minutes.

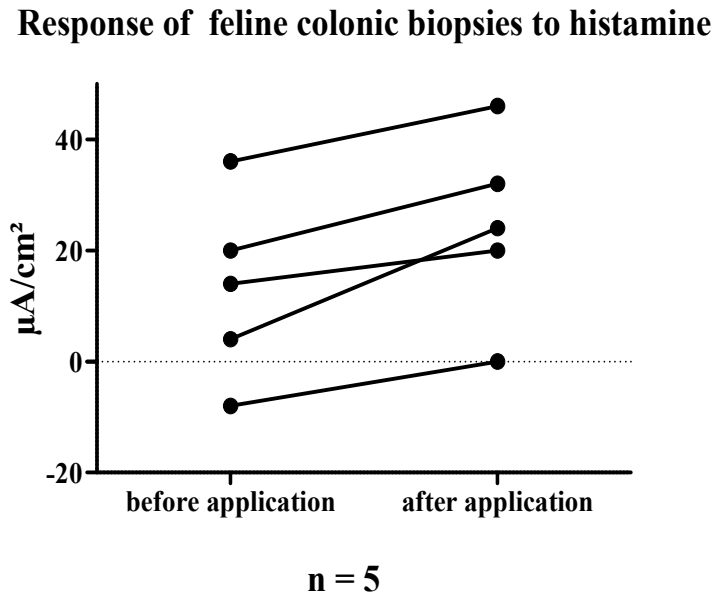

Figure 21: Response of feline colonic biopsies to histamine

This figure shows the response of five feline colonic biopsy samples to 200 µM histamine in the serosal buffer. Five of 16 exposed biopsies (31.3%) responded to the applied stimulus. Exposure to histamine increased the median short circuit current by 10 µA/cm² (range: 6-20 µA/cm^2). The time to reach the peak ranged from 49.2 to 213.2 s (median: 70.5 s).

4.2.3.2 Response to Serotonin

Seven biopsies responded to exposure to 200 µM serotonin (Figure 22). The mean increase of the short circuit current was 22 µA/cm², ranging from 12 to 58 µA/cm^2. The median time for this current change was 71.6 s (range 35.7-101.2 s). In six of these seven biopsies, the current reached pre-exposure values after 282.8-461.9 s. However, the short circuit current of one biopsy did not reach the pre-exposure values within 13.5 minutes.

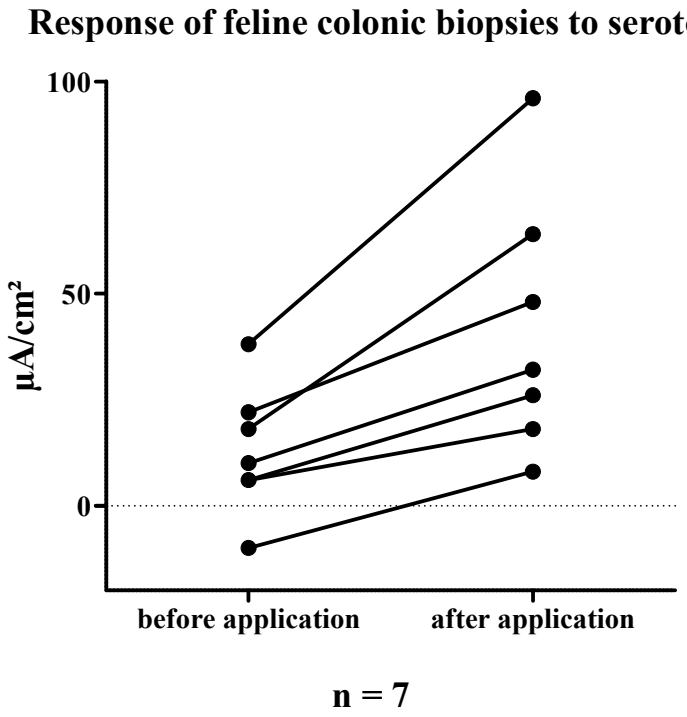

Figure 22: Response of feline colonic biopsies to serotonin

This figure shows the response of seven colonic biopsy samples to 200 µM serotonin in the serosal buffer. Seven of 16 exposed biopsies (43.8%) responded to the applied stimulus. Exposure to serotonin increased the median short circuit current by 22 µA/cm² (range: 12-58 µA/cm^2).

4.2.3.3 Response to Prostaglandin E_2

Of 16 biopsies exposed to 4 µM prostaglandin, five responded (Figure 23). The same five biopsies had also responded previously to both serotonin and histamine. The median rise of short circuit current was 16 µA/cm² (range: 12-40 µA/cm^2) and the median time for this change to occur was 46.0 s (range: 33.9-132.1 s). In four of the five cats, the graph quickly returned to baseline values (122.0-305.8 s). However, the current of one sample remained stable on its highest value for more than 7 minutes.

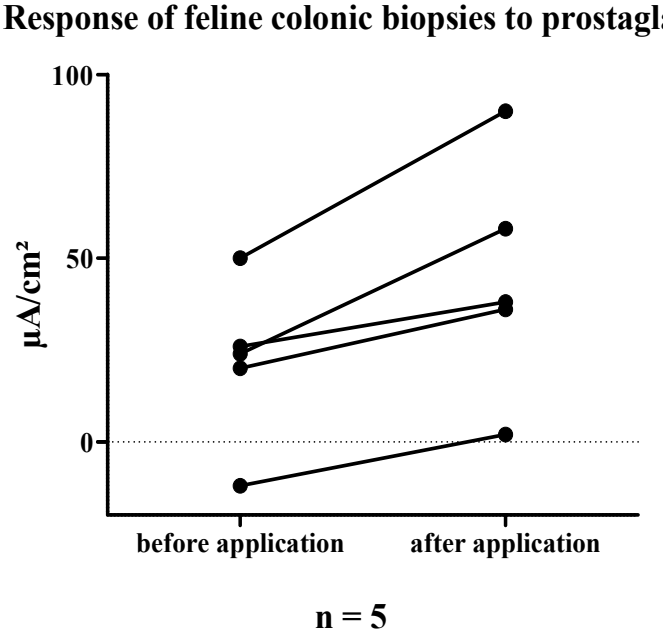

Figure 23: Response of feline colonic biopsies to prostaglandin

This figure shows the response of five colonic biopsy samples to 4 µM prostaglandin in the serosal buffer. Five of 16 exposed biopsies (31.3%) responded to the applied stimulus. Exposure to prostaglandin increased the median short circuit current by 16 µA/cm² (range: 12-40 µA/cm^2). The median time until maximum current values were reached was 46.0 s (range: 33.9-132.1 s).

4.2.3.4 Response to Forskolin

Of all tested biopsies (n=16), seven samples did respond to 5 µM forskolin (Figure 24). Six of these samples were the same biopsies that previously responded to 200 µM serotonin. The short circuit current of the various specimens increased by 6-110 µA/cm^2 (median value: 20 µA/cm²). Interestingly, values of most samples did not drop back to baseline values and the current of only one biopsy sample decreased to baseline values after 330.0 s, while the others remained stable at, or close to, their peak values.

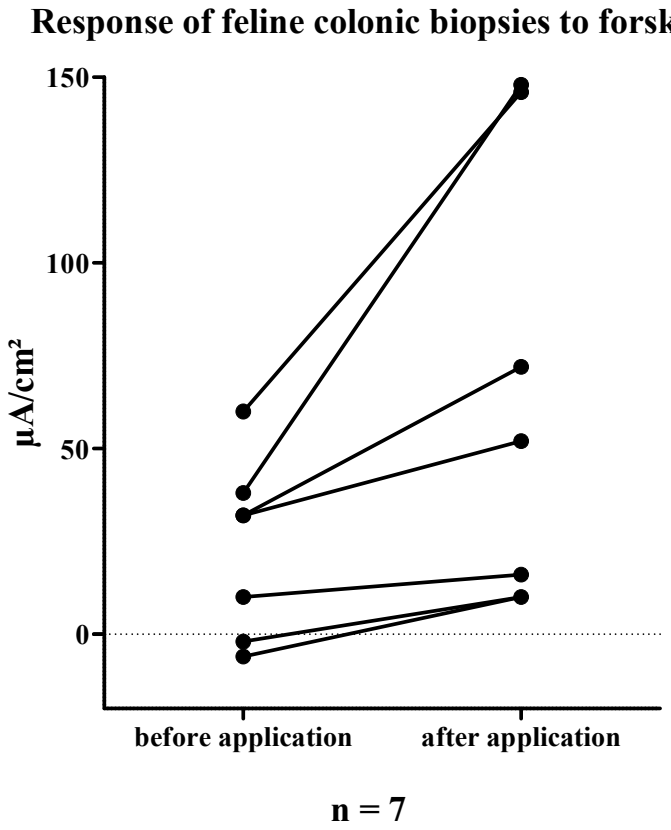

Figure 24: Response of feline colonic biopsies to forskolin

This figure shows the response of seven feline colonic biopsy samples to 5 µM forskolin in the serosal buffer. Seven of 16 exposed biopsies (43.8%) responded to the applied substance. Exposure to forskolin increased the median short circuit current by 20 µA/cm² (range: 6-110 µA/cm^2). Time until maximum current response was reached (Δt) was 88.9 s (range: 41.1-96.8 s).

4.2.4 Evaluation of Sample Viability

4.2.4.1 Response to Ouabain

Nine of 15 biopsies responded to 600 µM ouabain in the serosal bathing solution (Figure 25). While the short circuit current in some cases reached its lowest value after a short time (138.1 s), the current of other samples continuously dropped for up to 21.5 minutes. The median ΔI_{sc} was 32 µA/cm^2 and ranged from 4 to 128 µA/cm^2. While the current of eight biopsies dropped to values close to 0 µA/cm^2, the current of one biopsy was reduced from 4 µA/cm^2 to -44 µA/cm^2. Measurements were recorded for up to 40 minutes after ouabain was applied, but the observed current changes were nonspecific and therefore were not considered to be a direct result of ouabain application.

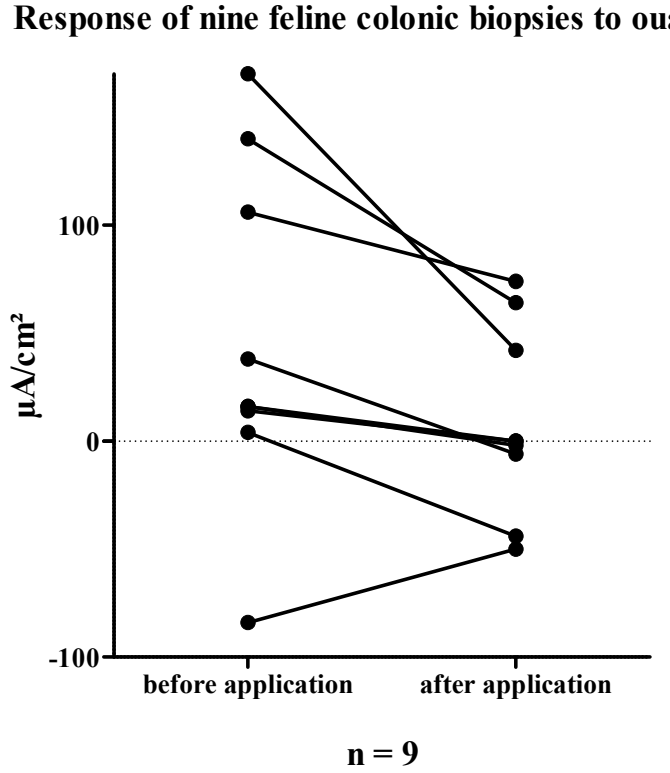

Figure 25: Response of feline colonic biopsies to ouabain
This figure shows the response of nine feline colonic biopsy samples to 600 µM ouabain in the serosal buffer. Nine of 15 exposed biopsies (60.0%) responded to the applied stimulus. Exposure to ouabain changed the median short circuit current by 32 µA/cm² (range: 4-128 µA/cm^2). Changes occurred within 2.2-21.5 minutes (median: 8.3 minutes).

Table 9: **Short circuit current measurements after exposure of feline colonic biopsies to various compounds**
This table summarizes the response of the feline colonic biopsies after exposure to various compounds. While none of the samples reacted to either glucose or phloridzin, at least five of them consistently responded to all secretory drugs and to ouabain. The reaction time varied widely between individual samples and a wide variability in response rate among the samples from the same patient could be observed.

stimulus	**n / N**	**%**	**median ΔI_{sc} (µA/cm²)**	**range (µA/cm²)**	**time (Δt) median (range)**	**number of cats, for which x biopsies showed a response**				
						x=0	**x=1**	**x=2**	**x=3**	**x=4**
glucose 40 mM	0/16	0 %	0	N/A	N/A	4	0	0	0	0
phloridzin 500 µM	0/16	0 %	0	N/A	N/A	4	0	0	0	0
histamine 200 µM	5/16	31.3 %	10	6-20	70.5 (49.2-213.2)	1	2	0	1	0
serotonin 200 µM	7/16	43.8 %	22	12-58	71.6 (35.7-101.2)	0	2	1	1	0
PGE 4 µM	5/16	31.3 %	16	12-40	46.0 (33.9-132.1)	1	2	0	1	0
forskolin 5 µM	7/16	43.8 %	20	6-110	88.9 (41.1-96.8)	0	2	1	1	0
ouabain 600 µM	9/15	60.0 %	28	12-128	8.3 min (2.2-21.5 min)	0	1	1	2	0
n = number of responding biopsies, N = number of exposed biopsies										

4.3 Canine Duodenal Biopsies

A total of 14 dogs were enrolled into the study. Fifty-one duodenal biopsies were transferred into Ussing chambers and 24 samples served as histopathological controls. For 44 of the 51 biopsies all experiments could be completed. Reasons of incomplete experiments included technical difficulties (electrode breakdown, free floating biopsies in the chamber, and amplifier overload). The median duration for completed experiments was 90.9 minutes (range: 75.1-153.0 minutes). A total of 19 biopsies did not respond to any substance. Three of those samples were from dogs that exhibited clinical signs of gastrointestinal disease, but no histopathological changes of the duodenal biopsy samples (group 2) and 16 samples were from animals with clinical signs of gastrointestinal disease and histopathological changes of the duodenal biopsies (group 3). None of the biopsies from three of the eight dogs of group 3 (12 biopsy samples total) responded to any substance applied. A summary of all results, as well as the results for each individual group including information how many samples per animal responded to the compound applied are displayed in tables 10, 11, 12, and 13 at the end of this section.

4.3.1 Baseline Observations

Forty-four canine duodenal biopsies were evaluated (Figure 26). At the beginning of the experiment, mean (±SD) conductance was 89.9 ±71.7 mS/cm^2 (resistance: 17.3 ±10.3 Ω•cm^2). During the experiment, the conductance rose until reaching values of 126.5 ±111.2 mS/cm^2 (resistance 14.5 ±10.5 Ω•cm^2; p-value: <0.0001).

The findings for the 44 biopsies were separately analyzed for each of the three groups. Group 1 consisted of two healthy dogs without any history of gastrointestinal disease and a WSAVA grading score of <2.7. Group 2 consisted of four dogs with clinical signs of gastrointestinal disease, but an unremarkable duodenum histopathologically (WSAVA score <2.7). Group 3 consisted of eight dogs with clinical signs of gastrointestinal disease and significant histopathological lesions (WSAVA score >3.0). For each group, the median conductance values were evaluated at the beginning and at the end of all experiments. Additionally, a large variation of the short circuit current could be observed in each of the three groups (Figure 18, 19, 20).

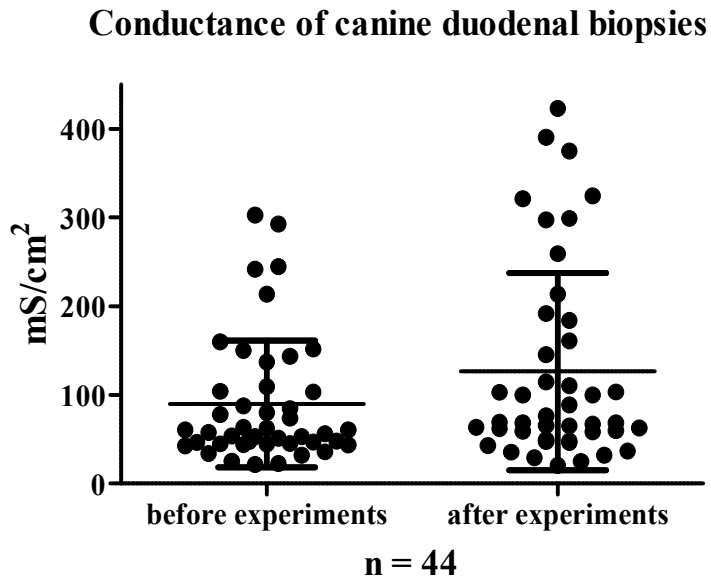

Figure 26: **Conductance of canine duodenal biopsies**
This scatter dot plot shows the conductance of 44 canine duodenal biopsies before and after performing the experiments. Lines represent mean ±SD. During the experiments the mean conductance rose significantly from 89.9 to 126.5 mS/cm^2 (p-value <0.0001).

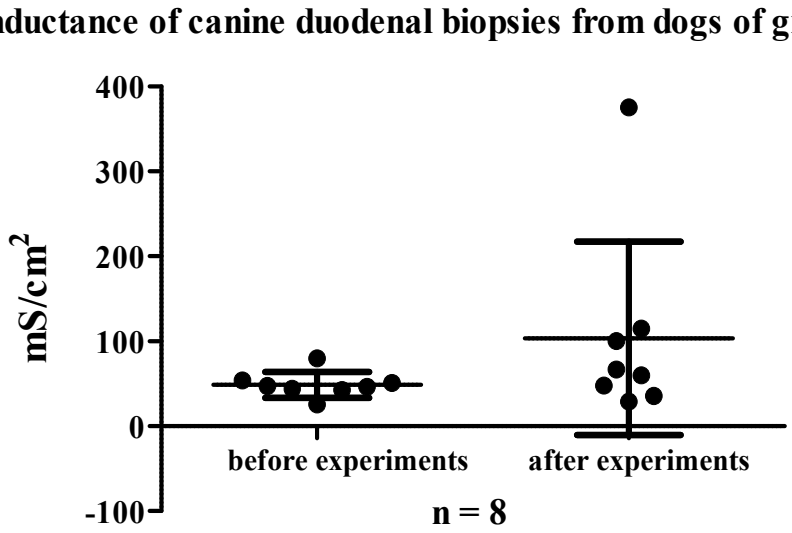

Figure 27: Conductance of canine duodenal biopsies from dogs of group 1
This scatter dot plot shows the conductance of eight duodenal biopsies from 2 healthy dogs before and after performing the experiments. Lines represent mean ±SD. During the experiments the mean conductance rose significantly from 48.9 to 103.6 mS/cm^2. The highest individual value observed was 375.2 mS/cm^2.

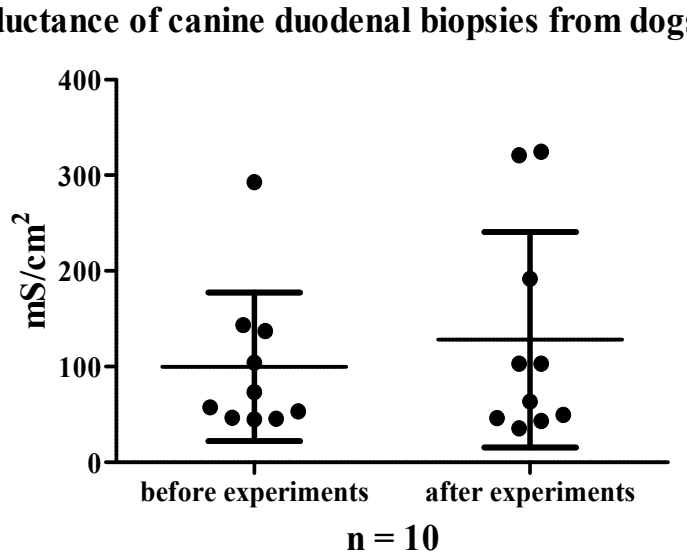

Figure 28: Conductance of canine duodenal biopsies from dogs of group 2
This scatter dot plot shows the conductance of ten canine duodenal biopsies before and after performing the experiments. Lines represent mean ±SD. Biopsies were taken from four dogs that were exhibiting clinical signs of gastrointestinal disease, but showed only minor histopathological abnormalities. During the experiments the mean conductance rose significantly from 99.9 to 128.1 mS/cm^2.

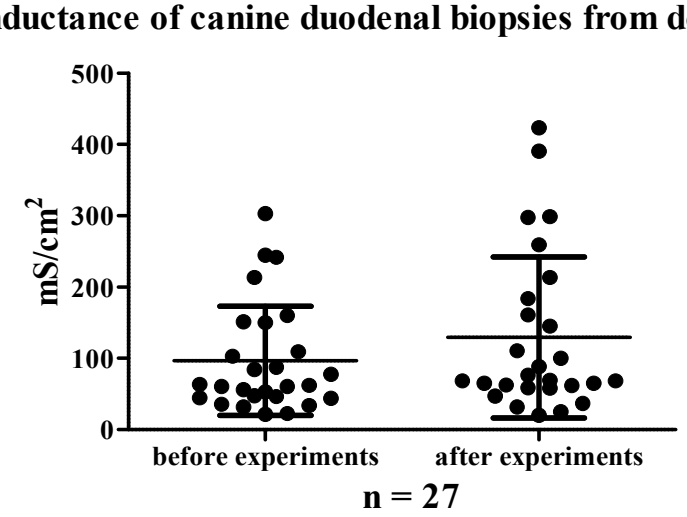

Figure 29: Conductance of canine duodenal biopsies from dogs of group 3
This scatter dot plot shows the conductance of 27 canine duodenal biopsies before and after performing the experiments. Lines represent mean ±SD. Biopsies were taken from eight dogs that were exhibiting clinical signs of gastrointestinal disease, and showed histopathological abnormalities. During the experiments the mean conductance rose significantly from 96.7 to 129.2 mS/cm^2. The highest individual value observed was 423.36 mS/cm^2.

4.3.2 Absorptive Function

4.3.2.1 Response to Glucose

The mucosa of 51 canine duodenal biopsies was exposed to 40 mM glucose. Twenty-one samples showed a response, demonstrated by a rise of their short circuit current (Figure 30). The median amplitude was 34 µA/cm², and ranged from 6 to 170 µA/cm². The median reaction time was 60.0 s (range: 2.5-177.3 s).

Results from these 21 biopsies were analyzed after classification (group 1: healthy individuals; group 2: dogs with clinical signs, but histopathologically unremarkable duodenum; group 3: dogs with clinical signs and accompanied with histopathological lesions of the duodenum). Figures 31, 32, and 33 provide detailed information about the changes in current that occurred within each group. In summary, there was a large degree of variation of baseline current, reaction time, as well as ΔI_{sc}, regardless of the prevalence of histopathological lesions and/or clinical signs of the patients.

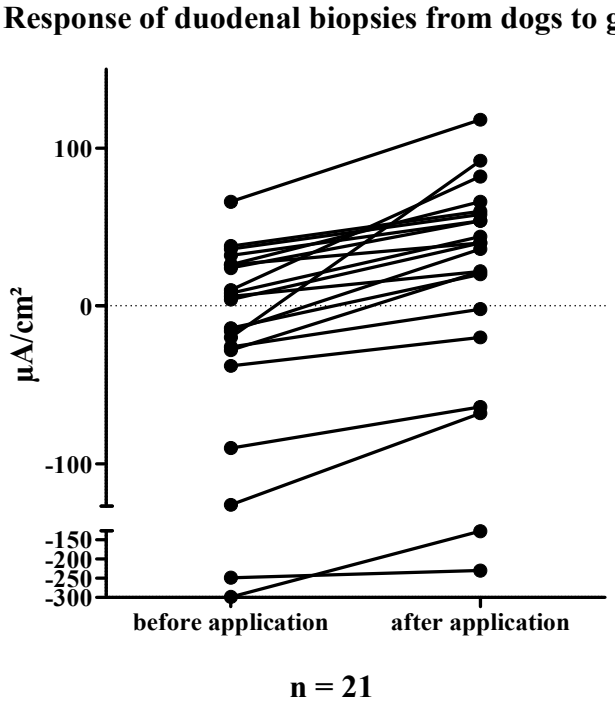

Figure 30: Response of duodenal biopsies from dogs to glucose

This figure shows the response of 21 duodenal biopsy samples to 40 mM glucose in the mucosal buffer. Exposure to glucose increased the median short circuit current by 34 µA/cm² (range: 6-170 µA/cm^2).

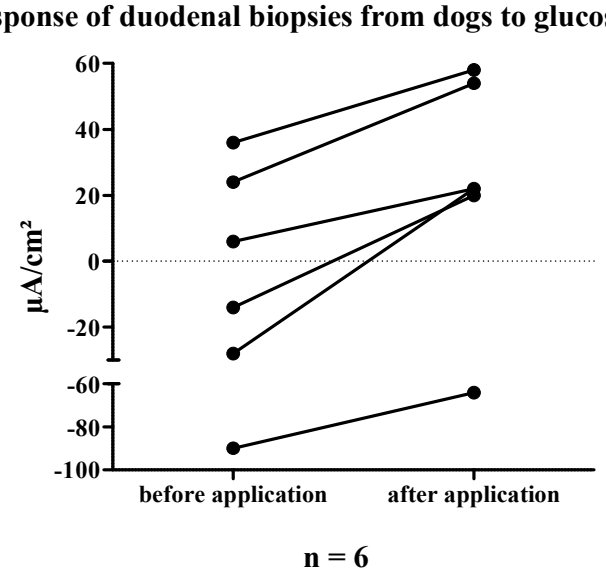

Figure 31: Response of duodenal biopsies from dogs to glucose: group 1
This figure shows the response of six duodenal biopsy samples from healthy dogs to 40 mM glucose in the mucosal buffer. Exposure to glucose increased the median short circuit current by 28 µA/cm² (range: 16-50 µA/cm^2) within 93.1 s (27.7-108.6 s). The baseline current exhibited a large degree of variation, ranging from -90 to 36 µA/cm^2. The short circuit currents of those biopsies after contact with 40 mM glucose ranged from -64 to 42 µA/cm^2.

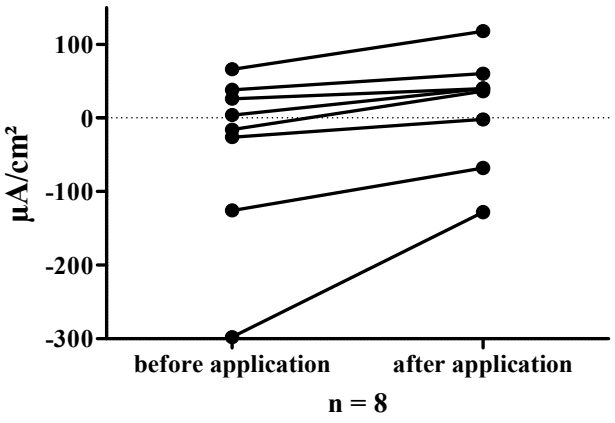

Figure 32: Response of duodenal biopsies from dogs to glucose: group 2
This figure shows the response of eight duodenal biopsy samples from dogs of group 2 to 40 mM glucose in the mucosal buffer. Eight of 15 (53.3 %) biopsies taken from dogs that exhibited only minor intestinal histopathological changes, but clinical signs of gastrointestinal disease, responded to 40 mM glucose. Exposure to glucose increased the median short circuit current by 52 µA/cm² (range: 14-170 µA/cm^2) within 50.8 s (33.1-146.0 s). No correlation between the duration and the amplitude of the current change could be observed.

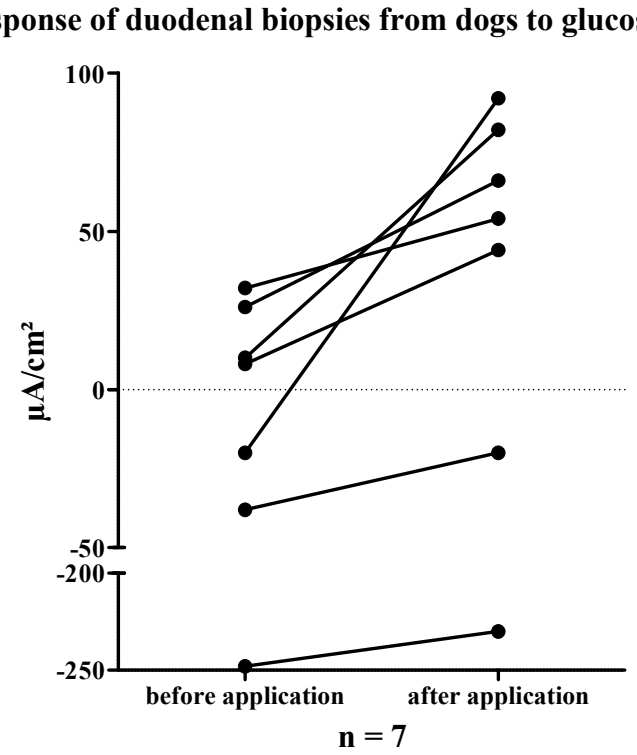

Figure 33: Response of duodenal biopsies from dogs to glucose: group 3
This figure shows the response of six duodenal biopsy samples to 40 mM glucose in the mucosal buffer. Seven of 32 (21.9%) of biopsies taken from dogs with clinical signs of gastrointestinal disease and histopathological lesions of duodenal biopsies responded to 40 mM glucose. Exposure to glucose increased the median short circuit current by 36 µA/cm² (range: 6-58 µA/cm^2) within 31.6 s (2.5-177.3 s). The baseline current exhibited a large degree of variation, ranging from -248 to 32 µA/cm^2. The short circuit currents of those biopsies after contact with 40 mM glucose ranged from -230 to 92 µA/cm^2.

4.3.2.2 Response to Phloridzin

Fifty-one canine duodenal biopsies were exposed to 500 µM phloridzin (Figure 34). The ΔI_{sc} of 25 specimens decreased immediately (range: 14-168 µA/cm²; median value: 32 µA/cm²). Median time until the peak current change was reached was 60.0 s (range: 9.3-169.6 s). Figures 35, 36, and 37 show the response to phloridzin for biopsies collected from dogs of groups 1, 2, and 3 separately. Similarly to the exposure to glucose, a higher percentage of samples from dogs in group 1 than from those in group 2 responded, and more samples from dogs in group 2 responded than did those from dogs in group 3.
An example of the effects of glucose and phloridzin on short circuit current is shown in Figure 38. The response to glucose and phloridzin on the short circuit current and voltage is shown in Figure 39.

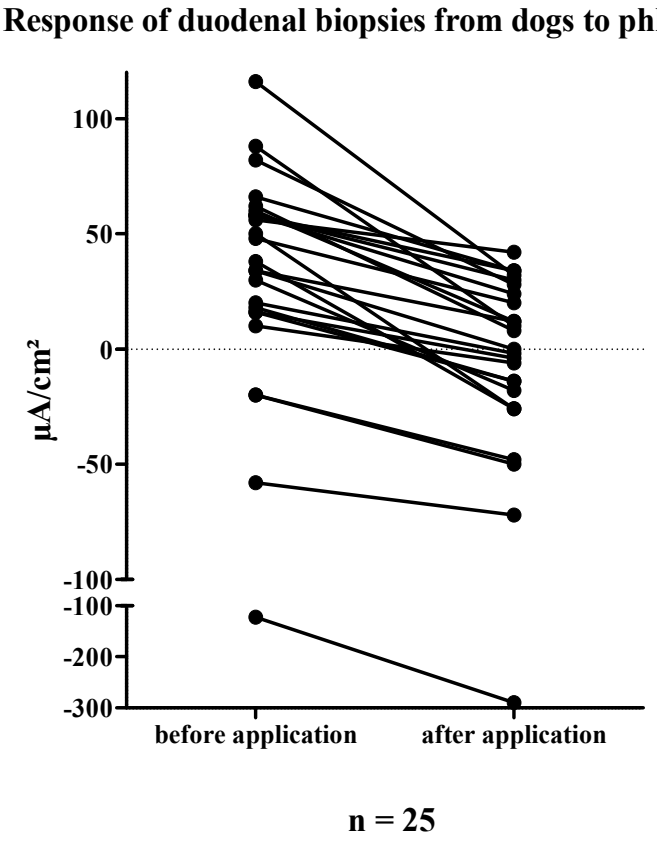

Figure 34: Response of duodenal biopsies from dogs to phloridzin
This figure shows the I_{sc} of 25 canine duodenal biopsies before and after exposure to 500 µM phloridzin. The short circuit current of all responding biopsies decreased between 14 and 168 µA/cm² (median value: 32 µA/cm²). Median duration of the current change (Δt) was 60.0 s (range: 9.3-169.6 s).

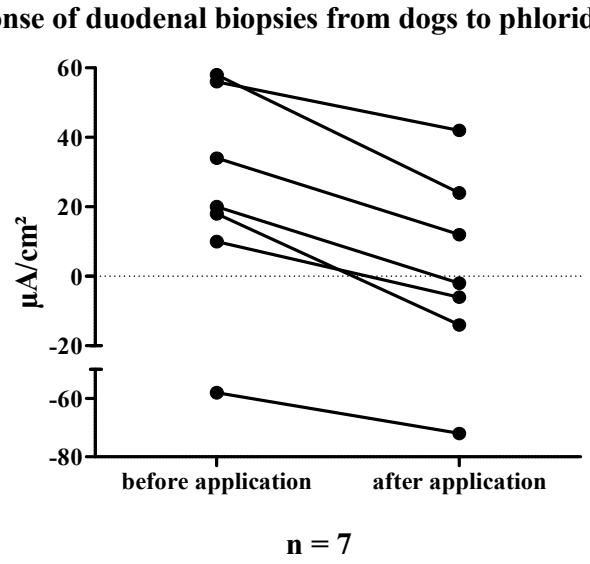

Figure 35: Response of duodenal biopsies from dogs to phloridzin: group 1
Seven of eight (87.5%) exposed duodenal biopsy samples from healthy dogs responded to phloridzin exposure. The figure shows the short circuit currents before and after exposure to phloridzin. Median ΔI_{sc} of the seven intestinal biopsies was 22 µA/cm² (range: 14-34 µA/cm²). The peak short circuit current was reached in a median of 88.6 s (range: 49.7-169.6 s). Baseline values ranged from -58 to 58 µA/cm² and decreased to values of -72 to 42 µA/cm².

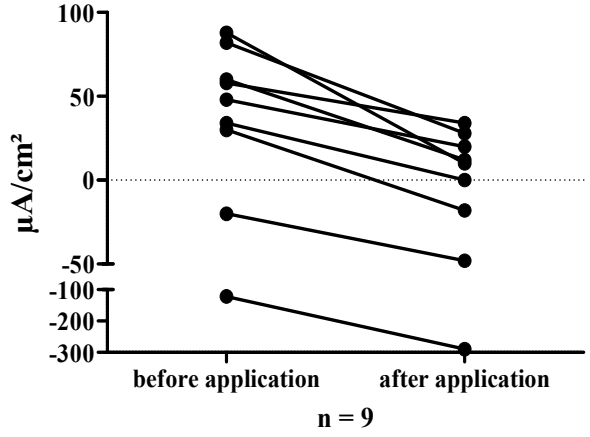

Figure 36: Response of duodenal biopsies from dogs to phloridzin: group 2
Nine of 15 (60.0%) exposed duodenal biopsy samples from dogs with clinical signs of gastrointestinal disease but unremarkable histopathologic findings responded to phloridzin exposure. The figure shows short circuit currents before and after exposure to phloridzin. Median ΔI_{sc} for the nine intestinal biopsies was 48 µA/cm² (range: 24-168 µA/cm²). The peak short circuit current was reached in a median of 74.0 s (range: 9.3-143.0 s). Baseline values ranged from -122 to 88 µA/cm² and decreased to values from -290 to 34 µA/cm².

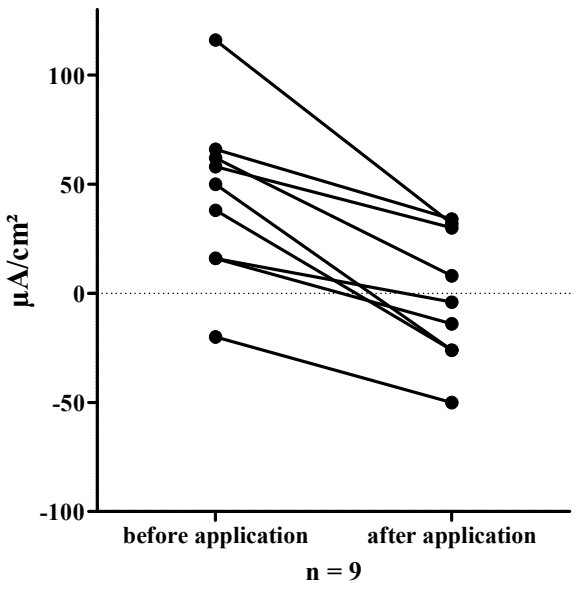

Figure 37: Response of duodenal biopsies from dogs to phloridzin: group 3
Nine of 32 (28.1%) duodenal biopsy samples from dogs with clinical signs of gastrointestinal signs and histopathologic changes responded to phloridzin exposure. The figure shows short circuit currents before and after exposure to phloridzin. Median ΔI_{sc} of the nine intestinal biopsies was 32 µA/cm² (range: 20-84 µA/cm²). The peak short circuit current was reached in a median of 53.7 s (range: 40.6-105.9 s). A large degree of variation of baseline, as well as post-exposure short circuit current values was observed.

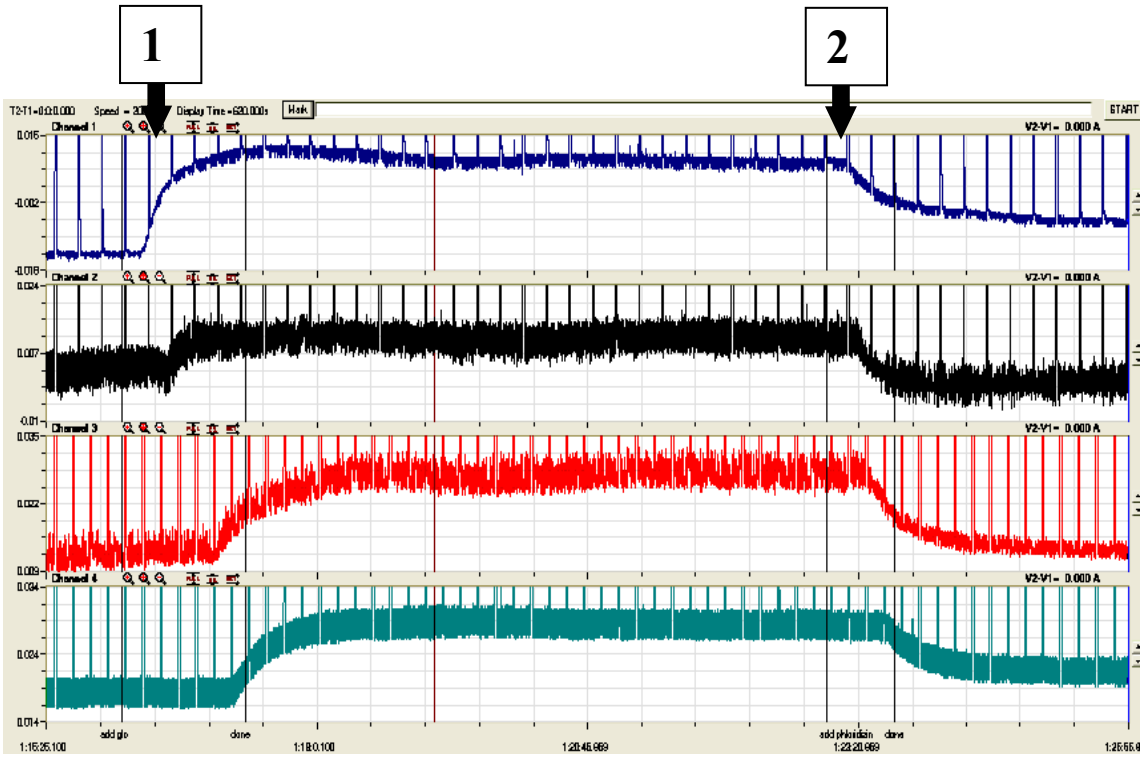

Figure 38: Short circuit current of four canine duodenal biopsies before, during, and after exposure to glucose and phloridzin

This figure shows the raw current data as recorded during the experiment. Four biopsies from the same animal were inserted into four different Ussing chambers. The current from each chamber is displayed in a separate color. There is an immediate response to 40 mM glucose that shows a saturation curve (arrow 1). After saturation, the current stayed stable at maximum values until it decreased in response to the glucose transporter inhibitor phloridzin (arrow 2). Short circuit currents are shown to decrease immediately until they reach baseline values.

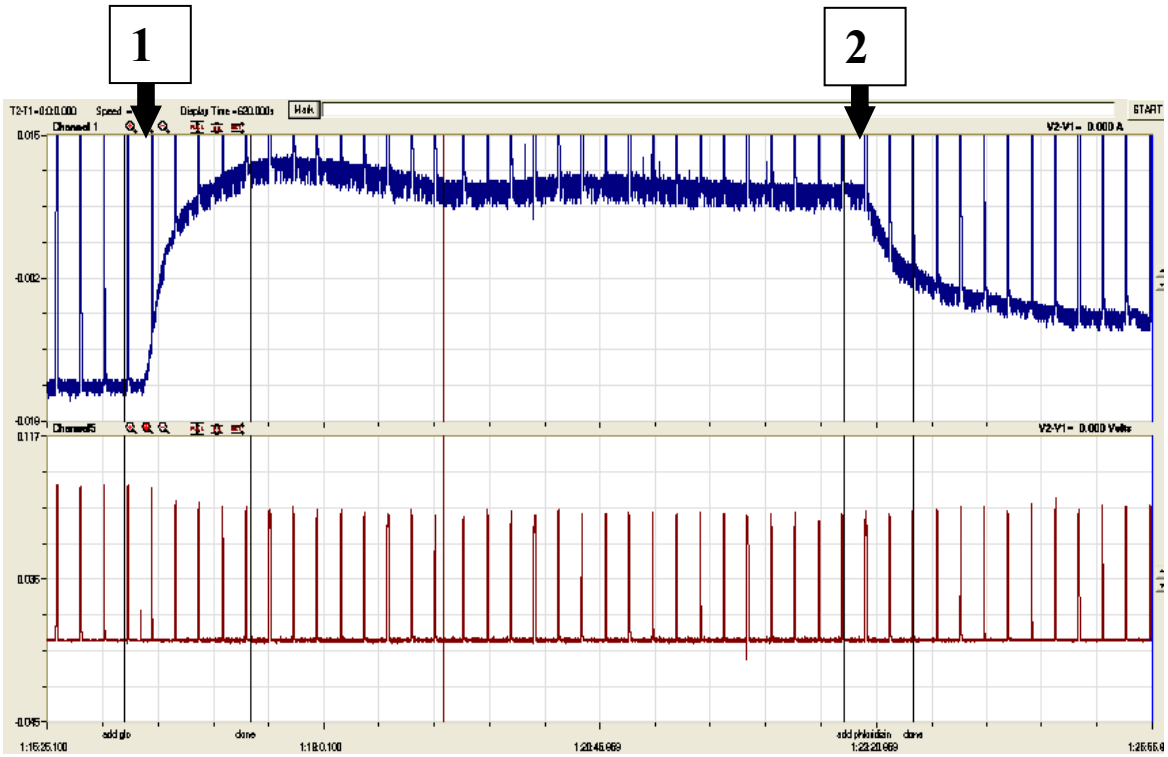

Figure 39: Short circuit current and voltage from evaluation of one canine duodenal biopsy before, during, and after exposure to glucose and phloridzin

Current and voltage values were recorded simultaneously. In this example, the current is shown in blue and the voltage in maroon. The short circuit current was increased immediately after glucose stimulation (arrow 1), and reduced after application of phloridzin (arrow 2). Voltage remained at zero during this voltage clamping condition.

4.3.3 Secretory Function

4.3.3.1 Response to Histamine

In total, 51 canine duodenal biopsies were exposed to 200 µM histamine, but only five biopsy samples responded (Figure 40). Two samples came from dogs of group 1 and three from dogs of group 3. The median change of short circuit current was 8 µA/cm² (range: 2-18 µA/cm²), and the median time until the peak was reached was 8.7 s (range: 3.6-32.5 s). The curve of short circuit current change can be described as hill-shaped, with a steep rise and a slow and long decline. The I_{sc} of four biopsies decreased to baseline values within 8.6 minutes. However, one current remained increased for longer than 11.6 minutes.

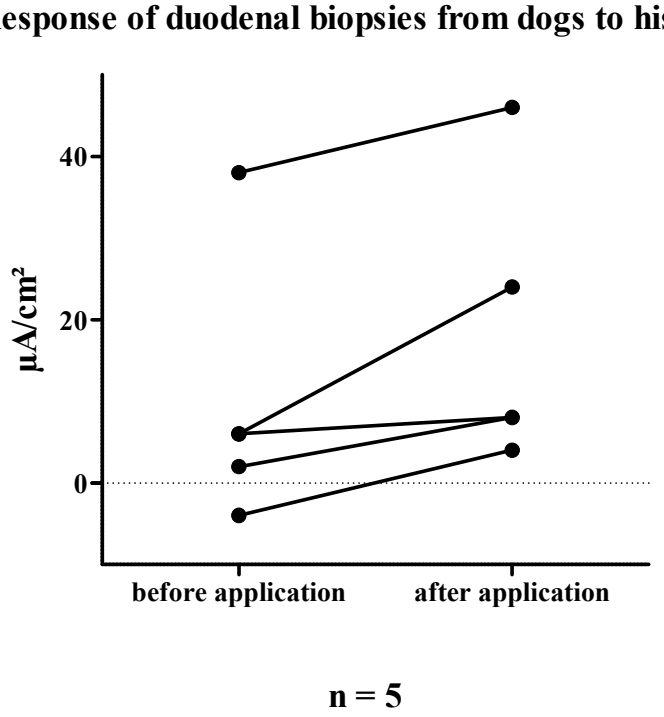

Figure 40: Response of duodenal biopsies from dogs to histamine
This figure shows the change of short circuit current (µA/cm²) of canine duodenal biopsy samples in response to 200 µM histamine. The short circuit current of the biopsies increased to 2-18 µA/cm² within 3.6-32.5 s. The median ΔI_{sc} of the five intestinal biopsies was 8 µA/cm².

4.3.3.2 Response to Serotonin

In total, 51 canine duodenal biopsies were exposed to 200 µM serotonin, but only two biopsy samples responded (Figure 41). The ΔI_{sc} in both biopsies was 6 µA/cm², and times until the peak was reached were 9.6 s and 10.6 s, respectively. Both of the biopsy samples had also shown response to exposure to histamine and phloridzin.

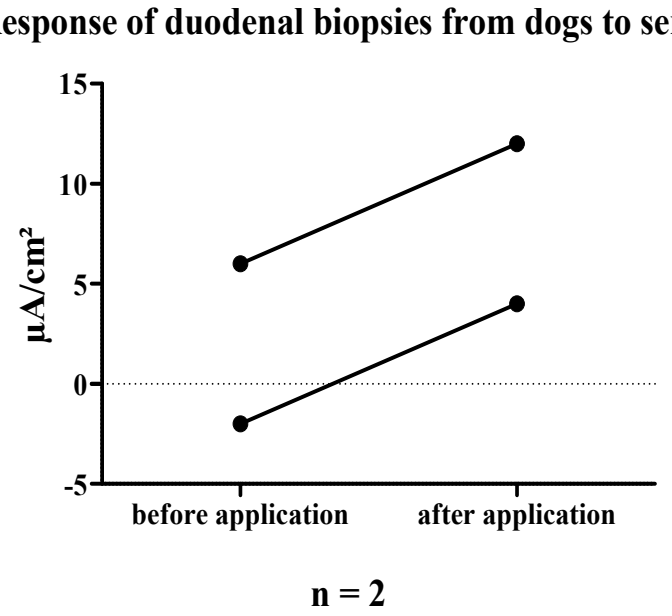

Figure 41: Response of duodenal biopsies from dogs to serotonin

The figure shows the change of short circuit current (µA/cm²) of canine duodenal biopsy samples in response to 200 µM serotonin. Median ΔI_{sc} for the five intestinal biopsies was 6 µA/cm². Time until the current peak was reached differed merely by one second. Both specimens that responded to 200 µM serotonin were from a dog of group 1 and group 3 each.

4.3.3.3 Response to Prostaglandin E_2

There was no response to exposure to 4 µM prostaglandin of any of the duodenal biopsies from dogs.

4.3.3.4 Response to Forskolin

As shown in Figure 42, five of 51 biopsies tested responded to 5 µM forskolin with a rise in short circuit current (median ΔI_{sc}: 6 µA/cm²; range: 6-16 µA/cm²). One of the samples came from a dog of group 2, while four samples came from dogs of group 3. While the current of three samples slowly returned to baseline values after 6.1 -11.4 minutes, two specimens from one animal kept a stable current at maximum values for more than 4.2 minutes.

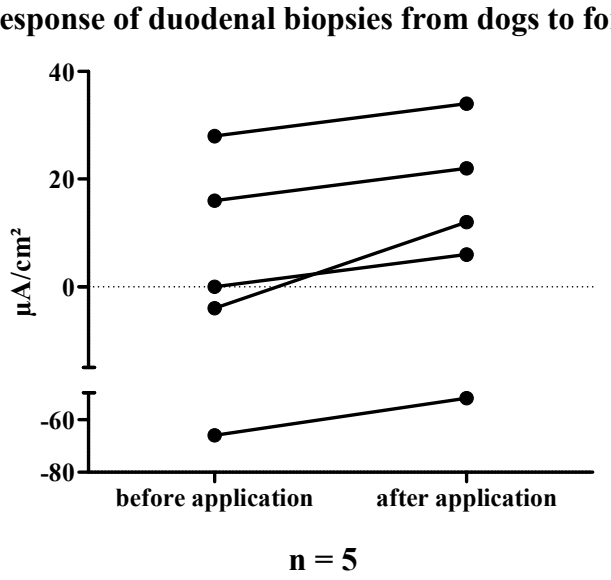

Figure 42: Response of duodenal biopsies from dogs to forskolin

This figure shows the change of short circuit current (µA/cm²) of five canine duodenal biopsy samples in response to 4 µM forskolin. The short circuit current of the biopsies increased to 6-16 µA/cm² within 23.3 s (range: 3.8-56.5 s). Median ΔI_{sc} of the five intestinal biopsies was 6 µA/cm².

4.3.4 Evaluation of Sample Viability

4.3.4.1 Response to Glucose after Completion of all Experiments

After completing the entire experimental protocol, which required 42.3 to 94.5 minutes (median value: 64.5 minutes), the 47 duodenal biopsies were once again exposed to 40 mM glucose. Nine of them responded with a rise of their short circuit current (median value: 20 µA/cm²; range: 8-66 µA/cm^2) within 3.9-180.4 s (median: 9.9 s). Results are shown in Figure 43. Three of those samples were from dogs of group 1, three samples were from dogs of group 2, and three samples were from dogs of group 3. Interestingly, the currents of all samples could not be abolished by 500 µM phloridzin and stayed at their maximum values for at least ten minutes.

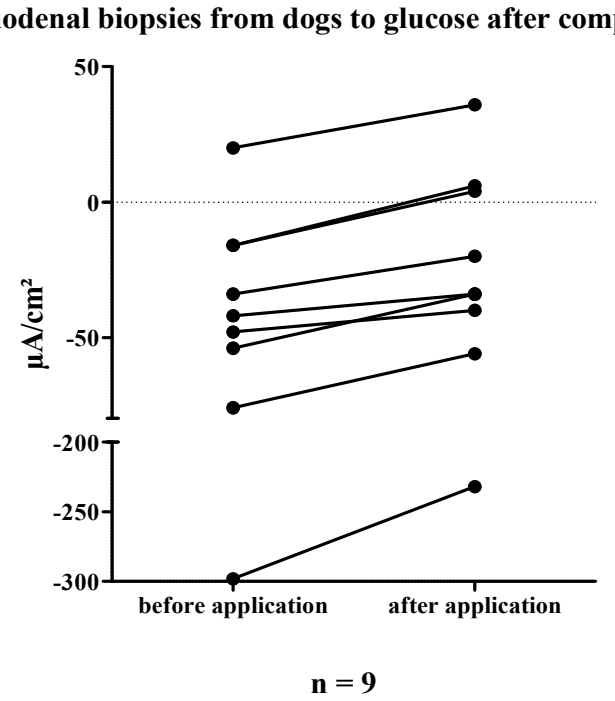

Figure 43: Response of duodenal biopsies from dogs to glucose after completion of experiments

This figure shows the short circuit current values of nine canine duodenal biopsies before and after exposure to 40 mM glucose. The median change in I_{sc} was 20 µA/cm^2 (range: 8-66 µA/cm^2). As observed for experiments discussed above, a wide variety of baseline short circuit current values could be observed. The median time to reach peak short circuit current values was 9.9 s (range: 3.9-180.4 s).

4.3.4.2 Response to Ouabain

A total of 44 duodenal biopsies were exposed to ouabain and 16 of the samples (36%) responded with a change in short circuit current (Figure 44). The median time to reach peak effects on short circuit current was 10.6 minutes (range: 2.4-12.5 minutes). The median change of the short circuit current was 17 µA/cm^2, ranging from 4 to 42 µA/cm^2. While the current of nine samples decreased, I_{sc} of seven samples increased.

Figures 45, 46, and 47 display responses of the short circuit current in response to ouabain exposure for the three individual groups. A wide variation of baseline currents, times until current change, and ΔI_{sc} could be observed in all groups.

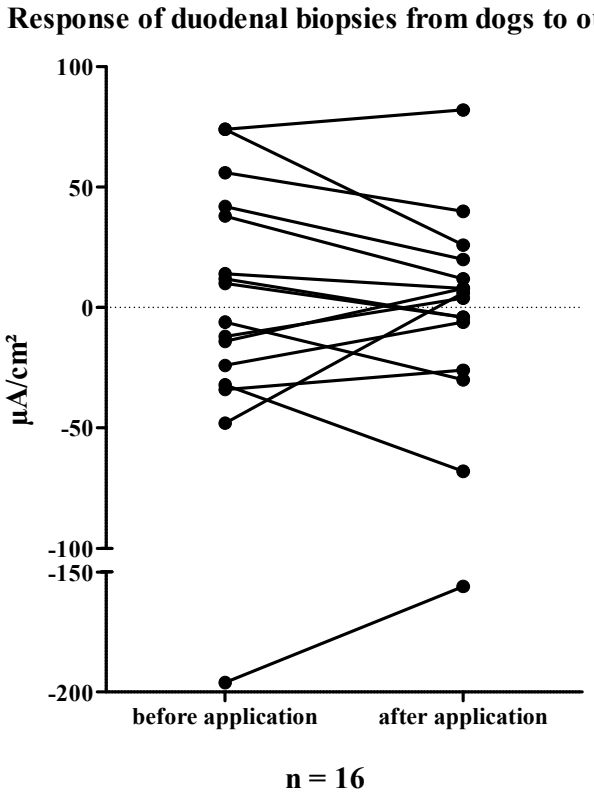

Figure 44: Response of duodenal biopsies from dogs to ouabain

This figure shows the short circuit current values of 16 canine duodenal biopsies before and after exposure to 600 µM ouabain in the serosal buffer. While the current of nine samples decreased, I_{sc} of seven samples increased. The median change of short circuit current was 17 µA/cm^2 (range: 4-42 µA/cm^2) and the median time until the maximum current was reached was 10.6 minutes (range: 2.4-12.5 minutes).

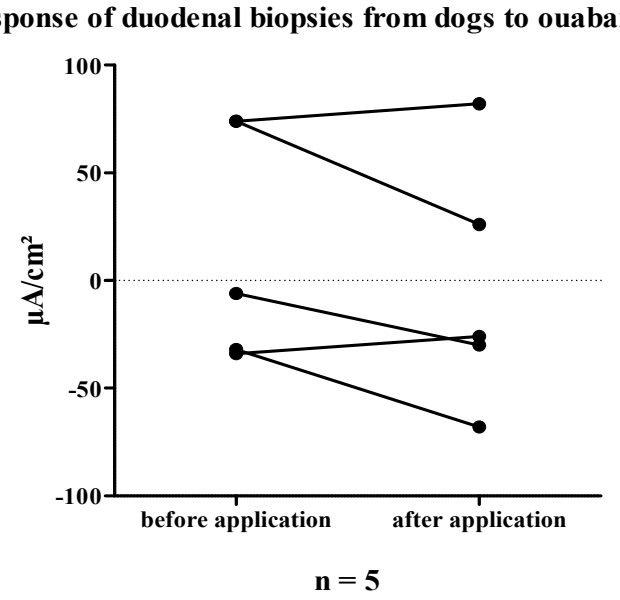

Figure 45: Response of duodenal biopsies from dogs to ouabain: group 1
This figure shows the short circuit current values for five duodenal biopsies from healthy dogs before and after exposure to 600 µM ouabain in the serosal buffer. While the current of three samples decreased, I_{sc} of two samples increased. The median change of current was 10 µA/cm^2 (range: 4-36 µA/cm^2) and the median time until maximum current values were reached was 10.5 minutes (range: 6.2-12.5 minutes). Baseline, as well as maximum current values varied from -68 µA/cm^2 to 82 µA/cm^2.

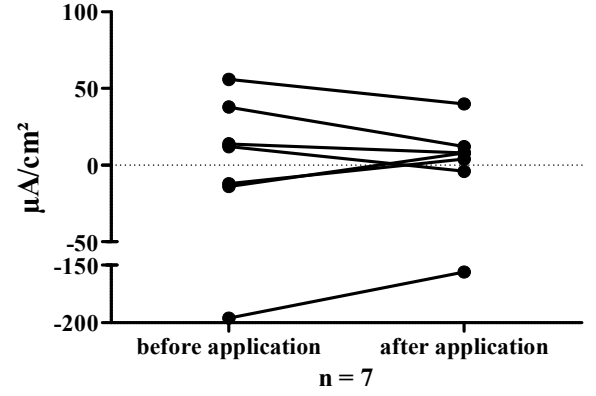

Figure 46: Response of duodenal biopsies from dogs to ouabain: group 2
This figure shows the short circuit current values for seven duodenal biopsies from dogs with clinical signs of gastrointestinal disease, but no histopathological changes before and after exposure to 600 µM ouabain in the serosal buffer. While the short circuit current for four samples decreased, I_{sc} for three other samples increased. However, short circuit currents diverged towards zero in all dogs. The median change of current was 16 µA/cm^2 (range: 6-40 µA/cm^2) and the median time until maximum current values were reached was 5.1 minutes (range: 2.3-12.5 minutes).

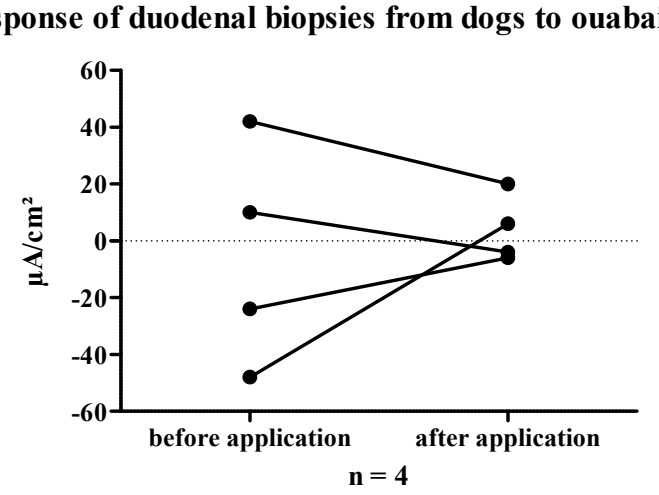

Figure 47: Response of duodenal biopsies from dogs to ouabain: group 3
This figure shows the short circuit current values for four duodenal biopsies from dogs with clinical signs of gastrointestinal disease and histopathologic lesions before and after exposure to 600 µM ouabain in the serosal buffer. While the current of two samples decreased, I_{sc} of two samples increased. However, short circuit currents diverged towards zero in all dogs. The median change of current was 20 µA/cm^2 (range: 6-42 µA/cm^2) and the median time until maximum current values were reached was 11.9 minutes (range: 11.1-12.3 minutes).

Table 10: **Short circuit current measurements after exposure of canine duodenal biopsies to various compounds**
This table summarizes the response of the canine duodenal biopsies after exposure to various compounds. Similarly to the duodenal biopsies collected in cats, none of the tissue samples responded to prostaglandin. The reaction time varied widely between individual samples. Also, a wide variability in response rates among the samples from the same patient could be observed.

stimulus	n / N	%	median ΔI_{sc} (µA/cm²)	range (µA/cm²)	time (Δt)	number of dogs, for which x biopsies showed a response				
						x=0	x=1	x=2	x=3	x=4
glucose 40 mM	21/51	41.2 %	34	6-170	60.0 (9.3-177.3)	6	1	4	0	3
phloridzin 500 µM	25/51	49.0 %	32	14-168	60.0 (9.3-169.6)	4	3	2	2	3
histamine 200 µM	5/51	9.8 %	8	2-18	8.7 (3.6-32.5)	10	3	1	0	0
serotonin 200 µM	2/51	3.9 %	6	6 in both	10.1 (9.6-10.6)	12	2	0	0	0
PGE 4 µM	0	0 %	N/A	N/A	N/A	14	0	0	0	0
forskolin 5 µM	5/51	9.8 %	6	6-16	23.3 (3.8- 56.5)	10	3	1	0	0
glucose 40 mM	9/47	19.2 %	20	8-66	9.9 (3.9-180.4)	6	3	3	0	0
ouabain 600 µM	16/44	36.4 %	17	4-42	10.6 min (2.3-12.5 min)	3	3	3	1	1
n = number of responding biopsies, N = number of exposed biopsies, N/A = not available										

Table 11: **Short circuit current measurements after exposure of canine duodenal biopsies to various compounds: group 1**
This table summarizes the response of duodenal biopsies from healthy dogs after exposure to various compounds. The percentage of responding samples was by far highest in this group compared to other groups. Response to substances stimulating absorption was more reliable than response to substances that stimulate secretion. The reaction time varied widely between individual samples and a wide variability in response rates among the samples from the same patient could be observed.

stimulus	n / N	%	median ΔI_{sc} ($\mu A/cm^2$)	range ($\mu A/cm^2$)	time (Δt)	number of dog, for which x biopsies showed a response				
						x=0	x=1	x=2	x=3	x=4
glucose 40 mM	6/8	75.0 %	28	16-50	93.0 (27.7-108.6)	0	0	1	0	1
phloridzin 500 µM	7/8	87.5 %	22	14-34	88.6 (49.7-169.6)	0	0	0	1	1
histamine 200 µM	2/8	25.0 %	13	8-18	5.8 (3.6-7.9)	0	2	0	0	0
serotonin 200 µM	1/8	12.5 %	6	N/A	10.6 (N/A)	1	1	0	0	0
PGE 4 µM	0/8	0 %	N/A	N/A	N/A	2	0	0	0	0
forskolin 5 µM	0/8	0 %	N/A	N/A	N/A	2	0	0	0	0
glucose 40 mM	3/8	37.5 %	14	8-22	4.2 (3.9-13.3)	0	1	1	0	0
ouabain 600 µM	5/8	62.5 %	10	4-36	10.5 min (6.2-12.5)	0	0	1	1	0
n = number of responding biopsies, N = number of exposed biopsies, N/A = not available										

Table 12: **Short circuit current measurements after exposure of canine duodenal biopsies to various compounds: group 2**
This table summarizes the response of the duodenal biopsies from dogs with clinical signs of gastrointestinal disease, but no histopathological evidence of duodenal lesions after exposure to various compounds. As for group 1, response to substances stimulating absorption was more reliable than response to substances that stimulate secretion. Similar to feline duodenal samples, phloridzin and ouabain appeared to lead to reliable responses. The reaction time varied widely between individual samples and a wide variability in response rates among the samples from the same patient could be observed.

stimulus	n / N	%	median ΔI_{sc} (µA/cm²)	range (µA/cm²)	time (Δt)	number of dogs, for which x biopsies showed a response				
						x=0	x=1	x=2	x=3	x=4
glucose 40 mM	8/15	53.3%	52	14-170	50.8 (33.1-146.0)	1	0	2	0	1
phloridzin 500 µM	9/15	60.0 %	48	24-168	74.0 (9.3-143.0)	0	1	2	0	1
histamine 200 µM	0/15	0 %	N/A	N/A	N/A	4	0	0	0	0
serotonin 200 µM	0/15	0 %	N/A	N/A	N/A	4	0	0	0	0
PGE 4 µM	0/15	0 %	N/A	N/A	N/A	4	0	0	0	0
forskolin 5 µM	1/15	6.7 %	16	N/A	23.3 (N/A)	3	1	0	0	0
glucose 40 mM	3/11	27.3 %	20	20-66	7.6 (6.4-9.9)	1	1	1	0	0
ouabain 600 µM	7/11	63.6 %	16	6-40	5.12 min (2.35-12.50)	0	1	1	0	0
n = number of responding biopsies, N = number of exposed biopsies, N/A = not available										

Table 13: **Short circuit current measurements after exposure of canine duodenal biopsies to various compounds: group 3**
This table summarizes the response of the duodenal biopsies from dogs with clinical signs of gastrointestinal disease and histopathological evidence of duodenal disease after exposure to various compounds. Compared to the total number (n=32) of tested biopsies from dogs in this group, only a small fraction of samples showed a response to any of the applied substances. The reaction time varied widely between individual samples and a wide variability in response rates among the samples from the same patient could be observed.

stimulus	n / N	%	median ΔI_{sc} (µA/cm²)	range (µA/cm²)	time (Δt)	number of dogs, for which x biopsies showed a response				
						x=0	x=1	x=2	x=3	x=4
glucose 40 mM	7/32	21.9 %	36	6-58	31.6 (2.5-177.3)	5	1	1	0	1
phloridzin 500 µM	9/32	28.1 %	32	20-84	53.7 (40.6 -133.0)	4	2	0	1	1
histamine 200 µM	3/32	9.4 %	6	2-8	17.3 (8.7- 32.5)	6	1	1	0	0
serotonin 200 µM	1/32	3.1 %	6	N/A	9.6 (N/A)	7	1	0	0	0
PGE 4 µM	0/32	0 %	N/A	N/A	N/A	8	0	0	0	0
forskolin 5 µM	4/32	12.5 %	6	6-14	34.5 (3.8-56.5)	5	2	1	0	0
glucose 40 mM	3/25	12.0 %	16	8-20	126.7 (19.3-180.4)	5	1	1	0	0
ouabain 600 µM	4/22	12.5%	20	6-42	11.9 min (11.1-12.3)	3	2	1	0	0
n = number of responding biopsies, N = number of exposed biopsies, N/A = not available										

4.4 Canine Colonic Biopsies

Twenty-one colonic biopsies were collected from four canine patients, thirteen of which were transferred into Ussing chambers. Twelve of the 13 biopsies did respond to at least one substance. With nine biopsies all experiments could be completed, but four samples from one animal had to be excluded because the amplifier failed just before the last substance was applied. The time period required for all experiments varied between 105.4 and 143.4 minutes (median: 129.2 minutes). Table 14 at the end of this section summarizes the results including information how many samples per animal responded to the compound applied.

4.4.1 Baseline Observations

Mean (±SD) conductance of eleven canine colonic biopsies was 62.7 ±35.9 mS/cm^2 (resistance: 19.7 ±8.3 Ω•cm^2). After completing all experiments conductance was 87.2 ±38.0 mS/cm^2 (resistance: 13.8 ±7.0 Ω•cm^2). Mean conductance was significantly higher after completion of all experiments when compared with baseline values (p-value=0.0010).

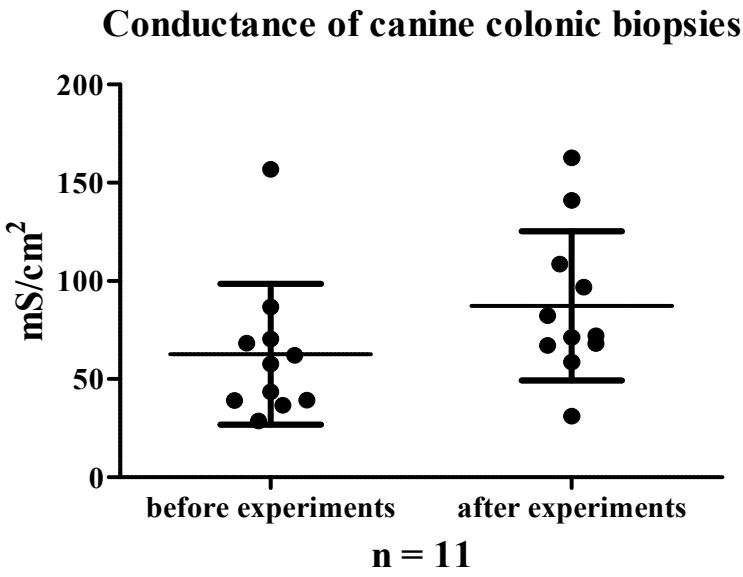

Figure 48: Conductance of canine colonic biopsies

This scatter dot plot displays the conductance of canine colonic biopsies before experiments were started and after completion of all experiments. Mean conductance increased significantly from 62.7 mS/cm^2 to 87.2 mS/cm^2 (p-value=0.0010).

4.4.2 Absorptive Function

4.4.2.1 Response to Glucose

All colonic biopsies were exposed to 40 mM glucose, but only four samples (30.8%) showed a response (Figure 49). The median change of the short circuit current was 25 µA/cm² and ranged from 14 to 88 µA/cm². The time until peak effect was reached (Δt) varied between 23.4 s and 52.2 s (median: 36.9 s).

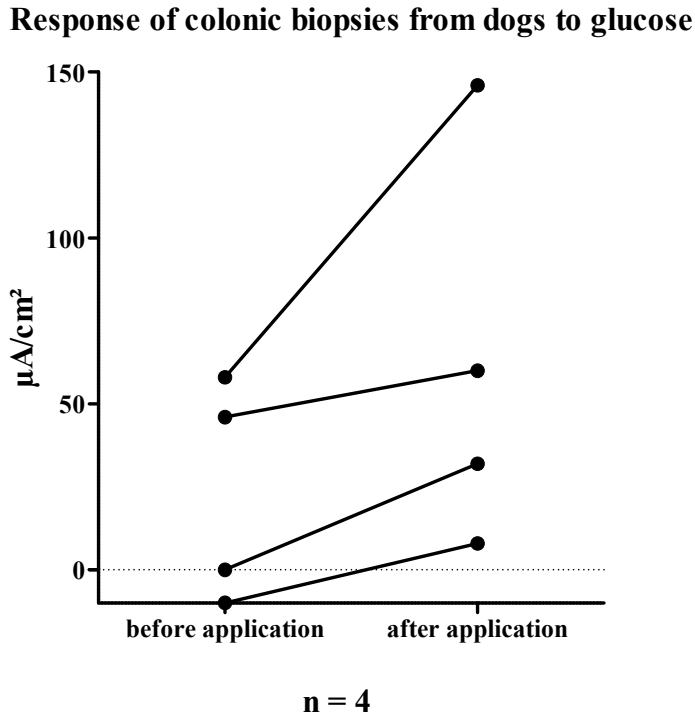

Figure 49: Response of colonic biopsies from dogs to glucose
This figure shows the response of four colonic biopsy samples to 40 mM glucose in the mucosal buffer. Exposure to glucose increased the median short circuit current by 25 µA/cm² (range: 14-88 µA/cm^2). Baseline values ranged from -10 to 58 µA/cm² and values increased after the tissues had been in contact with the substance. Post-exposure values ranged from 8 to 146 µA/cm².

4.4.2.2 Response to Phloridzin

Thirteen biopsies were exposed to 500 µM phloridzin and seven of those showed a response. The median ΔI_{sc} was 30 µA/cm², with values ranging from 28 to 74 µA/cm² (Figure 50). The short circuit current of all responding samples decreased with a median Δt (the time until the peak effect was reached) of 60.3 s (range: 34.9-80.9 s).

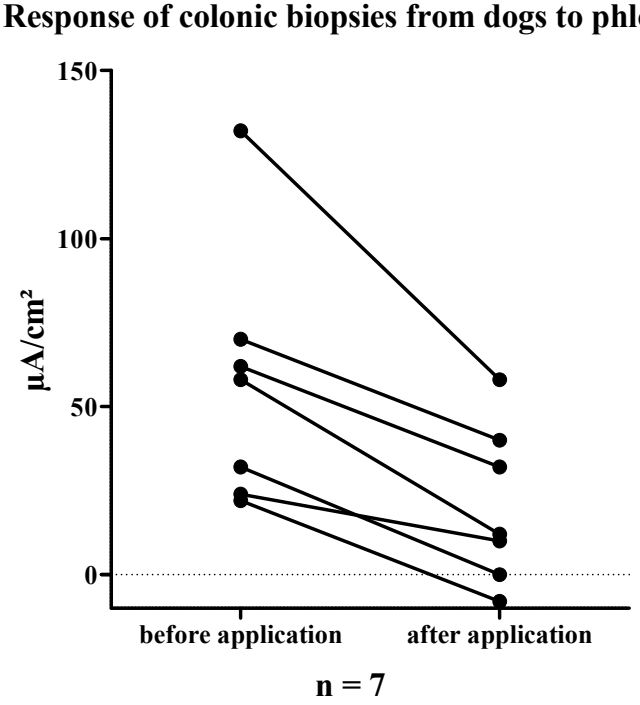

Figure 50: Response of colonic biopsies from dogs to phloridzin
This figure shows the response of seven colonic biopsy samples to 500 µM phloridzin in the mucosal buffer. A total of 53.8% of the exposed biopsies responded to the applied stimulus. Exposure to phloridzin decreased the median short circuit current by 30 µA/cm² (range: 28-74 µA/cm^2).

4.4.3 Secretory Function

4.4.3.1 Response to Histamine

Twelve of 13 tested biopsies showed an increase of I_{sc} values of 8-364 µA/cm² (median: 45 µA/cm^2) after being exposed to histamine (Figure 51). The median reaction time until peak effect was reached (Δt) was 75.8 s, with values ranging between 14.9 s and 133.1 s. Interestingly, the raw recording from eight biopsy samples had a biphasic shape. After an initial rise the current settled on a plateau for a few seconds, before increasing again to maximum values. Figure 52 displays this characteristic change in short circuit current. After histamine stimulation, most samples decreased to baseline short circuit current values after 6.5 minutes. However, two biopsies kept their maximum I_{sc} values for more than 20.5 minutes.

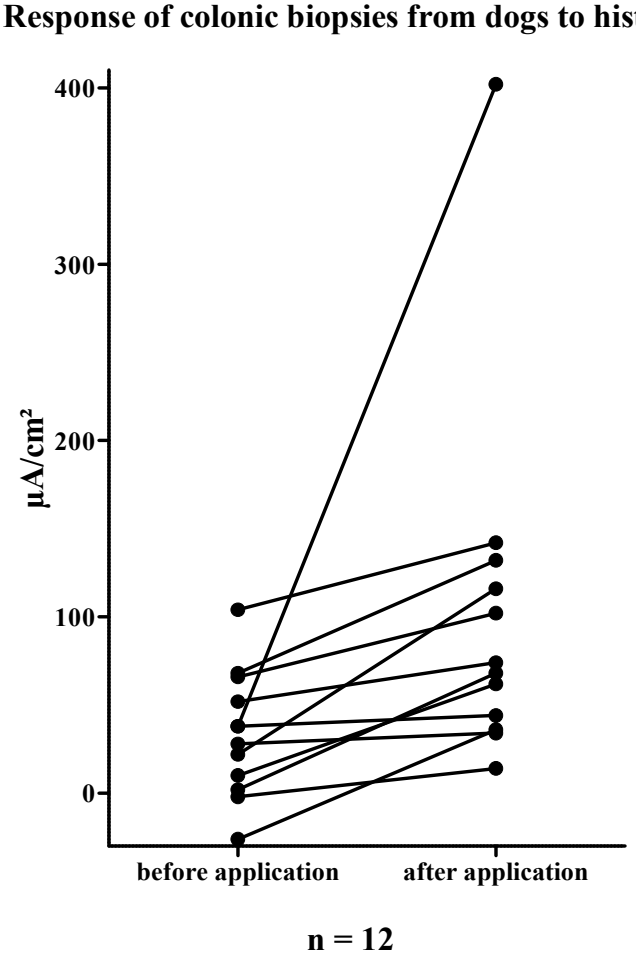

Figure 51: Response of colonic biopsies from dogs to histamine
This figure shows the response of twelve colonic biopsy samples to 200 µM histamine in the serosal buffer. The vast majority (92.3%) of the exposed biopsies responded to the applied stimulus. Exposure to histamine increased the median short circuit current by 45 µA/cm² (range: 8-364 µA/cm^2).

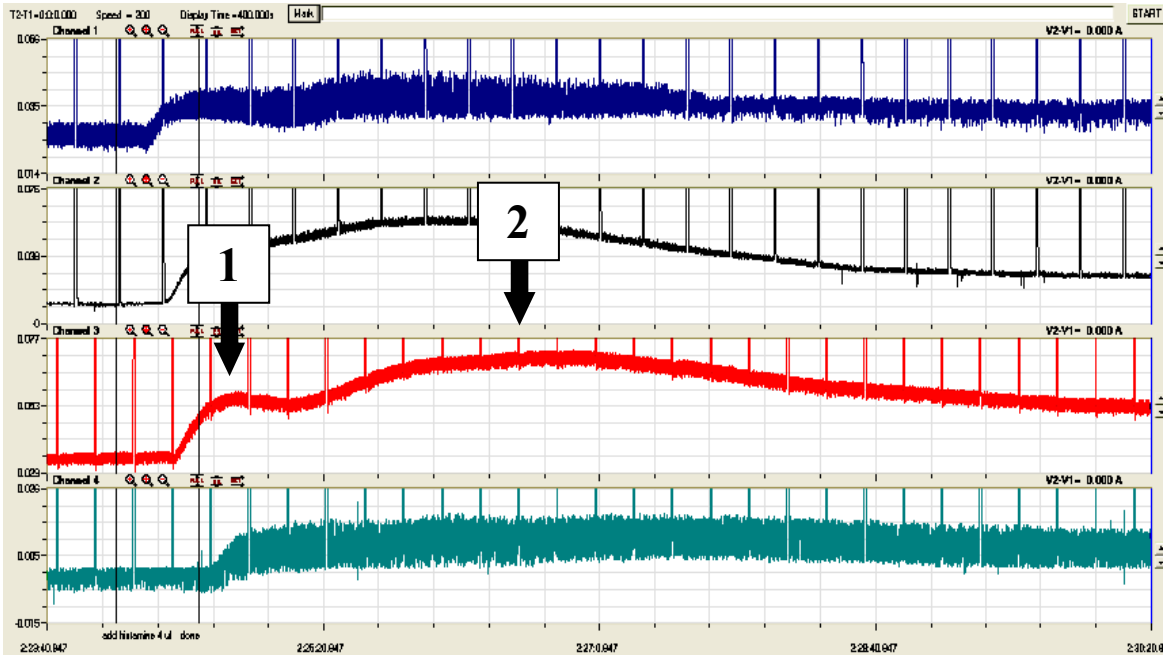

Figure 52: Raw short circuit current data for four canine colonic biopsies before, during, and after exposure to histamine

The computer software simultaneously displays the short circuit current for four individual biopsies evaluated in four Ussing chambers. The recording from each Ussing chamber is displayed with a different color. The red graph (current of chamber 3) shows a good example for the biphasic ΔI_{sc} response after exposure to histamine. Arrow 1 indicates the 1st peak of the short circuit current. After remaining on a plateau for a few seconds, the current continues to increase until reaching peak values (indicated by arrow 2). After reaching the maximum value, the current decreases slowly and steadily until settling close to pre-exposure baseline values.

4.4.3.2 Response to Serotonin

All samples that responded to histamine (as described in section 4.4.3.1) also reacted to serotonin (n=12). The change in short circuit current ranged from 6 to 60 µA/cm² with a median value of 44 µA/cm² (Figure 53). Peak values were reached within 70.6-190.5 s. The currents did not decrease to original baseline values for at least 10-22 minutes (Figure 54), except for a single biopsy, which reached pre-exposure values after 7.7 minutes.

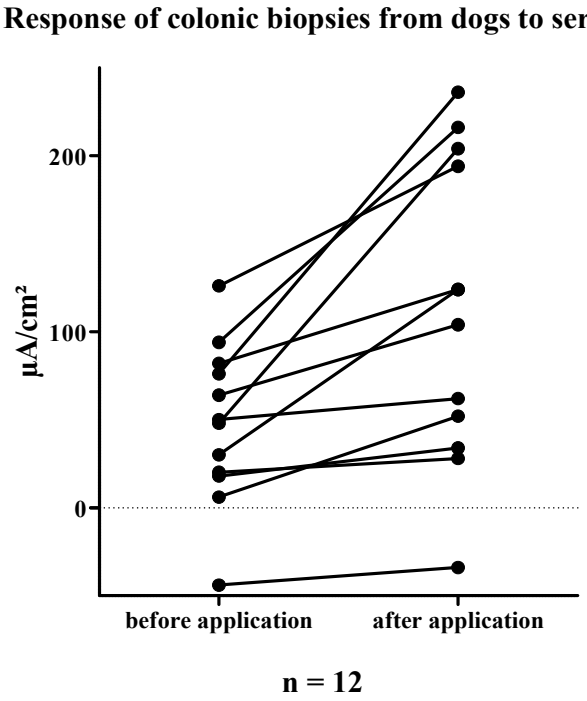

Figure 53: Response of colonic biopsies from dogs to serotonin

This figure shows the response of twelve colonic biopsy samples to 200 µM serotonin in the serosal buffer. Twelve of 13 exposed biopsies (92.3%) responded to the applied stimulus. Exposure to serotonin increased the median short circuit current by 44 µA/cm² (range: 6-60 µA/cm^2).

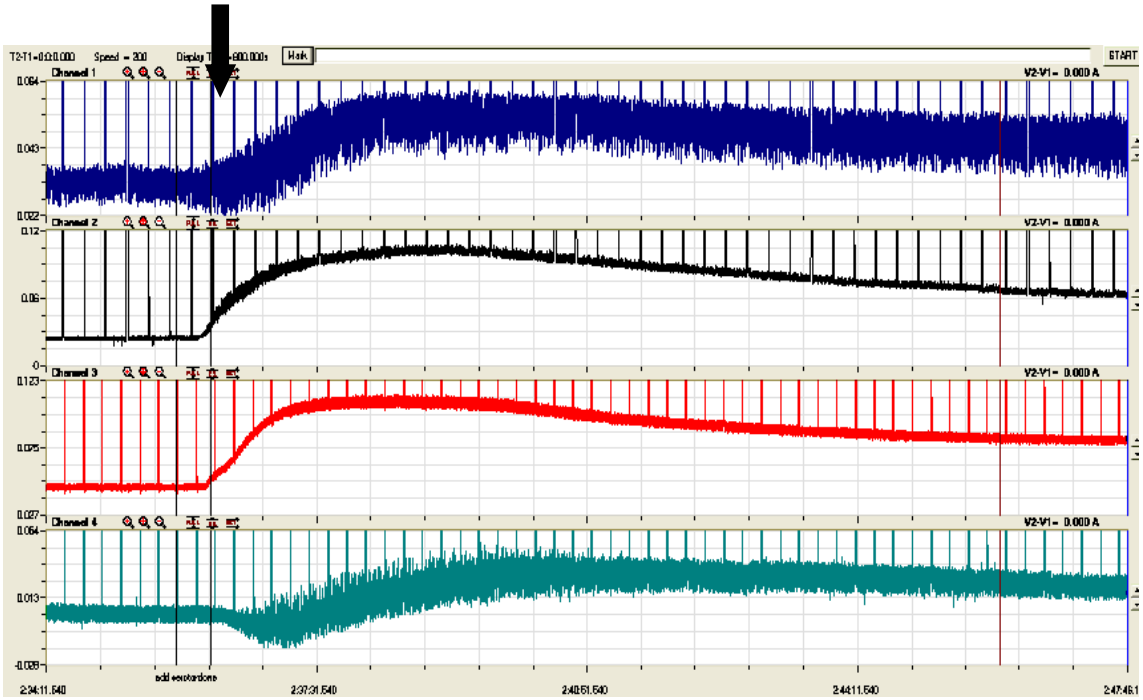

Figure 54: Raw short circuit current data for four canine colonic biopsies before, during, and after exposure to serotonin

The computer software simultaneously displays the short circuit current for four individual biopsies evaluated in four Ussing chambers. The recording from each Ussing chamber is displayed with a different color. The arrow above indicates the time of serotonin administration into the serosal bathing solution. After reaching the maximum value, the current decreased slowly and steadily until settling close to pre-exposure baseline values.

4.4.3.3 Response to Prostaglandin E_2

Fifty-four per cent of the investigated canine colonic biopsy samples responded to 4 µM prostaglandin that was added to the serosal buffer. The median ΔI_{sc} was 40 µA/cm², with values ranging between 6 and 46 µA/cm² (Figure 55). The median time to reach the peak short circuit current was 82.1 s (range: 34.3-144.3 s). While five samples returned to their baseline values after 3.5-16.5 minutes, two samples with a high peak after prostaglandin exposure (40 and 60 µA/cm²) failed to reach their baseline value in 20.2 minutes.

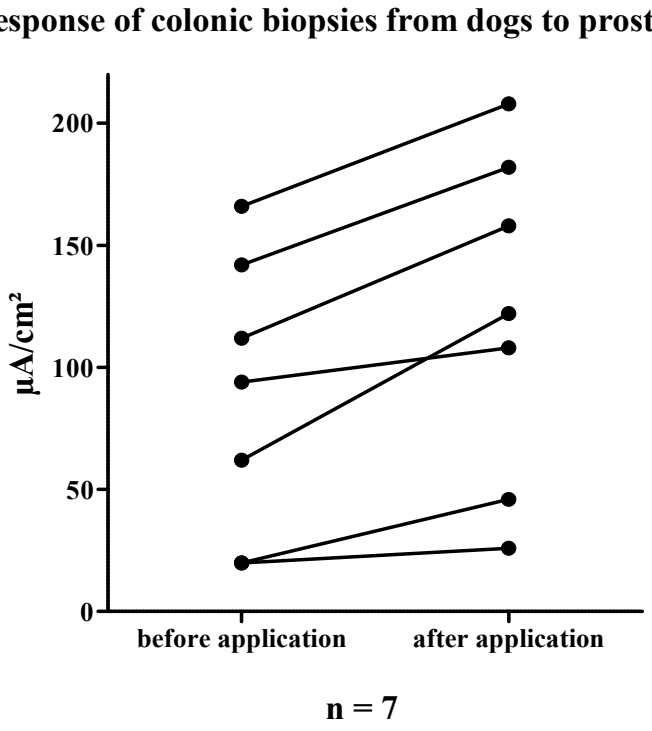

Figure 55: Response of colonic biopsies from dogs to prostaglandin
This figure shows the response of seven colonic biopsy samples to 4 µM prostaglandin in the serosal buffer. Seven of 13 exposed biopsies (53.8%) responded to the applied substance. Exposure to prostaglandin increased the median short circuit current by 40 µA/cm² (range: 4-46 µA/cm^2).

4.4.3.4 Response to Forskolin

In seven of the thirteen evaluated colonic biopsies, forskolin induced an increase in short circuit current (Figure 56). The median rise of the I_{sc} was 46 µA/cm² with values ranging from 20 to 196 µA/cm². Interestingly, the sample with the lowest ΔI_{sc} after forskolin exposure took 361.9 s until it reached the peak short circuit current, while the sample with the highest peak short circuit current only took 66.2 s to reach that peak.

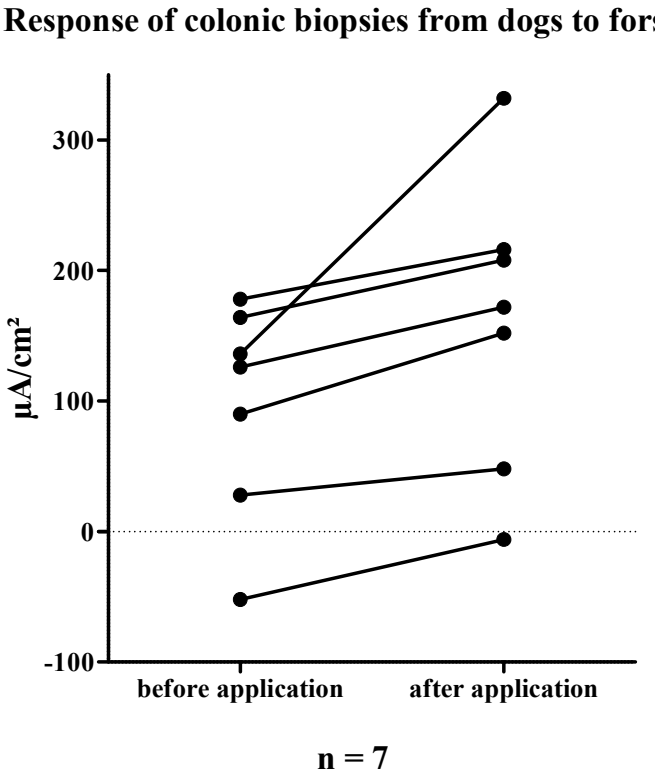

Figure 56: Response of colonic biopsies from dogs to forskolin

This figure shows the response of seven colonic biopsy samples to 5 µM forskolin in the serosal buffer. Seven of 13 exposed biopsies (53.8%) responded to the applied substance. Exposure to forskolin increased the median short circuit current by 46 µA/cm² (range: 20-196 µA/cm^2).

4.4.4 Evaluation of Sample Viability

4.4.4.1 Response to Glucose after Completion of all Experiments

None of the tested specimens responded to exposure to 40 mM glucose after completion of all experiments.

4.4.4.2 Response to Ouabain

Nine biopsies were exposed to 600 µM ouabain. The short circuit current of those seven samples that responded decreased immediately until it reached stable minimum values after a median of 11.6 minutes (range: 3.4-12.2 minutes) (Figure 57). The median amplitude of I_{sc} was 56 µA/cm² with values ranging from 8-344 µA/cm^2.

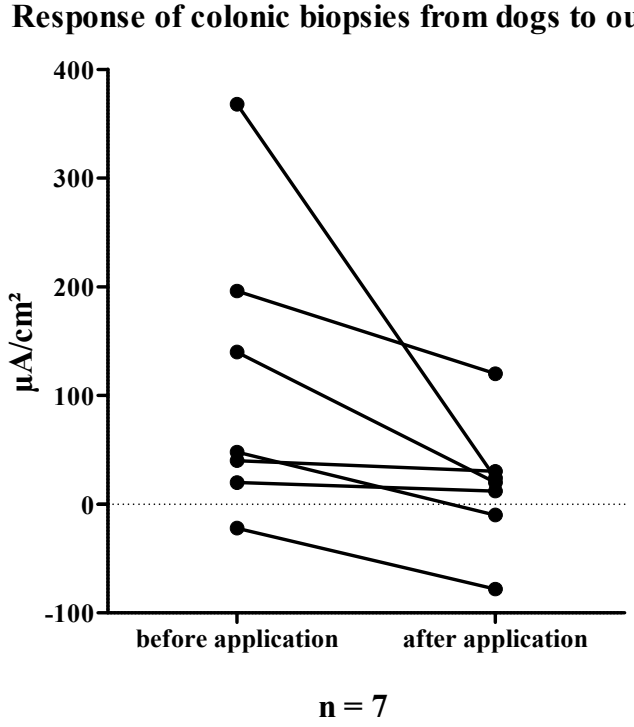

Figure 57: Response of colonic biopsies from dogs to ouabain
This figure shows the response of seven colonic biopsy samples to 600 µM ouabain in the serosal buffer. Seven of nine exposed biopsies (77.8%) responded to the applied substance. Exposure to ouabain decreased the median short circuit current by 56 µA/cm² (range: 8-344 µA/cm^2).

Table 14: **Short circuit current measurements after exposure of canine colonic biopsies to various compounds**
This table summarizes the response of the canine duodenal biopsies after exposure to various compounds. The majority of samples responded to all secretory drugs, but compared to the feline colonic biopsies, more specimens responded to glucose and phloridzin. The reaction time varied widely between individual samples and a wide variability in response rates among the samples from the same patient could be seen.

stimulus	n / N	%	median ΔI_{sc} (µA/cm²)	range (µA/cm²)	time (Δt)	number of dogs, for which x biopsies showed a response				
						x=0	x=1	x=2	x=3	x=4
glucose 40 mM	4/13	30.8 %	25	14-88	36.9 (23.4-52.2)	2	1	0	1	0
phloridzin 500 µM	7/13	53.8 %	30	28-74	60.3 (34.9-80.9)	0	3	0	0	1
histamine 200 µM	12/13	92.3 %	45	8-364	75.8 (14.9-133.1)	0	0	1	2	1
serotonin 200 µM	12/13	92.3 %	44	6-60	80.3 (70.6-190.5)	0	0	1	2	1
PGE 4 µM	7/13	53.8 %	40	6-46	82.1 (34.3-144.3)	1	1	1	0	1
forskolin 5 µM	7/13	53.8 %	46	20-196	210.7 (66.2-361.9)	1	0	2	1	0
glucose 40 mM	0	0 %	N/A	N/A	N/A	4	0	0	0	0
ouabain 600 µM	7/9	77.8 %	56	8-344	11.58 min (3.4-12.2)	0	0	2	1	0
n = number of responding biopsies, N = number of exposed biopsies, N/A = not available										

5. Discussion

In the present study, we evaluated the feasibility of the use of a modified Ussing chamber for investigation of endoscopically obtained intestinal biopsies from dogs and cats. Our results suggest that the modification of the conventional Ussing chamber method as described here provides a feasible tool for future studies of intestinal transport in companion animals.

5.1 Conductance

Conductance is a valuable parameter to evaluate intestinal integrity. In contrast to tight epithelia, changes of the transcellular conductance are difficult to detect in a leaky epithelia as the intercellular adherence complexes do not allow the clear separation between paracellular and transepithelia pathway.

Saunders et al. demonstrated the effect of acute stress factors on the intestinal membrane integrity in a rat model. He showed that psychological stress (restraining stress) and physical stress (cold environment) could increase tissue conductance from 30 mS/cm^2 in controls to 60 mS/cm^2 in stressed rats (SAUNDERS et al., 1994). The combination of reduced intestinal blood flow and hyperthermia appears to be responsible for the loss of tight junction integrity (DOKLADNY et al., 2006; LAMBERT, 2009). Thus, the broad variations in tissue conductance observed in biopsy samples collected from animals enrolled in this study may have been due to alterations of intestinal transport function due to the stress of hospitalization and/or separation anxiety.

Also, chronic stress has an impact on membrane permeability in many species (MEDDINGS and SWAIN, 2000; SOEDERHOLM and PERDUE, 2001). Thus, variations in our conductance might have occurred due to the long-term stress of the diseased study population in addition to the short-term stressors from being hospitalized.

Comparing the resistance recorded in our study to results obtained by other investigators, our values match previously reported values (POWELL, 1981).

Variations in biopsy thickness and biopsy size (folds of larger tissue pieces might have been folded over on itself impeding transport of ions) may have contributed to the large variation of our data.

The mean conductance did increase during the time of the experiments. These changes were significant for both feline and canine colonic samples and canine duodenal samples, but not for feline duodenal biopsies (p-values: 0.0006, 0.0010, <0.0010, and 0.1040, respectively). Since this increase in conductance was continuous and relatively small, it still allowed the evaluation of the short circuit current over time.

Significant increases or decreases in epithelial resistance are predominantly due to changes in paracellular resistance in leaky epithelia and to changes in transcellular resistance in tight epithelia (POWELL, 1981). The transepithelial conductance of the intestine is mainly determined by tight junctions and the volume of the lateral intercellular space. Several parameters may have an impact to the latter (GAWENIS et al., 2004). Besides the natural occurring loss of tissue integrity when tissues are maintained *ex vivo*, other factors may contribute to artificial conductance changes. Hypoosmotic conditions, were shown to cause an increased cell turgor associated with a collapse of the intercellular space, resulting in a decrease of conductance (BINDSLEV et al., 1974; LEVINE et al., 1978, SMULDERS et al., 1971). In order to prevent such interferences we carefully maintained isoosmotic conditions by adding the equivalent

concentration (10 mM) of sorbitol into the mucosal buffer as glucose was added to the serosal buffer solution of the Ussing chamber. The main purpose of applying glucose to the serosal buffer solution was to maintain tissue viability by providing an energy source, while sorbitol was added to the mucosal buffer solution to maintain osmotic balance.
An increase in tissue conductance (or decrease in resistance) during the experiments could also be attributed to edge damage of the mounted intestinal biopsy specimens. As air suction is applied to mount the tissue specimen securely in the modified Ussing chamber disc, strain is applied at the edges and cells could be crushed, dislocated, and/or stretched, which might increase tissue conductance. The effect becomes more significant as the size of the sample decreases. However, several studies have shown that conductance of full thickness biopsies can equal the conductance of endoscopic biopsy samples (STOCKMANN et al., 1999; WALLON et al., 2005).
The air suction applied to biopsies in our study was 400 mmHg, which has previously been reported to be used successfully in similar experiments with endoscopic biopsy samples from humans (LARSEN et al., 2001). This amount of suction also appeared to be the lowest amount of suction possible in our studies to hold the biopsy sample in place, preventing it from the convection forces of the oxygen-stirred buffer solution. Nevertheless, we lost four biopsies into the Ussing chamber lumen as the experiment was performed. However, any stronger suction forces might have led to membrane disruption, especially in case of fragile thin intestinal pieces from cats.
In order to eliminate artificial variations in the conductance, air bubbles were meticulously avoided in front of the electrode apertures, in the agarose bridges, or elsewhere in the circulating system. Any leaks were eliminated by use of Dow Corning high vacuum grease®. However, during the duration of the experiment, it was impossible to completely avoid the accumulation of small air bubbles, especially in the narrow connection tubes between the water reservoir and the Lucite Ussing chamber itself. Those air bubbles were periodically removed by manually squeezing the tubing. The inability to obtain any valid results in six cases might have been due to those or other technical difficulties, such as an improper connection due to broken agar in the salt bridge. These possible causes for poor connection in the circuit could not be eliminated, even though careful handling and diligent maintenance had been given highest priority.

5.2 Short Circuit Current

In our study, large variations of both the baseline current, as well as the ΔI_{sc} could be observed. Those variations occurred regardless of species, subject animal, sample site, or the substance tested. These variations might be due to physiologic differences, but may also be reflected of testing patients with a variety of gastrointestinal diseases. There are several explanations why the magnitude of chloride secretion might vary. Activation of the basolaterally located 1K/1Na/2Cl^- transporter can be affected by both the availability of intracellular chloride ions or by the cell volume (LYTLE and FORBUSH, 1996). Interactions of cytosolic electrolytes might interfere with the availability of substrates, and modifications of membrane proteins, such as CFTR receptors might alter transport function (BERGER et al., 1991).
Activation of potassium channels can be altered by the cellular concentration of cAMP, as well as the intracellular availability of cytosolic calcium (AL-JAHMANY et al., 2004, ISHII et al., 1997; LOMAX et al., 1995; MCROBERTS et al., 1985). Transport

functions can also be modified by the total number of available transporter proteins, which in turn are dependant on endo- or exocytosis of these transporter proteins as well as changes of gene transcription (MATTHEWS et al., 1995; MATTHEWS et al., 1992, BRADBURY and BRIDGES, 1994). In order to determine whether any of these factors played a role during our experiments further molecular studies would be necessary.

As mentioned earlier, stress also has a significant effect on epithelial transport. The jejunum of rats, exposed to restraining stress for several hours, exhibited markedly increased chloride secretion when compared to rats that did not undergo such stress (SAUNDERS et al., 1994). Baseline short circuit currents were increased by up to 100% in these studies, while the magnitude of the I_{sc} response to enteric nerve stimulation was significantly lower (SAUNDERS et al., 1994).

In addition to physical stress, psychological stress has also been shown to stimulate NaCl secretion, mediated by accumulation of histamine and tryptases that are being released by mast cells (BARCLAY and TURNBERG., 1987; BARCLAY and TURNBERG, 1988; SANTOS et al., 1998). The effect of hospitalization on the stress level of our study population cannot be estimated as our experiments could not be performed in animals that did not undergo the stress of hospitalization.

An additional possible reason for an inconsistent response of short circuit current to the stimuli applied is the unstirred water layer. As the physiologic intestinal epithelium has an effective diffusion barrier due to the glycocalix as well as the mucus secreted by goblet cells, the unstirred surface layer might lead to protracted contact with the dissolved chemicals. However, several chemicals have been reported to overcome an unstirred water layer (e.g., DL-dithiothreitol, tetrodotoxin) (SHELDON et al., 1988), though we did not test any of those agents.

Another reason for alterations in I_{sc} is the actual architecture of the epithelium. Alteration of the architectural structures during biopsy collection may have caused an abnormal response (DONOWITZ and MADARA, 1982; MEIJSSEN et al., 1991). Thus, architectural changes of either the villi and/or the crypts might have had an effect on the individual response of different biopsies to the same chemical. The total number of the available epithelial cells within the exposed area might contribute to the differences in the amplitude between canine and feline species, and between animals with histological lesions and those with a histopathologically unremarkable duodenum.

The gastrointestinal diseases in the patients enrolled into this study might also have caused an alteration in response the applied stimuli. For example, patients with small intestinal ulcerations have been reported to have an altered duodenal bicarbonate secretion (BUKHAVE et al., 1990). Additionally, in inflamed tissue I_{sc} values may have been underestimated, as the conductance is affected by a loss of tissue integrity (DOBSON et al., 1968).

As our study population was inhomogeneous, not only in age and disease, but also in histologic findings, more Ussing chamber experiments of clearly defined groups would be needed to gain reliable results regarding the baseline values and the amplitudes of short circuit current changes in normal and diseased dogs.

5.3 Tissue Viability

There are several reports about tissue viability during Ussing chamber experiments. While some studies report that murine intestinal biopsies show an appropriate response for up to three hours after being transferred into the chamber, others report an *ex vivo* lifespan of human intestinal tissue samples of only approximately two hours (CLARKE, 2009; LARSEN et al., 2001).

Complete loss of viability of the tissue does become immediately apparent as the ability for voltage clamping of the tissue gets lost. In our study, during experiments with six biopsies the amplifier showed a sudden electrical overload with short circuit current values reaching >400,000 $\mu A/cm^2$, suggesting complete loss of viability of those 6 biopsies.

The duration of tissue viability depends on several factors, including the substances applied during the experiments. For example, glucose enhances tissue vitality, whereas the application of secretory chemicals might cause stress related cell exhaustion. Finally, ouabain leads to cell death.

Overall, the response to absorptive substances was a lot greater in duodenal compared to colonic samples. While 54% of duodenal biopsies exhibited a response to either glucose and/or phloridzin, only 24% of colonic samples reacted to either of them. Also, interestingly all of the biopsies that responded were canine samples.

Reasons for these findings might be the physiologic functions of the colon. The large intestine is designed to absorb fluids and secrete mucus into the feces and thus contains less glucose transporters than the duodenum.

Thus, evaluation of tissue response to glucose is not a reliable parameter for the assessment of tissue viability with this chamber design. This is especially true for feline and colonic samples.

5.4 Correlation with Histological Findings

In order to correlate functional alterations of intestinal transport with morphological findings, canine duodenal samples were grouped into three categories. Group 1 included healthy dogs without histopathological evidence of gastrointestinal diseases (WSAVA grading score <2.7), group 2 included patients with clinical symptoms of gastrointestinal disease, but histopathologically unremarkable duodenal biopsies (WSAVA grading score <2.7), and group 3 included dogs with clinical signs of gastrointestinal disease and histopathological lesions (WSAVA grading score >3.0). Interestingly, the total rate of responsive biopsies was highest (87.5%) in biopsy samples from dogs from group 1, while only 28.1% of the biopsies from dogs in group 3 responded to any given stimulus. These observations confirm the importance of tissue integrity. Because many samples of group 3 showed crypt cell necrosis, villus blunting, and/or lymphocytic inflammation, intact transport function of these biopsies would have been surprising. Results of the Ussing chamber experiments in this study thus appear to be correlated to histological findings.

5.5 Response to Sampling Site

While the main function of the duodenum is the absorption of nutrients, the colon has a large secretory capacity. The results of this study reflect on the primary function of these different intestinal segments. The highest response rate for duodenal biopsy samples was observed when biopsies were evaluated for absorptive function. The application of phloridzin caused a decrease of the short circuit current in 49.0% of the canine and 70.6% of the feline specimens. Response of duodenal biopsy samples to secretory drugs also consistently led to alterations in I_{sc}, however to a lesser extent than that of substances that influence absorptive function. The highest response rate for canine duodenal specimens was 9.8% in response to histamine/forskolin, while the highest rate of response in feline duodenal samples could be observed after application of serotonin (50.0%).

Colonic biopsy samples responded to all secretory compounds tested. Reactions of feline colonic biopsy samples were quite consistent and varied between 31.3-43.8% for all four agents applied. Reactions of canine colonic biopsy samples varied between 53.8-92.3% for all four agents applied. While feline colonic samples did not respond to any absorptive stimulus, about 53.8% of canine colonic biopsies did respond to phloridzin indicating an ability for glucose uptake in the canine colon.

5.6 Response to Various Substances

The concentrations of substances used in this study were relatively high to increase the likelihood of a response. Previous studies tested the response of canine colonic samples to various secretagogues, but data were not published due to an insufficient response (RANGACHARI and MCWADE, 1986). Unfortunately, this fact hinders us from comparing our results to previously observed findings.

However, cellular secretion is dependent on basolateral electrolyte uptake. Application of several chemicals during one experiment may have led to unusual stress of the tissue specimen. Additionally, channels and transporters may already have been in a state of maximum conductance, resulting in an attenuated response to further stimulation by cAMP. Therefore, our findings must be evaluated cautiously.

5.6.1 Response to Glucose and Phloridzin

Especially strict carnivores like cats are known for their low tolerance of carbohydrate uptake and their inability of respond to varying carbohydrate levels in the diet (BUDDINGTON et al., 1991). In contrast, the SGLT-1 transporter function in the small intestine of carno-omnivores, like the domestic dog, is known to be 2-fold higher compared to cats (BATCHELOR et al., 2010). Hence, dogs have a higher capacity to digest and absorb carbohydrates than cats. In our measurements, the median ΔI_{sc} of canine duodenal biopsy samples exposed to glucose and phloridzin was 34 and 32 $\mu A/cm^2$, respectively, and therefore higher compared to the median ΔI_{sc} of feline duodenal biopsies samples exposed to the same compounds (28 and 19 $\mu A/cm^2$). Additionally, the maximum ΔI_{sc} of canine duodenal biopsy samples exposed glucose and phloridzin was 170 and 168 $\mu A/cm^2$, respectively, while maximum ΔI_{sc} of feline duodenal biopsy samples was 32, and 66 uA/cm^2 after exposure to glucose and phloridzin.

Our results indicate that glucose uptake in domestic dogs is not only limited to the small intestine but also takes place in the colon. The expression of sodium-glucose

cotransporter in the large intestine has previosly been reported in cows, chicken, and rats (BINDSLEV et al., 1997; DA LA HORRA et al., 2001; GONZALEV et al., 1998; YOSHIKAWA et al., 2011; ZHAO et al., 2011).
In our study, glucose failed to induce a change in I_{sc} in many cases. Not only did only a small number of samples respond to glucose at the end of the experiment, but also in the beginning of the experiment, many biopsies did not react to this stimulus. However, glucose-induced short circuit current has been suggested as a marker for villous integrity (INAGAKI et al., 2005).
Ferraris and Diamond demonstrated that the gradient for phloridzin binding along the intestinal wall paralleled the gradient for maximum glucose transport (FERRARIS and DIAMOND, 1986). Therefore, the normal positional gradient in glucose transport along the intestine arises from a gradient in transporter density, which in turn appears to be induced by the normal gradient in luminal glucose concentration. The amplitude of ΔI_{sc} therefore reflects not only villous integrity, but also the overall absorptive capacity of the animal's small intestine. The rate of phloridzin binding depends on the potential difference, as well as the sodium concentration of the surrounding solution (RESTREPO and KIMMICH, 1986). In our study, maintenance of the samples with physiologic buffer solution excluded bias due to an improper sodium concentration.
In canine and feline duodenum samples, as well as in the group of canine colon, a higher percentage of samples responded to phloridzin compared to glucose. The response to phloridzin proved an existing glucose uptake, although the sugar absorption could not reliable be demonstrated by adding 40 mM glucose. Possible explanations for this observation might be that glucose uptake was maximal even before the 40 mM glucose was applied mucosally. This would also be a possible explanation why phloridzin did show a much higher response rate, when compared to glucose.
As mentioned, 10 mM of glucose was applied to the serosal bathing solution, while 10 mM sorbitol was concurrently added to the mucosal buffer solution to maintain iso-osmolarity. While both sugars were dissolved in the buffer solution, they might have crossed the serosal/mucosal barrier due to heavy water movements when air bubbles entering the system stirred the bathing fluid. Finally, leakages between the chamber halves might have occurred. Also, the diameter of the air suction adapter aperture might have been too wide, allowing the biopsy to be held in place, but enabling an improper sealing at the edges. Indications for the latter might be the fact that in the feline duodenal study population, only two out of twelve phloridzin-positive samples exhibited a response to glucose, whereas in canine patients nearly all phloridzin-positive samples also responded to glucose (overall, 21 of 25 samples). According to the anatomic characteristics of the cat, the intestinal wall is thinner compared to dogs. Hence, the air suction space might have been appropriate for canine samples, but might have been too large for mounting the intestinal samples from cats.

5.6.2 Response to Histamine

As mentioned previously, in studies with rabbit duodenum histamine significantly increased I_{sc}, suggesting stimulation of electrogenic chloride secretion (HOGAN et al., 1995). In this study, 9.8% of canine and 47.1% of feline duodenal biopsy samples responded to the exposure to histamine. Increased histamine concentrations in the gastrointestinal tract can be found with a variety of gastrointestinal diseases and might have resulted in a lower short circuit current baseline value as well as an altered I_{sc}

response compared to healthy individuals.
However, the effect of histamine that is mediated through H_2 receptors is responsible for the inhibition of prostaglandin E_2 stimulated bicarbonate secretion in rabbit duodenum has previously been demonstrated (HOGAN et al., 1995). In the study by Hogan et al., the response to prostaglandin was significantly reduced when the tissue was pretreated with histamine when compared to the response to prostaglandin alone (HOGAN et al., 1995). As the same mechanisms likely would also occur in intestinal samples from companion animals, this might explain why none of the duodenal biopsy samples in our study in either cats or dogs responded to prostaglandin administration.
The effect of histamine itself on colonic epithelia has been tested in a human cell line, T_{84}, for example (WASSERMAN et al., 1988). In these studies, the application of 0.1 mM histamine increased the short circuit current by 12-36 $\mu A/cm^2$, but returned to baseline values within ten minutes after application (WASSERMAN et al., 1988). It has also been demonstrated that in canine colonic mucosa the application of 10^{-5} M histamine sharply increased the short circuit current of stripped intestinal samples from 106.6 ±21.9 (mean ±standard error of the mean) $\mu A/cm^2$ to 272.4 ±64.3 $\mu A/cm^2$ in intact mucosal samples, and from 77.0 ±12.2 $\mu A/cm^2$ to 212.2 ±47.4 $\mu A/cm^2$ in epithelia detached from the lamina muscularis mucosae (RANGACHARI and MCWADE, 1986). This response to histamine is higher when compared to that observed in our study. As the action of histamine via H_1-receptor is mediated by an increase of free cytosolic calcium, the availability of this messenger might have been limited due to stress or other unforeseen reasons. However, by applying histamine as the first of four secretory agents, this effect should have been prevented.

5.6.3 Response to Serotonin

Previous investigations of rabbit ileum and canine colonic epithelium in Ussing chamber experiments revealed a response of short circuit current when the samples were exposed to serotonin (DONOWITZ et al., 1980; RANGACHARI and MCWADE, 1986). Especially in canine epithelial samples that had been separated from submucosal nerves and the lamina muscularis mucosae, serotonin led to a consistent response, increasing the short circuit current from 51.0 ±17.3 $\mu A/cm^2$ to 390.3 ±47.9 $\mu A/cm^2$ (RANGACHARI and MCWADE, 1986). In this study, one individual responded disproportionally well with an increase of I_{sc} from 23.9 to 541.8 $\mu A/cm^2$, increasing its initial value by 2100%. In our study, ΔI_{sc} ranged from 6 to 60 $\mu A/cm^2$, but only in one canine colonic biopsy exposed to histamine, short circuit current increased from 38 $\mu A/cm^2$ to 402 $\mu A/cm^2$ (i.e., an increase by 1058%).
The maximal ΔI_{sc} in rabbit intestine was reported to be 50 $\mu A/cm^2$, which was recorded after stimulation with 0.26 mM serotonin. In all experiments the short circuit current decreased back to baseline values no later than 10 minutes after serotonin had been applied. It is interesting to note that responses were altered based on the severity of histological abnormalities (DONOWITZ et al., 1980).

5.6.4 Response to Prostaglandin E_2

The role of prostaglandins in maintaining gastroduodenal mucosal integrity is complex. In addition to stimulating mucosal bicarbonate secretion, prostaglandins enhance cell proliferation, mucus secretion, and mucosal blood flow. They also increase the synthesis of mucosal sulfhydryl groups, promote lysosome stability, and increase the formation of

mucosal phospholipids (MILLER, 1983). PGE_2 is a potent agonist of bicarbonate secretion, thus stimulating I_{sc}. The response of bicarbonate secretion to exogenous prostaglandin seems not to be affected by the destruction of afferent neurons, which would suggest a direct interaction with enterocytes (TAKEUCHI et al., 1999).
In our study, none of the duodenal samples exhibited a response prostaglandin. This is not surprising, since previous studies have shown that when the tissue samples were pretreated with histamine the bicarbonate secretory response to PGE_2 was almost completely inhibited (HOGAN et al., 1995). In Hogan's study, removal of histamine reversed this inhibitory effect, and reapplication of PGE_2 induced a secretory response. However, histamine specifically inhibits the secretion of bicarbonate, without affecting chloride efflux. In canine and feline colonic samples, PGE_2 did induce an increase of short circuit current, indicating active bicarbonate secretion.
Human patients with duodenal ulcers have been reported to have significantly less baseline and PGE_2 stimulated bicarbonate secretion in the proximal duodenum when compared to healthy individuals (BUKHAVE et al., 1990; ISENBERG et al., 1987). In contrast, bicarbonate secretion in the distal duodenum of those same patients was not different from normal subjects. PGE did not enhance the bicarbonate secretory response in the proximal duodenum, there was a significant increase in transmucosal potential difference in the distal duodenum, which was not different from that found in normal subjects. This increase in the potential difference was attributed to increased chloride secretion in patients with duodenal ulcers (HOGAN et al., 1990). These findings suggest that patients with duodenal ulcers have an impaired mucosal bicarbonate response to endogenous PGE_2 in the proximal duodenum (BUKHAVE et al., 1990).
Therefore, response to prostaglandin in our study may have been affected by the gastrointestinal diseases present, but may also have been secondary to species-specific differences or a variable thickness of tissues in our endoscopically obtained biopsies.

5.6.5 Response to Forskolin

The effect of 5 µM forskolin on the samples of our study population confirms previous findings: Many samples responded to this substance by an elevation of the short circuit current, while magnitude and duration of response varied a lot between individual samples. No conclusions could be drawn regarding the magnitude of ΔI_{sc}, as the height of response might have been altered due to exposure of chemicals applied earlier during the experiments.

5.6.6 Response to Ouabain

The effect of 600 µM ouabain on intestinal samples in our study caused equivocal results. While the short circuit current of most samples decreased, some samples increased their current after ouabain exposure. Once again, the observed changes could not be associated with a certain groups of patients or type or severity of histopathological changes. It is also noteworthy that the time it took for a change of the short circuit current was relatively long (up to 21.5 minutes) compared to the chemicals applied earlier during the experiments. Previous studies in various species reported an effect of ouabain that lasted for 20 minutes, but only one study on canine colonic samples has been reported (RANGACHARI and MCWADE, 1986). In some of the biopsies we investigated, I_{sc} continued to change until 45.9 minutes. However, it remained unclear if those changes were due to the influence of ouabain, or whether these

changes reflected deteriorating tissue viability while mounted in the Ussing chamber. Rangachari and McWade observed an effect of ouabain on stripped epithelium, which produced a decrease in current indicating inhibition of the sodium pump, while ouabain applied to mucosa that was still attached to the lamina muscularis mucosae and the plexus submucosus resulted in an increase of I_{sc}, followed by a decline (RANGACHARI and MCWADE, 1986).

5.7 Limitations of the Study

5.7.1 Study Population

The number of individuals enrolled in this feasibility study was relatively small, and evaluation of a greater number of individuals would be required before conclusions about the usefulness of this new analytical technique can be made.

The inhomogeneous study group may have had a significant impact on our results. Our study population included dogs and cats of different breeds. Some breeds are known to be pre-disposed to gastrointestinal disorders, such as Siamese cats, Soft Coated Wheaten Terrier, Boxers, and German Shepherds (HALL et al., 1994; JERGENS et al., 1992; LITTMAN et al., 2000). Breeds that are pre-disposed to gastrointestinal disease that were represented in our study included one Siamese cat, one Soft Coated Wheaten Terrier, two German Shepherds, and one Boxer. All, except two healthy dogs, underwent diagnostic endoscopy for the routine work-up of their suspected gastrointestinal disease. Many of these animals had severe disease and were treated aggressively during the time period the biopsy samples were collected. Diagnosis, based on a complete clinical work up, including clinical history, clinical examination, blood chemistry, complete blood count, histopathologic evaluation of intestinal biopsies, and/or response to treatment, identified several different diseases, such as IBD, protein-losing enteropathy, intestinal adenoma, and others. Each of these disorders may have affected intestinal transport physiology in a different fashion.

Also, since most of the animals enrolled in our study were diseased, most of them likely received specific medications during the time they were hospitalized for the workup of their disease, which might also have affected mucosal transport physiology. It has previously been shown that glucocorticoids and mineralocorticoids upregulate the expression of ENaC and Na^+/K^+ ATPase, thus potentially affecting sodium absorption (BATT et al., 1982). Furthermore, it has been shown in humans and rats, that low doses of glucocorticoids induce electroneutral sodium absorption, whereas high doses additionally increase electrogenic sodium absorption (BASTL, 1987; BINDER et al., 1989). Polyene antibiotics (e.g., amphoterin B, nystatin), are known to decrease tissue resistance by leading to a marked increase of sodium transport (FRIZZELL and TURNHEIM, 1978). Since the group of animals studied was rather inhomogeneous, conclusions on a single parameter such as a specific medication could not have been drawn from this study. However, this was also not the goal of the current study.

There also may have been an effect of age on epithelial function. The age of the animals enrolled in this study ranged widely between 7 and 149 month. The affect of age on ion transport has previously been studied in several species (FREEMAN and QUAMME, 1986; GREIG et al., 2003; KOSIK-BOGAKA et al., 2004; VENKATASUBRAMANIAN et al., 2000). Reported findings are consistent across species studied in that cAMP-dependent chloride secretion is significantly lower in adult individuals than in individuals that are not adult (BRAATEN et al., 1988). Furthermore, chloride secretion stimulated by cAMP and cholinergic nerves decreases gradually over the life span of individuals and is accompanied by reduced colonic motor function, reduced colonic water permeability, as well as alterations in colonic endocrine cell population (MARIN and APERIA, 1984; MCDOUGAL et al., 1984; SANDSTROM et al., 1998).

5.7.2 Sample Collection

During biopsy collection, mechanical damage of the epithelium might have occurred. Especially the fragile duodenal villi can easily be damaged by the endoscopic biopsy forceps. An impact of the collection procedure on tissue resistance has previously been reported by Stevens and may also have played a role in our experiments (STEVENS, 1964). Endoscopy and biopsy collection was performed by several clinicians with varying degrees of experience. More specifically, operators included both residents in their first year of training and highly specialized clinicians with years of experience in small animal endoscopy. This might have had an impact on sample quality. For example, the duration of the collecting procedure varied between operators. Damaged tissue may exhibit a higher conductance compared to good quality biopsies. Another factor that may have affected the overall response rate is the influence of the sensitive neural network of the intestinal wall, which might have been damaged during biopsy collection, resulting in the induction of phospholipase C or A_2 activity as previously been shown in stripped rodent intestine (BUKHAVE and RASK-MADSEN, 1980). Stripping of the intestinal biopsy describes the procedure of removing the visceral peritoneum from a full thickness intestinal biopsy. This procedure may result in prostanoid secretion, which might have an impact on the function of the NaCl transporter as well as the CFTR receptor, as intracellular Ca^+ and cAMP pathways become activated (BUKHAVE and RASK-MADSEN, 1980). However, these effects of submucosal damage can be minimized by incubating the pre-stripped sample in ice cold, oxygenated buffer containing indomethacin (CLARKE, 2009). Pre-treating endoscopic biopsy samples in this fashion is obviously not feasible *in vivo*. However, since the biopsy samples collected during this study were transferred into a beaker of Meyler's buffer solution, we speculate that most of the prostanoids were washed away into the buffer solution. Also, by using the transport medium at room temperature, rather than cooled, we intended to reduce the stress on the biopsy samples. However, trauma-induced hormonal stimulation of the samples cannot be completely excluded.

5.7.3 Hydrostatic Pressure

The design of the standard Ussing chamber calls for use of 10 ml of buffer solution on either side of the tissue membrane. This relatively large volume of 10 ml buffer solution in each half of the chamber has the advantage of being able to dose the substances added to the buffer solutions more accurately. However, the large volume of buffer solution also leads to a relatively large hydrostatic pressure, which might have affected the transport functions of the biopsy specimens. These effects might be further intensified by the sudden pressure changes during the manual filling of the buffer reservoirs. To minimize these pressure changes on the biopsy samples, a special filling procedure was used. Twelve ml syringes connected to long tubing were used, so the buffer could be gently placed into the bottom of the glass basins, rather than being poured from the top of the reservoir, which would have caused large pressure changes during the filling procedure. An indication of excessive pressure changes might have been the fact that in four cases the biopsy specimen was lost into the lumen of the Ussing Chamber. Hydrostatic pressure imbalances may also affect electrical measurements by an artificial increase of the transepithelial conductance. The possible impact of pressure changes on conductance are previously discussed in section 5.1.

5.7.4 Experimental Protocol

Physiologic Meyler's buffer was used as the basic perfusion solution. This solution has a composition very different from intestinal digesta. Therefore, the obtained values do not necessarily reflect mucosal transport *in vivo*. Furthermore, the transport characteristics of each segment are subjected to regulatory control in response to whole body homeostasis (HOLTUG et al., 1996).

The substances applied have been shown to have distinct effects on the intestinal transport physiology. We therefore expected electrochemical changes following their application. Since dogs and cats have not been used in many Ussing chamber studies before, previously unreported species-specific variations in the tissue response might have occurred. Differences in receptor expression, as well as in pathways and mechanisms of absorption/secretion are possible and therefore, we tested a variety of chemical agents, expecting at least some of them to cause a consistent response. The capacity to stimulate a secretory response is strongly correlated to the ATP level in the cell. However, the application of four different secretagogues chemicals in less than 120 minutes might have exhausted the tissue, causing uncharacteristic secretory patterns and an unphysiologic response. However, due to concerns over tissue viability, prolonging the experiments was not considered to be an option.

By the end of the experiments, ATP-dependent transporters and channels, like the CTFR or Na^+/K^+ ATPase might not have had enough energy to become activated. The same principles may be true for the opening of the calcium dependent potassium channels on the basolateral membrane of the enterocytes. Thus it is important to note that the aim of this study was to assess the feasibility of the use of an adapter modified Ussing chamber, but not to study the response of intestinal biopsies to a variety of different stimuli.

5.7.5 Cross-Contamination

In order to avoid drug contamination between the Ussing chamber experiments, all instruments were washed with soapy water and rinsed thoroughly with distilled water after each use. The inner surface of the chamber was cleaned and dried with cotton buds. Tubing material was replaced periodically. However, the soft plastic tubes were not replaced between each experiment due to financial constraints. This might have led to some interferences as drugs that were dissolved in ethanol, like phloridzin or forskolin, could potentially have penetrated into the plastic surfaces. Lane L. Clarke impressively demonstrated the possibility of such effects in experiments with murine intestine (CLARKE, 2009). In his studies Clarke showed that forskolin contamination can increase the baseline short circuit current, and therefore significantly reduce the amplitude of ΔI_{sc} after application of stimulating chemicals. However, the response to glucose was not affected by forskolin contamination. Clarke therefore advised to rinse the Ussing chamber with 70% ethanol, and to soak the acrylic chamber in soapy water for 15-20 minutes as well as in 20% dimethylsulfoxid after each use. However, since these data were not available at the time of our experiments (experiments were performed in 2008 and 2009, while the paper by Clarke was published in 2009), we did not follow those recommendation. Thus, in retrospect, a possible contamination of the Ussing chamber or components of the chamber with some of the substances used for the experiments cannot be excluded and further experiments should be performed using the recommendations by Clarke to exclude such contamination. Theoretically, contaminations might have had an effect on our experiments and, in the extreme case, in

case of contamination with ouabain, might even have been an explanation why several biopsy specimens did not respond at all to any of the chemicals through the entire experiment.

5.7.6 Method

Ussing chamber experiments allow for precise measurements of electrical parameters on sections of intact intestinal epithelium. Over decades, Ussing chamber experiments have proven valuable and they are still considered to be the gold standard for investigating transmembrane transport. The use of a biopsy adapter disc enables the unique approach of investigating endoscopically collected intestinal biopsy specimens from diseased companion animals. This technique is minimally invasive and provides functional data rather than morphological data based on histopathological evaluation.

However, it should be noted that in living animals overall intestinal function is achieved by the complex interactions of immune cells, neurons, endocrine cells, mucosal cells, and smooth muscle cells. Thus, *ex vivo* studies may not be entirely representative of the situation *in vivo*. Due to this limitation, conclusions concerning effects observed in Ussing chamber experiments have to be interpreted cautiously.

Some studies have demonstrated certain shortcomings using small biopsy samples for the diagnosis of gastrointestinal diseases (MILLARD et al., 1986). Small tissue samples have the disadvantage of providing only limited diagnostic information. Ideally, a series of biopsies is collected as many morphological and functional changes show a patchy distribution. Therefore, we evaluated four biopsies from each animal at the same time. However, comparing and summarizing the four different values observed in experiments with the different samples from one animal was not found to be very useful as we observed large variations of the response rate of biopsies from the same patient. As displayed in table 8, 9, 10, 11, 12, 13, and 14, in several instances one or two tissue samples from a patient did not respond to some or all chemicals, while the other tissue samples responded with a clear change in short circuit current. Only in rare cases, all samples of an animal responded adequately to the applied stimulus, or none of the samples exhibited any response. There are several possible reasons for these observations. The patchy distribution of chronic enteropathies might have affected the response of the different biopsy samples from the same patient. Also, any of the interferences discussed above may have affected the response of some biopsies, but not of others. For future studies, a larger sample size may be of great advantage, but this would also delay the tissue transfer from the animal into each of the chambers as this transfer would have to occur in parallel for each biopsy sample, which may negatively impact the lifespan of the samples. Handling of even four biopsy samples simultaneously as done for this study was challenging at times and the viability assessed of the samples did vary, and the impact of those variations needs to be determined through further studies. In our study, the percentage of biopsies responding to at least one chemical ranged between 92.3-0% depending on the species, tissue section, and chemical stimulant used. However, Ussing chamber experiments show great variability even when conducted with regular chambers and big tissue samples and performed by experienced scientists (personal communication with Judith M. Ball, Texas A&M University, Jody L. Gookin, North Carolina State University, and Mark W. Musch, University of Chicago, 2009). Unfortunately, no publication on the rate of overall success of Ussing chamber experiments is available.

Several studies showed the feasibility of evaluating endoscopically-collected biopsy samples from human patients with gastrointestinal disease in Ussing chambers. Understanding the dysfunction of intestinal epithelial transport functions, such as transporter up- or downregulation in companion animals affected by chronic enteropathies might allow further characterization of these diseases by the electrophysiologic parameters and might improve our understanding of those diseases or even might allow the development of new treatments of such diseases.

5.8 Conclusion

In summary, we can conclude that duodenal and colonic biopsies from dogs and cats maintain their transport ability *ex vivo* when transferred into the modified Ussing chamber.

Additionally, our results reflect characteristic intestinal functions. The total responding rate correlates to histopathological damage. The adapter modified Ussing chamber permits the evaluation of responses of intestinal tissue to specific substances and thus allows for the investigation of intestinal ion transport. The advantage of evaluating endoscopic biopsy samples, rather than surgically obtained tissue, cannot be overemphasized. The use of this method for large study populations, follow-up studies, or long-term studies is feasible due to its minimal invasiveness. Further studies in a larger number of healthy dogs and cats are needed to better characterize intestinal transport in those species. Such studies are especially important as a foundation for further studies of intestinal transport in diseased dogs and cats, but also to better understand intestinal transport physiology in dogs and cats. However, the wide variability of response within samples from the same patient limits the clinical use of the adapter modified Ussing chamber.

In summary, the present study has, for the first time, evaluated the feasibility of using a modified Ussing chamber for the evaluation of transport physiology in endoscopically-collected canine and feline intestinal biopsy sections.

6. Summary

Chronic enteropathies in companion animals occur frequently. Due to a lack of information regarding the pathophysiology of these disorders, better characterization of these disorders is warranted.

Ussing chambers have been used to study intestinal membrane transport in many species. Since studies using a conventional circulating Ussing chamber require the collection of large tissue specimens, such studies have rarely been undertaken in small animal patients with spontaneously occurring gastrointestinal diseases. In order to overcome this disadvantage, an adapter modified Ussing chamber was investigated for evaluation of endoscopically obtained duodenal and colonic biopsies from dogs and cats in this study. Seventeen duodenal biopsies from five cats, 16 colonic biopsies from four cats, 51 duodenal biopsies from 14 dogs, and 13 colonic biopsies from four dogs were analyzed in the adapter modified Ussing chamber. Canine duodenal samples were grouped according to the WSAVA scoring system in combination with the presence of clinical symptoms. Group 1 was made up for healthy individuals (WSAVA score <2.7; n=8), group 2 was made up for animals exhibiting clinical signs of gastrointestinal disease, accompanied with histopathologically unremarkable biopsy samples (WSAVA score <2.7; n=15), group 3 was made up for dogs that showed clinical signs of gastrointestinal diseases and moderate to severe histopathological lesions (WSAVA score >3.0, n=32).

All biopsies were sequentially exposed to 40 mM glucose, 500 µM phloridzin, 200 µM histamine, 200 µM 5-hydroxytryptamine (5-HT), 4 µM prostaglandin (PGE_2), and 5 µM forskolin. If the intestinal biopsy showed a response to the initial glucose stimulus, 40 mM glucose was once again applied at the end of the study in order to assess biopsy viability. Finally, the experiment was terminated by application of 600 µM ouabain.

Voltage (mV) and the membrane-generated short circuit current (I_{sc}) were constantly recorded. To calculate the resistance, current pulses until reaching 18 µA were induced every 10 s for 0.5 s. I_{sc} was measured in microamperes (µA), and conductance (mS/cm^2) was calculated as the reciprocal value of resistance.

78.6% of all feline duodenal samples , 60.0% of all feline colonic samples, 49.0% of all canine duodenal samples, and 92.3% of all canine colonic samples responded to at least one chemical reagent by an appropriate change in I_{sc}. In the canine duodenal biopsy subgroups, the rate of overall response ranged from 87.5% (group 1), to 63.3% (group 2), and 28.1% for biopsies of dogs of group 3.

Mean conductance of biopsies of all groups increased as the study progressed, indicating loss of tissue integrity over time. A high variation of conductance, baseline current, and the amplitude of current change could be observed within every group, within samples from the same animal, and for all substances that were applied.

It can be concluded, that duodenal and colonic biopsies of canine as well as feline patients maintain their transport ability *ex vivo* when quickly transferred into the modified Ussing chamber. The adapter modified Ussing chamber permits the use of distinct substances and the investigation of intestinal ion transport. This study demonstrates that the adapter modified Ussing chamber has the potential for becoming a valuable research tool in the study of duodenal and colonic transport in both healthy and diseased dogs and cats, but, because of the large variability, is of limited clinical use. Investigation of larger study populations, follow-up studies, as well as long term studies is warranted.

7. Zusammenfassung

Evaluierung einer Adapter-modifizierten Ussingkammer zur Untersuchung von endoskopisch entnommenen Duodenal- und Kolonbioptaten von Katze und Hund

Kleine Haustiere erkranken häufig an chronischen Enteropathien. Da die Pathophysiologie dieser Erkrankungen nicht ausreichend erforscht ist, ist eine genauere Untersuchung dieser Problematik von Interesse.

Ussingkammern werden verwendet, um den intestinalen Membrantransport zu studieren. Herkömmliche Ussingkammern benötigen relativ große Gewebeproben, weshalb solche Untersuchungen bei Hunden und Katzen nur selten durchgeführt werden. Um diesen Nachteil zu umgehen, war das Ziel dieser Studie die Evaluierung einer Adapter-modifizierten Ussingkammer für die Untersuchung endoskopisch entnommener Duodenal- und Kolonbioptate von Hund und Katze. Siebzehn Duodenalbioptate von fünf Katzen, 16 Kolonbioptate von vier Katzen, 51 Duodenalbioptate von 14 Hunden und 13 Kolonbioptate von vier Hunden wurden in der Adapter-modifizierten Ussingkammer analysiert. Die Duodenalproben von Hunden wurden mit Hilfe des WSAVA-Klassifizierungssystems in Kombination mit dem Auftreten gastrointestinaler Symptome in drei verschiedene Gruppen unterteilt. Gruppe 1 bestand aus gesunden Hunden (WSAVA-Index <2.7; n=8), Gruppe 2 bestand aus klinisch erkrankten Hunden ohne besondere histopathologische Befunde (WSAVA-Index <2.7; n=15), Gruppe 3 bestand aus klinisch erkrankten Hunden mit moderaten bis hochgradigen histopathologischen Veränderungen (WSAVA-Index >3.0, n=32).

Alle Bioptate wurden nacheinander 40 mM Glukose, 500 µM Phloridzin, 200 µM Histamin, 200 µM 5-Hydroxytryptamin (5-HT), 4 µM Prostaglandin (PGE_2) und 5 µM Forskolin ausgesetzt. Im Falle, dass das Bioptat auf die initiale Glukosezugabe reagierte, wurde gegen Ende des Experiments nochmals 40 mM Glukose verabreicht. Der Versuch wurde jeweils durch die Zugabe von 600 µM Ouabain beendet.

Die elektrische Spannung (mV) und der von der Membran produzierte Kurzschlussstrom (I_{sc}) wurden kontinuierlich gemessen. Um den Widerstand berechnen zu können, wurden im zehn Sekunden-Takt über die Dauer von jeweils 0.5 s Strompulse bis zu einer Stärke von 18 µA verabreicht.

I_{sc} wurde in Mikroamper (µA) gemessen und die Leitfähigkeit (mS/cm^2) der Gewebeprobe wurde aus dem reziproken Wert des Widerstandes errechnet.

78.6% aller felinen Duodenalbioptate, 60.0% aller felinen Kolonbioptate, 49.0% aller caninen Duodenalbioptate und 92.3% aller caninen Kolonbioptate reagierten mit einer angemessenen Änderung des I_{sc} auf mindestens eine Substanz. Diese Reaktionsrate variierte je nach Untergruppe der kaninen Duodenalproben zwischen 87.5% (Gruppe 1), über 63.3% (Gruppe 2), bis hin zu 28.1% bei Bioptaten von Hunden der Gruppe 3. Die mittlere Leitfähigkeit der Bioptate erhöhte sich in allen Gruppen während der Dauer des Versuchs. Dies lässt auf einen zunehmenden Verlust der Zellintegrität der Bioptate schließen.

Eine große Variation der Leitfähigkeit, der ursprünglichen Stromstärke, sowie der Amplitude der Stromstärkenänderung konnte in jeder der untersuchten Gruppen, in den Bioptaten von jedem Tier, sowie bei jeder applizierten Substanz beobachtet werden.

Aufgrund dieser Ergebnisse kann folgende Schlussfolgerung gezogen werden: Sowohl canine als auch feline Duodenal- und Kolonbioptate halten ihre Transportfähigkeit nach Verbringen in die modifizierte Ussingkammer *ex vivo* aufrecht. Die Adapter-modifizierte Ussingkammer ermöglicht die Verwendung unterschiedlicher Substanzen

zur Analyse des intestinalen Ionentransports. Diese Studie demonstriert die Möglichkeit des Einsatzes der Adapter-modifizierten Ussingkammer zur Untersuchung der intestinalen Transportfunktion bei gesunden und kranken Hunden und Katzen. Das Instrument könnte sich besonders für wissenschaftliche Fragestellungen eignen, der klinische Gebrauch ist aufgrund der großen individuellen Ergebnisvariation eingeschränkt. Die Untersuchung größerer Studienpopulationen, verlaufskontrollierte Untersuchungen, sowie Langzeitstudien sollten mit dieser Methode durchgeführt werden.

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9. Appendix

9.1 List of Figures

9.2. List of Tables

10. List of Publications

Ruhnke I, DeBiasio JV, Suchodolski JS, Musch MW, Steiner JM
Evaluierung einer modifizierten Ussingkammer auf die Anwendbarkeit bei endoskopisch gewonnenen Dünndarmbioptaten von Hunden.
56. Jahreskongress DGK-DVG, Düsseldorf, Germany, October 2010 - oral presentation

Ruhnke I, DeBiasio JV, Suchodolski JS, Musch MW, Steiner JM
Evaluation of an adapter-modified Ussing chamber for assessment of endoscopically obtained colonic biopsies from cats and dogs.
20th ECVIM-CA Congress, Tolouse, France, September 2010 - oral presentation

Ruhnke I, DeBiasio JV, Suchodolski JS, Musch MW, Steiner JM
Feasibility of a modified Ussing air suction chamber for the evaluation of intestinal mucosal function in endoscopic biopsies from dogs and cats.
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Isabelle Ruhnke

12. Selbständigkeitserklärung

Hiermit bestätige ich, dass ich die vorliegende Arbeit selbstständig angefertigt habe. Ich versichere, dass ich ausschließlich die angegebenen Quellen und Hilfen in Anspruch genommen habe.

Berlin, den 19.1.2011 Isabelle Ruhnke