COMMERCIAL DIETS DO NOT AFFECT THE COLONIC ULTRASTRUCTURE OF NORMAL DOGS

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(ABSTRACT)

Commercial and homemade diets are currently used to treat many canine patients with acquired disorders of the colon. Clinically, the efficacy of diets has been found to be unpredictable. Only one study to date has evaluated the effect of diet on the colonic mucosa. This study showed that diet did not observably alter the colonic mucosa of normal dogs, when biopsy samples were evaluated by light microscopy. The effect of diet on colonic ultrastructure in the dog, using transmission electron microscopy, has not previously been investigated.

To determine the effect of diet on colonic ultrastructure, cell height, cell area, microvillus height, number of microvilli/apex width and basement membrane width were measured. Ten cells per animal were evaluated. Six dogs were assigned to the control group and fed the control diet for the duration of the study. Six dogs were fed each of the three test diets at four week intervals. The test diets used included a high fiber diet, a hypoallergenic diet and a highly digestible diet. These diets were selected because they are the diets most often recommended for the canine patient with colonic disorders. The value for cell height for the highly digestible group was significantly greater than the other groups, as measured by ANOVA and Duncan's multiple comparison test. No other significant differences were found. The biological relevance of a significantly different value for cell height alone is difficult to evaluate, as other parameters that would indicate an alteration in maturation or proliferation of the colonic epithelial cells did not change.

value for cell height alone is difficult to evaluate, as other parameters that would indicate an alteration in maturation or proliferation of the colonic epithelial cells did not change. Therefore, we conclude that commercial diets do not have an effect on the colonic ultrastructure of normal dogs. Although no effect of diet was found, this study does provide morphologic measurements that can be used as a basis for future ultrastructural studies of the colonic mucosa.

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I. INTRODUCTION

In the dog, dietary management is often the initial therapy used to treat inflammatory bowel disease (IBD) and fiber-responsive large bowel diarrhea. Sometimes there is either no response or partial response to diet alone, and medical treatment is necessary. Currently there is no way of predicting which animals will respond to dietary management based on clinical signs, endoscopic examination or histologic changes. This may be due to either a lack of understanding of the pathology of the disease and/or to a lack of understanding how diet actually affects the disorder.

A few studies have examined the clinical response of animals with large bowel diarrhea to diet, but no attempt has been made to evaluate the effects of diet on the colonic mucosa in these animals [1,2]. A recent study evaluated the effects of various diets on the microscopic anatomy of colonic mucosa in normal dogs, but found no difference in the parameters measured [3].

Although colitis in the dog is well classified by light microscopy, ultrastructural descriptions have not been extensive. Canine histiocytic ulcerative colitis is the only disease that has been evaluated ultrastructurally [4]. Evaluation of other large bowel disorders by electron microscopy may be beneficial in understanding the pathophysiology, and also provide information that may influence treatment. This is especially true in fiber-responsive large bowel diarrhea, where light microscopy of colonic mucosa is normal, and a high fiber diet is effective in controlling the clinical signs. In this disorder, either the clinical signs are associated with dysfunction and not structure, or the structural changes may only be apparent at the ultrastructural level.

The purpose of this study was to evaluate the effects of diet on the colonic ultrastructure of normal dogs. The results could be applied to clinical medicine by providing

a standard of ultrastructural parameters to which other biopsy specimens could be compared. In addition, any changes found in the colonic ultrastructure related to diet might provide a better understanding of the use of diet in the management of large bowel diseases, and may allow for more effective control of these disorders.

The following literature review includes a discussion of the gross, microscopic and ultrastructural anatomy of the colon, and a description of colonic epithelial renewal and maturation. A brief discussion of colonic motility follows, as this is important in understanding some of the effects diet may have on colonic function. This is followed by a general summary of dietary effects on the colon, the pathogenesis of canine inflammatory bowel disease, and the potential role of food as an antigen. The different types of canine colonic disorders, the current dietary management of these disorders, and a comparison of human and canine IBD are discussed next. The selection of morphometric parameters used to evaluate the samples in this study was based on the research of others, both at the light and electron microscopic level; therefore a summary of those findings is presented. The intention of the literature review is to provide the reader with the background information needed to understand the purpose for and evaluation of this study.

II LITERATURE REVIEW

A. Gross and Microscopic Description of Normal Canine Colon

The canine large intestine from orad to aborad consists of the cecum, ascending colon, transverse colon, descending colon, rectum and anus (Figure 1). The canine colon is relatively short when compared to the colon of herbivores, as its primary function is to absorb water and electrolytes. However, studies have shown that the absorptive cells of the adult canine colon have sodium-dependent, active transport mechanisms for absorption of sugar and amino acids [5]. In addition, the colonic epithelial cell has the capability to absorb short chain fatty acids produced by colonic bacteria, and uses one of these fatty acids, butyrate, as a main source of energy [6].

Estimated length of the colon ranges from 0.2 to >0.6 meters [7] depending on the size of the dog and is considered to be 10%-20% of the length of the small intestine. This estimate (0.2 -0.6 m) is probably low as endoscopic experience has found the colon in large breed dogs to be in excess of 1 meter. Two flexures separate the three segments of the colon: one between the ascending and transverse colon (the right colic or hepatic flexure), and the other between the transverse and descending colon (the left colic or splenic flexure) [8]. There are no villi or folds in the large intestine. Folding that is seen postmortem is an artifact due to contraction of the muscularis mucosae. Blood flow to the large intestine is through the cranial and caudal mesenteric arteries and venous drainage is via the mesenteric veins to the portal vein. Lymphatic drainage is through mesenteric lymph nodes.

The ganglia of the gastrointestinal tract are organized into three separate plexuses, which are referred to as the intrinsic nervous system [7,9,10] (Figure 2). The subserosal plexus is located at the point of attachment of the mesentery to the intestinal wall. This plexus acts as a relay between the extrinsic nerves and the internal plexuses. The myenteric plexus is found between the circular and longitudinal layer of the muscularis, and the submucosal

plexus lies within the submucosa. These plexuses are interconnected by neurons both within and among the plexuses.

Afferent and efferent innervation of the colon via sympathetic and parasympathetic pathways is referred to as the extrinsic nervous system. Sympathetic efferent nerves travel through thoraco-lumbar vertebrae (approximately T13-L4) and are carried to the intestines by the splanchnic nerves. Since synapses occur in a number of sympathetic ganglia that are found paravertebrally, the sympathetic fibers found in the splanchnic nerves are considered postganglionic. Parasympathetic efferent nerves are supplied via the vagus for the proximal large bowel and from the sacral spinal cord via the pelvic nerve for the distal portion. These fibers synapse within ganglia located on the wall of the intestine (the subserosal plexuses), and are considered to be preganglionic fibers. Parasympathetic innervation is primarily responsible for motility and is more extensive than sympathetic innervation which has a modulating effect. Sympathetic and parasympathetic afferent fibers from the colon travel in their respective nerve trunks to local and central synapses. The afferent fibers respond to changes within the intestinal lumen, such as distension, and elicit colonic reflexes [7,9].

The layers of the colon are the mucosa, submucosa, muscularis, and serosa [11]. The mucosa can further be divided into epithelium, lamina propria and muscularis mucosae, and is organized into crypts or glands of Lieberkuhn, which are straight tubular structures, approximately 0.5-0.7 mm deep [12] (Figure 3). The epithelial layer consists of epithelial or absorptive cells, goblet or mucus cells, and enteroendocrine cells.

The lamina propria is the area between and underneath the crypts. It contains lymphocytes (primarily T- helper cells), plasma cells, eosinophils, macrophages and mast cells. Increased numbers of lymphocytes and plasma cells in this layer are one of the diagnostic criteria for inflammatory bowel disease. Lymphoid follicles are foci of lymphoid cells located in the lamina propria and the submucosa [13]. Lymphoid follicles are especially prominent in young animals and during endoscopic examination can be seen as circular,

umbilicated areas, 2-3 mm in diameter, on the mucosal surface [14]. Other components of the lamina propria include a loose areolar connective tissue matrix which supports blood vessels, lymphatics and unmyelinated nerve fibers [11]. The blood vessels and lymphatics receive substances absorbed from the mucosa into the general circulation and they also provide nutrients and immune components to the mucosa proper [12].

The muscularis mucosae separates the mucosa from the submucosa [7]. It is composed of smooth muscle fibers, and in humans is approximately 8-12 cell layers thick. The muscularis mucosae in dogs is slightly thicker [15]. Contraction of this layer causes the folding noted postmortem.

The submucosa is composed of connective tissue with elastic fibers. It provides a loose framework through which arteries, veins, lymphatics and nerves pass [11, 12]. The submucosal plexus is a collection of nerve fibers and ganglia and is diffusely spread throughout the submucosa. Nerve fibers extend from the submucosa into the lamina propria of the mucosa. These are primarily parasympathetic fibers, with occasional sympathetic fibers.

The muscularis is made up of two layers of smooth muscle, an outer longitudinal and an inner circular layer [11, 15]. The circular layer is responsible for segmental contraction and the longitudinal layer is responsible for progressive movement. As with most smooth muscles, the muscularis acts as a syncytium which allows propagation of electrical activity throughout the smooth muscle layers. The myenteric plexus is located between the circular and longitudinal layers and innervates these muscles [9]. The serosa is the external layer of the colon [11, 12]. It consists of a single layer of squamous epithelial cells.

B. Endoscopic Evaluation of the Colon

Endoscopy is a relatively noninvasive method used to obtain intestinal biopsies. Because of the size of the endoscopic forceps, usually only the mucosal layer can be

evaluated. Biopsies of deeper layers can be obtained endoscopically by serial biopsies at the same site [16]. However this latter technique usually is used only when a specific lesion is being sampled.

One of the first studies evaluating endoscopically-obtained colonic biopsies from normal dogs concluded that 1) there was considerable variability noted in normal dogs, especially for lamina propria cell populations; and 2) endoscopic biopsies provide adequate samples for accurate mucosal evaluation [16]. The number of inflammatory cells noted in the lamina propria varied substantially among dogs. The crypts were lined with cuboidal and columnar epithelial cells. The columnar cells were located at the top of the crypts with a gradual transition to cuboidal cells in the bottom of the crypts. At the very bottom of the crypt, immature cells were noted. Mucin producing cells were also noted, these cells being more abundant in the bottom portion of the crypts. Lymphoid follicles were frequently noted endoscopically and occasionally distorted the epithelial surface. Microscopically, the lymphoid follicles were found primarily within the submucosa. This information is of particular relevance to the study presented here, as all samples were collected endoscopically.

C. Epithelial Renewal and Maturation

The cells along the crypts undergo a process of mucosal cell renewal and maturation. The cells at the bottom of the crypts are undifferentiated and are the precursor for the three populations of cells that make up the mucosal epithelium: epithelial, goblet and enteroendocrine cells [17]. The precursor cells are cuboidal, and have the ability to proliferate and secrete water and electrolytes. The cells mature as they move up the crypt. Approximately 2/3 the way up the crypt, the cells become fully differentiated and mature, losing the ability to replicate and attaining maximum absorptive capabilities. The final fate of the mature epithelial cell, as it reaches the top of the crypt, is to be sloughed into the gut

lumen. Average turnover time for human mucosal epithelial cells (both absorptive and goblet cells) is 3-8 days [17].

Mature cells are found at the top 1/3 of the crypt. Maturity is indicated by a change of cellular shape from cubiodal to columnar. Morphometric changes that occur in the epithelial cells as they mature include increase in cell height and cell area (reflecting the change from cuboidal to columnar), increase number and height of microvilli, and increase number of intracellular organelles [11, 18, 19] (Figure 4). There are apparently more absorptive cells than goblet cells at the top of the crypt. However, this observation is somewhat misleading as most goblet cells are depleted of mucus by the time they reach the top of the crypt, thus appearing more like absorptive cells. Maturation changes that occur along the crypt were noted by Canfield in a study of endoscopic biopsies [16]. The increased numbers of cuboidal cells in the bottom of the crypts, and the increased numbers of columnar cells and decreased numbers of goblet cells at the top of the crypts, reflect the normal maturation process of colonic epithelial cells. Therefore changes in goblet cell population and absorptive cell conformation can indicate a change in maturation and proliferation. In addition, change in proliferation rate can be determined by calculating the mitotic index of the epithelial cells located at the bottom of the crypts, with an increased mitotic index reflecting an increased rate of proliferation [16]. These factors are important in the discussion of morphometric parameters used to evaluate colonic structure (See Morphometric Evaluation of Colonic Biopsies).

The series of events occurring within the colonic epithelial cells during proliferation is referred to as the cell renewal cycle [17, 20, 21, 22]. After mitosis, or M phase, the cells enters first interphase. This portion of interphase is referred to as postmitotic-presynthetic gap, or G_1 phase. During G_1 phase, RNA, enzymes, and other proteins used for nucleic acid metabolism are synthesized. Synthesis and replication of DNA is the next event in the cell renewal cycle and is called synthetic or S phase. S phase is the only phase of the cell cycle in

which DNA is synthesized. The postsynthetic-premitotic gap, or G_2 phase, follows S phase, and allows the cell to prepare for mitosis. Protein is synthesized and duplication of centrioles is completed during G_2 phase, which is also referred to as the second interphase. The cell then proceeds to mitosis, and the cell cycle is completed. During mitosis, RNA synthesis stops and protein synthesis is decreased. If G_1 phase is prolonged to the point where DNA and protein synthesis are suspended, this is referred to as G_0 phase. At the completion of G_0 or resting phase, the cell resumes activity at S phase and follows the sequence of events as described above. The length of time devoted to the different phases of the cell renewal cycle can be determined by radioisotope labeling. In humans, estimates for colonic epithelial cells are as follows: S phase, 11-15 hours; G_1 phase, 10-30 hours; and G_2 /M phase, 1 hour. This results in a 1-2 day duration for the average cell cycle of a single colonic epithelial cell [22]. Although the average time for a colonic epithelial cell to replicate is 1-2 days, this should not be confused with the average life span, which is 3-8 days [17]. Results of studies conducted to find out which cell cycle phase is affected by different nutrients are discussed below (Dietary Effects on Colonic Mucosa).

Rate of proliferation of epithelial cells along the crypt varies. The precursor cells located in the crypt base replicate at a slightly slower rate than the most rapidly proliferating cells which are located in the area just above the precusor cells [23]. From that point, cell proliferation slows progressively as the cells move up the crypt, until the top third of the crypt, where the cells lose their ability to proliferate altogether.

Although the change in rate of proliferation that occurs along the crypt is the same for all sections of the colon, cell kinetics within a particular segment vary from that of another segment. Research in rats and mice showed that the descending colon had the highest cell/crypt ratio, crypt length, mitotic index, and birthrate (i.e. the greatest number of cells reproduced over a period of time); and the longest cell cycle duration [24, 25]. Changes noted in the cell populations moving orad included a decrease in both mitotic index and cell

cycle duration. The cell/crypt ratio and crypt length also decreased progressively from the descending colon to the ascending colon, but increased at the cecum. Therefore, the descending colon has the highest cell production rate of all the sections of the colon studied. Because of regional differences in cell cycle and cell number, it is important to note the specifiic section of colon biopsied to allow for adequate comparison between studies.

Pericryptal fibroblasts and enteroendocrine cells also have specific maturation processes that occur along the crypt. It is generally accepted that the fibroblasts below the basement membrane, referred to as pericryptal, migrate along the crypts with the epithelial cells [26]. (Fibroblasts have been shown to migrate in rabbits [26], but not in rats [27].) Both fibroblasts and epithelial cells secrete collagen (Type IV by the epithelial cells), and together form the basement membrane [19]. It has not been determined if the basement membrane migrates or remains stationary. The final fate of the fibroblasts and basement membrane is also unknown, but it is speculated that they are either sloughed into the lumen with the epithelial cells or recycled into the lamina propria [11, 19, 26]. Enteroendocrine cells follow a slower pattern of migration and maturation than the absorptive and goblet cells, with an average lifespan of approximately 2-3 weeks [28]. As the enteroendocrine cells mature and reach the top of the crypt, they are eventually sloughed into the lumen.

The continual renewal process of the colonic mucosa is in some ways protective, as it allows for rapid replacement of mucosal surface when damaged. However, in some circumstances the rapid rate of proliferation predisposes the intestinal mucosa to injury. Processes such as chemotherapy, radiation therapy [11], and certain infectious organisms, such as parvovirus, [29] that have a predilection for rapidly dividing cells, may affect the colon in a deleterious manner. In particular, irradiation stops the proliferation of cells in the crypts, but does not affect the rate of sloughing. Cells are sloughed but not replaced, resulting in epithelial loss, mucosal atrophy and ulceration. Clinically this manifests as diarrhea. If enough quiescent stem cells remain, the epithelium will eventually be repopulated, and the

mucosal surface repaired. (This series of events, initial cell death and sloughing with eventual mucosal repair, occurs in patients undergoing radiation therapy, which explains the gastrointestinal signs experienced by these patients.)

D. Ultrastructural Description of Normal Canine Colonic Epithelium

The mucosal surface is lined with columnar epithelial cells which have microvilli on the luminal surface and rest on a basement membrane on the basal surface [30] (Figure 5). The basement membrane separates the epithelium from the lamina propria. The microvilli increase surface area. Microvilla in the colon are shorter than those found in the small intestine. In addition, microvilli found on the epithelial cells at the bottom of the colonic crypts are shorter, wider and fewer in number when compared to the microvilli on epithelial cells at the top of the crypts [19]. Microvilli contain no tubules and are covered by a trilaminar plasma membrane. A filamentous glycoprotein coat covers the microvilli and is referred to as the glycocalyx [31]. Unlike the glycocalyx of the small intestine, the glycocalyx of the colonic epithelium has no digestive enzymes. The glycocalyx is constantly renewed and, in humans, has a turnover rate of 16-24 hours [32]. C-Proteins are small membrane encapsulated vesicles that are interconnected by the glycocalyx. R-bodies, found at the apical surface of the immature epithelial cells, fuse with the plasma membrane, are extruded from the cells, and form the C-bodies noted in mature epithelial cells [33]. The glycocalyx is thought to provide protection to the epithelial cells, as it has been shown that proteases cause greater destruction of the luminal epithelial cells when the glycocalyx is removed. Immunocytochemistry suggests that immunoglobulins (possible IgA) are found in some of the C bodies, and therefore the glycocalyx may play a role in gut immunity [34]. Filamentous extensions from the microvilli known as rootlets are embedded into the terminal web, which is a network of filaments located in the apical cytoplasm of the epithelial cell [34]. The web filaments and rootlets are actin fibrils and are part of the cytoskeleton. These actin fibrils

may facilitate transport of absorbed nutrients into the cell (e.g. free fatty acids, electrolytes, and water), as well as provide for movement of the microvilli therefore stirring intestinal contents [34]. The terminal web functions to stabilize the surface. The actin fibrils from the microvilli that extend into the terminal web attach to meromyosin fibrils of the tight junction and intermediate junction, which are part of the junctional complex [12].

The junctional complex is made up of tight junctions, intermediate junctions and desmosomes [35] (Figure 6). The tight and intermediate junctions surround the apical margin of the epithelial cells and allow for attachment of cells to each other. Tight junctions are made up of outer leaflets from opposing cells which fuse to become a single leaflet with punctate contacts. Intermediate junctions are located just below the tight junctions [35]. Myosin fibrils are attached to the tight junctions and intermediate junctions, and are associated with actin fibrils from the terminal web [36]. Tight junctions of the colon are impermeable to fluid and electrolytes. Active transport allows movement of electrolytes into the epithelial cell against the concentration gradient [34]. Desmosomes are button-like structures distributed intermittently along the lateral surface of the cell and do not completely encircle the cells as do tight junctions and intermediate junctions [35].

Intracellular organelles found in colonic epithelial cells include a nucleus, Golgi apparatus, mitochondria, lysosomes, endoplasmic reticulum (ER), and ribosomes [10, 12] (Figure 5+7). The nucleus of the epithelial cell is located close to the base of the cell. It is separated from the cytoplasm by the nuclear membrane. Protein synthesis is directed by the nucleus via RNA. In the ER, protein, triglycerides, and lipoprotein are transported and synthesized. The Golgi apparatus consists of an integrated complex of parallel membranes, cisternae, tubules and vesicles and is usually located above and around the nucleus. Protein synthesized by the granular ER as well as substances absorbed by the cell are transferred to the Golgi apparatus, where proteins may be converted to glycoprotein (a major component of mucus). Hormones (depending on cell type) are also are produced and modified in the

ER/Golgi system. All substances processed by the Golgi are encapsulated into secretory vesicles derived from the Golgi apparatus membrane, and some are eventually exocytosed from the cells. The Golgi apparatus is especially prominent in goblet cells, where it is involved in the synthesis of mucin [12, 19]. Ribosomes, which synthesize protein, are found attached to the granular endoplasmic reticulum as well as free in the cytoplasm. Mitochondria provide energy via oxidative phosphorylation for cell metabolism and function and are more numerous in the absorptive cells [10,12, 19]. Mitochondria are located both above and below the nucleus.

Lysosomes are vesicles that store various substances used in intracellular digestion [34]. Substances found in the lysosomes are synthesized in the endoplasmic reticulum, transported to the Golgi apparatus where they are encapsulated in vesicles and released into the cytoplasm. Materials digested by lysosomes include substances both made by the cell and absorbed by the cell.

The basement membrane consists of two layers [19, 37, 38]. The basal lamina is a thin layer of Type IV collagen which is adjacent to the epithelial cells and is secreted by these cells. A thicker layer, produced by fibroblasts, consists primarily of laminin, proteoglycans, and elastic, reticular, and collagenous fibrils that blend with the surrounding connective tissue of the lamina propria [37]. The function of the basement membrane is to act as a diffusion membrane for rapid transport of ions, to provide physical support for the epithelium, and to allow for stretch and recoil of the intestines [19, 37]. This latter property is provided by Type IV collagen which has both tensile strength and flexibility. Recently, fenestrations have been found in the basal lamina of humans and rats [39]. The purpose of the fenestrations, presumably, is to allow passage of inflammatory cells from the lamina propria to the epithelial surface. Occasionally extensions from the epithelial cells have been found to protrude through the fenestrations. The perception of the morphologic significance of the basal lamina fenestrations and the protrusion of epithelial cells through the fenestration has

recently changed. At one time these changes were thought to be preneoplastic. Since these changes can be seen in normal tissue, the idea that the fenestrations are indicators of neoplasia needs to be reevaluated.

The above summary of ultrastructural anatomy describes the absorptive cells of the epithelium. Goblet cells and enteroendocrine cells differ somewhat from the absorptive cells. Goblet cells follow the same maturation and migration pattern as the absorptive cells [39, 37]. The cytoplasm at the base of these cells is dense, and the cell proper, or theca, is swollen, being distended by mucin [19]. Most of the intracellular organelles are similar to those found in the absorptive cells [40]. Key features of this cell type are the elaborate supranuclear Golgi apparatus, the presence of mucin, and the short irregularly spaced microvilli. As the goblet cells distend with mucin, the microvilli become decreased in number [19] (Figure 7).

Mucin, a sulfated glycoprotein, is synthesized in the granular ER; is glycosylated, sulfated and packaged in the Golgi apparatus; and released from the Golgi into the basal portion of the cell [19, 40]. Mucin moves slowly up to the apical surface of the cell and is secreted into the gut lumen by exocytosis. Mucin production and secretion is a continual process which begins in the cells located in the mid portion of the crypt [19]. Rate of secretion increases in the cells at the luminal surface and eventually exceeds the rate of production. Therefore most of the goblet cells at the top of the crypt are depleted of mucin. Parasympathetic input increases the rate of secretion in goblet cells in the lower crypts, but cells in the upper portion of the crypts are unaffected [41]. Mucin secretion is also increased by chemical and physical irritation . Mucin is secreted as a viscoelastic gel and is one of the components of mucus, which is the free slime that covers the gut surface and protects the epithelium from caustic substances and physical trauma [40, 42]. (Other components of mucus include sloughed epithelial cells, salts, and leukocytes.) Mucin is also a source of energy for enteric bacteria [40].

Enteroendocrine cells are dispersed among the epithelial cells and are decreased in number in the colon [40]. They can be differentiated from absorptive cells by the presents of secretory granules within the cytoplasm [11]. They are not always exposed to the gut lumen, but have a broad base at the basal lamina. The enteroendocrine cells that do contact the lumen have a small number of microvilli resembling a tuft on the apical surface. The substances secreted by human colonic enteroendocrine cells include vasoactive intestinal peptide, somatostatin, enteroglucagon, neurotensin, substance P, bombesin, and enkephalin [43]. These are collectively known as regulatory peptides and are not secreted into the lumen. Regulatory peptides are hormones that either act distally (i.e. endocrine) or locally (i.e. paracrine, via diffusion across the intracellular space). They are also produced by the noncholinergic neurons of the enteric intrinsic nervous system and function as neurotransmitters [44]. Each regulatory peptide has a specific function, acting to either stimulate or inhibit colonic functions.

E. Normal Colonic Motility [7,45,46, 47]

Colonic motility is affected by diet and disease, therefore normal motility will be discussed briefly. The four types of motility in the colon are segmentation, peristalsis, retropulsion, and intense propulsive movement referred to as mass movement. All of these are related to slow wave activity except for mass movement or giant migratory contractions. Slow wave activity is the intrinsic electrical activity that exists in smooth muscle, independent of innnervation. In the colon, slow wave activity occurs at about 5cycles/min. This does not mean that there are 5 contractions of colonic smooth muscle per minute, because slow waves alone are not capable of stimulating contraction. In order for the colonic smooth muscle to contract, a combination of slow wave activity along with spike potentials from the intrinsic nervous system is needed. The intrinsic nervous system, which includes the colonic neural plexuses, secretes cholinergic (acetylcholine) and noncholinergic (VIP, serotonin, etc.) neuroregulatory peptides. The cholinergic peptides are stimulatory

and the noncholinergic peptides are inhibitory. The effect of the stimulatory peptides is to elevate the baseline migratory potential, thus sensitizing or priming the smooth muscle. When slow wave activity occurs, the primed smooth muscle should then reach threshold, resulting in an action potential and muscle contraction. Because the smooth muscle of the gut acts as a syncytium, this results in a generalized contraction of that segment of colon. The effects of the intrinsic nervous system are affected by the extrinsic nervous system (parasympathetic input is stimulating, sympathetic input is inhibiting), regulatory peptides, and intraluminal contents. Segmentation and peristalsis occur by this mechanism.

Retropulsion also occurs by slow wave activity. However, the direction of these slow waves is oral not aboral. It appears that these particular slow waves are under the control of pacemakers, areas of intrinsic nervous activity, located in the mid portion of the gut [45]. Retropulsive waves move the colonic contents orally and can be found from the transverse colon to the cecum.

Giant migratory contractions, or intense propulsion, is the result of migratory spike bursts. Migratory spike bursts are not related to slow waves and have also been referred to as long spike bursts. Migratory spike bursts orginate in the mid colon, migrate in an aborad direction, leading to defecation, and appear to affect the circular muscle layer of the muscularis externa. Pacemakers that evoke retropulsive activity also elicit propulsive activity, although these two events do not always occur together [45]. Retropulsive activity and mass movement are also modified by the extrinsic nervous system and regulatory peptides.

Peristalsis occurs throughout the length of the colon, and moves fecal content aborally, a short distance at a time. Segmentation and retropulsion occur in the ascending and transverse colon, where maximum absorption of electrolytes and water occurs. Segmentation slows the aboral movement of colonic contents and allows for mixing. Retropulsion inhibits the aboral movement of colonic contents. Together, these two forms

of motility provide resistance to movement and allow for increased absorption of electrolytes, water, etc. The primary function of the proximal colon is therefore enhanced by the type of motility that occurs there.

The distal colon, however, is responsible for fecal storage and defecation. Motility found in this segment of the colon consists of segmentation, providing resistance to flow, and mass movement, which causes the entire contents of the colon to move distally, preparing for defecation. During giant migratory contractions, segmentation stops in the area of the colon receiving the contents.

At one time, hypermotility of the colon was thought to be responsible for diarrhea. It is apparent however, from this discussion, that hypomotility of the proximal colon can lead to diarrhea [7]. If the proximal colon is inhibited from contracting, segmentation and retropulsion stop. Since these forms of motility provide resistance to movement, fecal transit time decreases. In addition, there is decreased absorption, which would increase fecal volume. These factors are important when considering the cause and treatment of large bowel diarrhea and the effects disease and diet have on colonic motility.

F. Dietary Effects on Colonic Mucosa

Response of the colon to the presence of food can best be described by studies that examine the effect of fasting on colonic mucosa. Mice fasted for 24, 48 and 72 hours showed decreased proliferation along the colonic crypts but not a decreased number of epithelial cells [48]. With refeeding, there was a rapid burst of proliferation and increased number of crypt cells. The effects of starvation and refeeding on colonic proliferation occurred within 24 hours and were thought to be due to the physical presence of food, nutrients provided by the food, changes in motility, or regulatory peptides. One synthetic hormone found to have a trophic effect on the colon was pentagastrin [49]. Pentagastrin is an exogenous form of gastrin, a regulatory peptide also shown to have a trophic effect on the

colon [50]. Rats that were fasted for 64 hours and then given pentagastrin showed increased proliferation (H^3-thymidine labeling)of colonic epithelial cells, an effect that was not seen in the non-fasted rats that were given pentagastrin.

Attempts have been made to correlate the changes in cell proliferation with cell cycle kinetics. One study showed that the G_1 phase of the cell renewal cycle was prolonged during starvation [48]. Refeeding induced colonic epithelial proliferation which was related to an accelerated progression from G_1 to S phase and a shortening of the G_1 phase .

In order to determine if the effects of starvation and refeeding were due to regulatory peptides or the physical presence of food, rats were fed normal diets, fed parenteral fluid orally, or fed parenterally [51]. DNA synthesis, colonic weights and gastrin levels were measured. The rats fed parenterally or fed the parenteral fluid orally had significantly decreased gastrin levels, colonic weights, and DNA synthesis when compared to the rats fed the normal diet. Pentagastrin was then given to some of the animals receiving the liquid diet, and non-nutritive bulk (cellulose) was added to the diet of others. The animals receiving pentagastrin had increased gastrin levels. Both of these groups had increased colonic weights and DNA synthesis when compared to rats not supplemented with pentagastrin or non-nutritive bulk, but the difference was substantially greater for the rats receiving the non-nutritive bulk. The authors concluded that a combination of the physical presence of food and the release of regulatory hormones is responsible for the effects that food has on the colon. However it appears from these results that the physical presence of food may have a more dramatic effect.

Regulatory peptides have been suggested as being moderators of colonic proliferation after surgical resection of the small bowel [52, 53]. Extensive small intestinal resection in rats not only resulted in proliferation of the small intestine but also was associated with hypertrophy and hyperplasia of the colonic mucosa. The response in the large intestine was greater than that seen in the small bowel. The changes in the colon occurred within 2 days

to 1 week, and the effects were presumably due to unidentified regulatory peptides, although the effects of intraluminal contents (i.e. "food bathing") could not be excluded.

Attempts to determine the effects of specific nutrients on colonic mucosa have resulted in several studies. Strang and Hageman [54] looked at the effects of specific diets on colonic proliferation and found that diets with sugar, protein and mineral salts induced hyperproliferation after starvation. Diets lacking these components did not . Lack of mineral salts appeared to have the most effect and it was proposed that mineral salts are needed to induce a transition from G_1 to M phase.

Dobesh et al [55] found that dogs fed meat-based protein diets had decreased colonic mucosal surface area when compared to dogs fed a cereal-based protein diet. However these diets were not evaluated for nutrient content. Pigs that were fed a low protein diet had significantly higher surface area when compared to a group of pigs fed a high protein diet [56]. These studies indicate that the concentration of protein, and perhaps the source of protein, have a significant effect on the colonic mucosa. The authors concluded that the change in surface area may be directly related to absorption, i. e. an increase in surface area indicates an increase in absorption, and vice-versa. Since protein is not absorbed in significant amounts by the colon, perhaps the effects of the different diets on the colon were due to the intraluminal effect of food, or the result of regulatory peptides. Dobesh's group also found that in dogs, dietary induced changes in colonic mucosa were apparent by day 3, and completed by day 10-14 [55].

Dietary fiber is currently an active area of study because of its association with colonic cancer in humans. An association between high fat/low fiber diet and colonic cancer was first proposed by Birkitt and Trowell [57]. Since that time intensive research has been conducted to identify factors that explain this effect. The effect of fiber on the colon is of particular interest in veterinary medicine, as high fiber diets are often used to treat large bowel diarrhea in both dogs and cats [2, 58].

The effect of fiber on the colon depends, in part, on the fiber type [59]. Fiber can be classified into soluble or insoluble or classified based on water holding capacity. Sources of soluble fiber include fruits and vegetables, psyllium, legumes, oat and oat bran. Soluble fiber has a high capacity for holding water and is digested anerobically by colonic bacteria into three short chain fatty acids (SCFA): acetate, butyrate and propionate [60]. Once soluble fiber is digested, compounds bound to it are released, including bile acids, minerals, and water [61].

The effect of soluble fiber on the colon is thought to be due to the production of short chain fatty acids, release of bile acids, and increased numbers of bacteria. As a source of energy for colonic bacteria, short chain fatty acids increase bacterial numbers. Increased in bacterial numbers result in increased colonic bulk as bacteria make up > 50% of fecal dry matter [62]. As will be discussed later, this can have an effect on colonic motility. One of the short chain fatty acids, butyrate, serves as an energy source for colonic epithelial cells as well as colonic bacteria [6]. Short chain fatty acids have been shown to have a trophic effect on the colonic epithelium [63]. Proposed mechanisms for the trophic effect of short chain fatty acids include: 1) providing energy for the epithelial cells; 2) possibly stimulating release of regulatory peptide; or 3) possibly stimulating neurologic input. Short chain fatty acids also cause a decrease in lumen pH which may have a protective effect against bile acid-induced injury [64]. Studies have shown that elevated lumen pH has been associated with increased incidence of colonic cancer presumably because of bile acid effects. At increased luminal pH, bile acids are protonated, and then bound to calcium and other salts, which eliminates their toxic effect on the colon.

Short chain fatty acids may enhance absorption of sodium by monoionic diffusion, and are associated with the secretion of bicarbonate ion [7, 65, 66]. It has also been suggested that short chain fatty acids provide a source of nutrition for patients with malabsorption syndromes and malnourishment related to IBD [67]. It has been recently suggested that defects in short chain fatty acid absorption may be a cause of ulcerative colitis [65]. One study

found that human beings with this disorder had decreased absorption of butyrate. Whether this was the cause or effect of the disease is not known.

Soluble fiber may have a deleterious effect on the colon by causing an increase in the number of bacteria that metabolize and deconjugate bile acids [68]. Certain bacterial enzymes, including 7-alpha hydroxylase, convert primary bile acids to secondary bile acids. Secondary bile acids have been implicated as potential carcinogenic promoters and have been found to increase the rate of proliferation along the colonic crypts. A study showed that rats fed high soluble fiber diets had an increased incidence of distortion and damage to the epithelial cells and loss of microvilli when compared to rats fed diets high in insoluble fiber [69]. Changes were presumably related to the increased binding of bile acids to soluble fiber and subsequent release by colonic bacteria when compared to insoluble fiber.

Insoluble fiber is not broken down by colonic bacteria and has a low capacity for holding water [57]. However, because this type of fiber is not fermented by bacteria, it has fecal water retention equal to or greater than that of soluble fiber [61]. Insoluble fiber sources include cereal grain, seed hulls and cellulose. A protective effect of insoluble fiber against colon cancer in rats and humans has been shown [64]. Insoluble fiber is thought to have a dilutional effect, especially important when toxic substances are in the colon such as ammonia from colonic bacterial digestion of protein [70].

Another important aspect of diet on colonic function is the effect food has on colonic motility. The gastrocolic reflex is stimulated by high caloric intake and a high fat meal. However a high protein meal decreases or eliminates this response [71]. Proximal colon activity is increased due to the gastrocolic reflex. Since retropulsion and segmentation are the forms of motility in this segment of colon, an increase in activity does not cause an increase in fecal movement but results in increased absorption of water and electrolytes.

Studies measuring effects of low and high fiber diets (using bran as a fiber source) on colonic motility in dogs and pigs found that the low-fiber-diet group had increased

segmental motility in the proximal colon, whereas the high-fiber-diet group had increased propulsive motility in the distal colon [72, 73]. This would indicate that the high-fiber group had decreased transit time. Electromyographic studies of the proximal colon indicate that insoluble fiber (alpha-cellulose) decreased duration of spike activity short bursts, but had no effect on slow wave activity [74]. Further studies showed that high levels of alpha-cellulose decreased transit time [75]. Insoluble fiber may alter colonic motility by increasing fecal bulk [57, 62]. Because insoluble fiber is not digested by canine colonic bacteria, the resulting increase in luminal contents and distension acts as a stimulus for colonic motility. In this manner, insoluble fiber may help restore normal colonic segmentation. A final note: the effect of insoluble fiber depends upon the size of the fiber particles. For example, finely ground bran does not increase fecal bulk, but coarse bran and insoluble fiber high in lignin do have the ability to increase fecal bulk [76].

Soluble fiber has also been shown to increase motility of the colon [65]. The increase in motility seen with soluble fiber may in part be due to the release of bile acids by colonic bacteria. Bile acids act as secretogogues which stimulate activity. Soluble fiber also increases fecal nitrogen content. The source of increased fecal nitrogen is the colonic bacteria, which use intraluminal ammonia for protein synthesis and reproduction [66]. Increased fecal nitrogen reflects increased colonic bacterial growth causing an increase in fecal bulk, which results in decreased transit time [65].

Unanswered questions remain regarding the effects of fiber on the colon. Soluble fiber has been shown to be both protective and potentially carcinogenic [62, 65, 66, 68, 69]. Conflicting information on this subject may arise either from the species of animal being studied, from the source of fiber being used, from the different concentration of fiber used in the test diets or from a combination of these factors. However, certain conclusions can be made. Fiber does appear to have an effect on colonic structure and function. The effects of soluble fiber are most likely due to changes in bacterial population, bacterial enzyme

concentrations, bile acid concentration and metabolism, and changes in colonic motility [62, 65, 66]. Insoluble fiber has protective effects on the colon and modifies colonic motility [61,62, 70].

Diet has a major effect on the colon, even though the carnivore colon does not absorb substantial amounts of nutrients. The results of starvation and refeeding studies as well as intestinal resection studies suggest that both the physical presence of food as well as the release of regulatory peptides are responsible for these effects [48, 49, 50, 51, 52, 53]. Dietary protein, dietary fiber and other dietary components have also been shown to affect colonic structure and function [54, 55, 56, 57, 62, 66].

G. Pathogenesis of Inflammatory Bowel Disease

Dietary management is often the first therapeutic modality used for treatment of Inflammatory Bowel Disease (IBD) of the colon in dogs [77, 78]. Because of this, and the fact that we are interested in the specific effects of diet on the colon, a review of the pathogenesis of IBD is in order.

Diseases that are commonly grouped under the general term canine inflammatory bowel disease of the colon include lymphocytic plasmacytic colitis, eosinophilic colitis and histiocytic colitis [79, 80]. Inflammatory bowel disease of the colon or colitis manifests clinically as diarrhea characterized by tenesmus, hematachezia, and excess mucus production. Affected animals often defecate more frequently, but produce decreased amounts with each bowel movement; less often is vomiting and weight loss associated with colitis [81]. Diarrhea associated with colitis is due to both a change in electrolyte and water absorption and defects in motility, which are usually secondary to inflammation [78]. Changes in mucosal permeability caused by changes in the electrical potential across the absorptive cell plasma membrane lead to decreased absorption. Tight junctions are important in maintaining normal mucosal permeability [31]. Tight junctions of the colon are impermeable to fluid and electrolytes and active transport allows movement against the concentration

gradient into the epithelial cell. Factors that disrupt the tight junctions or interfere with the active transport mechanism will obviously affect mucosal permeability. The end result is increased net secretion and decreased net absorption of sodium, chloride and water [78].

Reversed peristalsis and segmentation are the normal motility patterns in the proximal colon and result in increased transit time of fecal material through the colon. This allows for the increased absorption of water and electrolytes. The distal colon acts as a reservoir for feces and controls defecation. In colitis, reverse peristalsis and segmentation are reduced and the proximal colon is hypomotile, leading to decreased resistance to flow, decreased transit time, and decreased absorption [78]. In addition, the distal colon can be hypermotile, due to effects of the products of inflammation. This may result in increased stimulation of the defecation reflex which is often abnormally stimulated when the colon is empty [81]. These changes explain some of the clinical signs of colitis, i. e. increased frequency of defecation, decreased fecal amounts, and tenesmus.

Other clinical signs can be explained by microscopic changes noted in the colonic mucosa in IBD. Breakdown of the mucosal barrier results in ulceration and increased friability [80]. Engorgement of mucosal blood vessels, ulceration and increased friability of the mucosal epithelium result in frank blood, or hematachezia, often noted with large bowel diarrhea. Ulceration can also be a result of increased destruction without a compensatory increase in proliferation of epithelial cells. Because mucus is the major secretory component of the colon, excess fecal mucus is often noted in colitis due to excessive mucus secretion [81].

Canine inflammatory bowel disease is thought to occur because of a defect in the immune response and/or a breakdown of the mucosal barrier and alterations in permeability [79]. Possible causes that have been implicated, but not yet proven to be directly related, include dietary allergens, genetic predisposition, parasites, defects in the immune system, and bacteria. A change in diet sometimes relieves the signs of colitis, therefore implicating diet

as a potential cause [1, 2, 77]. Certain types of IBD have been shown to have a genetic predilection by affecting specific breeds; an example is histiocytic colitis in boxers [82, 83].

Several intestinal parasites have been associated with IBD. Visceral larval migrans has been associated with eosinophilic colitis in German Shepherds experimentally, although it has not been reported in clinical cases [84, 85]. Other potential infectious causes of eosinophilic enterocolitis include *Trichuris spp.* and *Ancylostoma sp.* [86]. *Giardia sp.* and *Trichruis sp.* have been associated with lymphocytic plasmacytic colitis [87].

A coccoid to coccobacillus organism resembling *Chlamydia sp.* has been associated with histiocytic colitis in boxers [4]. However, it is more likely that this association is opportunistic, and that *Chlamydia* does not cause histiocytic colitis. A positive response to antibiotic therapy suggests that other unidentified infectious agents exist. However, response to antibiotics is most likely an indication of change in the colonic bacterial population [77, 80]. Response to antibiotic therapy may indicate control of a condition that is secondary to IBD, but may not have any effect on the primary underlying cause.

Normal gastrointestinal flora may contribute to IBD because of changes in the immune system, changes in bacterial numbers and changes in bacterial metabolism. Normal colonic bacteria are usually present in numbers of 10^{10} and 10^{11} per gram [88]. The colon has the highest bacterial population of any segment of the intestinal tract and contains anaerobic as well as aerobic bacteria. The anaerobic bacteria are approximately 1000 times more numerous in actual numbers than aerobic bacteria. Common bacteria include *Enterobacter sp., Klebsiella sp., Bacteroides sp., Staphylococcus sp., Streptococcus sp., Lactobacillus sp., Corynebacterium sp., Bacillus sp., and Clostridium sp.*. Yeasts are also found in large numbers. Although bacterial populations are different, depending upon the section of intestine, each area maintains the numbers and distribution of its bacterial population at a steady state. Factors influencing maintenance of the bacterial populations include gut motility, gastric and bile acid production, interaction between bacteria, luminal pH, the

mucosal barrier, and diet. Changes that disrupt any of these factors can disturb the bacterial population balance, resulting in bacterial overgrowth of the small intestine or colon. Factors that have the greatest impact on colonic bacterial homeostasis are bacterial interactions and administration of antibiotics [89]. Defects in the mucosal barrier can allow bacteria exposure to the epithelial surface, which can then stimulate an inflammatory response. Motility and diet, which are important factors in maintaining the normal bacterial population in the small intestine, are less influential in the colon, but still can have a marked effect. Bacterial effects implicated in IBD include stimulating peristalsis, inducing an increase in the thickness of the mucosal surface, accelerating transit time of epithelial cells along the crypts (therefore affecting cell renewal), and increasing the lymphocyte population [89]. Bacteria may also contribute to IBD indirectly, by converting primary bile acids to secondary bile acids, and altering colonic mucosa as previously discussed [68, 69].

Changes in mucosal lymphocyte population in patients with IBD suggest a defect in the immune system, or possibly an immune mediated response. In humans with IBD, the mucosal B- and T- lymphocyte populations are increased [90]. However, the B-lymphocytes are predominant, with an increased number of IgG-producing B-cells when compared to the normal population. The normal population of B-cells in the mucosal epithelium is primarily IgA-producing. In IBD, IgA-producing B-Cells still predominate, but there is a greater percentage of IgG-producing B-cells [90]. The T-lymphocyte population in patients with IBD, although increased, has a normal distribution of T-cell types [91]. Evaluation of circulating and intraluminal antibodies shows elevated serum IgA levels, but lumen concentration of IgA is decreased [91]. Whether the changes in mucosal B-cell and T-cell population, and IgA levels are due to an immune defect or an immune response is not known.

Specific serum immunoglobulin concentrations have not been evaluated in dogs with IBD. However, a recent study evaluated the plasma cell population in normal dogs [92]. This study identified IgA- producing cells as the primary type of plasma cell in the lamina

propria, IgG-producing cells as the next most common cell type, and IgM-producing as the least frequently noted cell type. The results of this study provide a basis by which other studies evaluating lymphocyte populations in the colon of dogs with IBD can be compared. It is interesting that the distribution of B-cells in normal dogs differs from that of normal humans, where IgM-producing plasma cells are normally found in higher concentrations than IgG-producing plasma cells [93]. However, the ratio of IgM-producing cells to IgG-producing cells in humans changes within the different segments of the gastrointestinal tract and becomes more equal in the distal portions of the colon.

Hypersensitivity reactions, as a form of an abnormal immunologic response, have been associated with different forms of IBD [94]. The Type I (immediate, IgE mediated) hypersensitivity has been proposed as a mechanism of action for eosinophilic gastroenteritis and colitis. Allergens implicated in this reaction include dietary proteins and other food allergens, parasites and bacteria. Because Type I hypersensitivity reactions are immediate, antigens would have to be continuously present in order to incite a chronic reaction [80]. Both Type II (cytotoxic) and Type III (immune complex) reactions have been associated with lymphocytic plasmacytic enterocolitis in dogs [80]. Type IV (delayed) hypersensitivity, may be involved with granulomatous enteritis and inflammatory bowel disease [80].

Finally, increased mucosal permeability has been identified as a possible cause of IBD [94]. Changes in permeability may be due to defects in the mucosal barrier. Defects in the permeability/mucosal barrier allow luminal antigens chronic access to the lamina propria. Chronic antigenic stimulation of the mucosal immune system may elicit the inflammatory response noted in IBD. In humans, defects in the mucosal barrier and permeability are thought to be genetic, as healthy relatives of patients with inflammatory bowel disease also have permeability defects [94]. This information supports the theory that the changes in permeability may be primary and not secondary.

H. Food as an Antigen

Because food hypersensitivity has been implicated as a potential cause of IBD, the role of food as an antigen must be considered. Adverse reactions to food can be classified into three general categories, 1) food allergy, or hypersensitivity, which is immune mediated; 2) food intolerance and 3) dietary indiscretion [95]. Neither of the last two are immune mediated, and only food allergy will be discussed here.

The antigenic effects of food in humans have been well documented and are typically associated with certain proteins and glycoproteins. In particular, proteins between 10,000 and 70,000 daltons have been identified as having strong antigenic capabilities [96]. The antigenic properties of proteins can be eliminated by denaturation, which can be accomplished either by heat or by digestion [97]. However, most antigenic food proteins are heat resistant, and/or are not easily digested. It also appears that partial digestion can increase the antigenicity of food by revealing hidden antigenic determinants [97].

Foods identified as being antigenic in the dog include soybean protein, beef, cow's milk, and wheat gluten [98]. Other foods suspected of being antigenic are chicken, pork, eggs, horsemeat, corn meal, oats, and yeast [99]. Food groups, such as seafood, usually show cross reactivity, i. e. sensitivity to one substance in a food group usually means sensitivity to all items of the food group [97]. This phenomenon is appreciated more in humans than animals .

The role of food additives in food allergy is controversial. Most additives are small, nonantigenic molecules [98]. However, anitigenicity can be acquired by binding to carrier proteins. The extent to which this happens has not been fully investigated, but some believe that the inability of some commercial hypoallergenic diets to alleviate clinical signs of food hypersensitivity may, in part, be due to food additives. However, additives are more likely to be associated with food intolerance than true food allergy [95].

The pathophysiology of food hypersensitivity is regulated by the immune system. In order to understand how food can adversely affect the body, a review of the gastrointestinal immune system and how food is normally tolerated is necessary. Because tolerance of food and bacteria have similar mechanisms, and since colonic bacteria are suspected of contributing to the inflammatory process noted in IBD, tolerance of bacteria will also be discussed.

Antigens elude the immune system by being present in too high a concentration or too low a concentration, by presenting incorrectly to the immunocyte, or by activating T-suppressor cell receptors [100]. (T-suppressor cell activity is one of the mechanisms of tolerance.) If the immune system responds to antigens, the results are either tolerance, immunity or hypersensitivity [100, 101]. Oral tolerance of antigens presented to the gastrointestinal tract is usually mounted against antigens that are constantly present, i.e. bacteria and commonly ingested proteins [102]. Tolerance, also referred to as unresponsiveness, is an active attempt by the immune system not to mount an inflammatory response to an antigen. Tolerance prevents chronic inflammatory response to persistent, non-harmful antigens, therefore avoiding deleterious effects. Tolerance is a local as well as a systemic phenomenon. Antigens that are tolerated orally will also be tolerated if systemic exposure occurs. A classic example of oral tolerance is seen in the American Indians of the Southwest who feed their children leaves from the poison ivy plant in an effort to prevent contact dermatitis when exposed to the plant later in life [103].

The first step in oral tolerance is preventing exposure of the antigen to the surface epithelium and lamina propria [100, 102, 104]. This is a non-specific immune response and its success is dependent upon having normal gut digestive processes (gastric secretions-acid and pepsin, and intestinal secretions-lysozymes, lactoferrin, lactoperoxidase and bile salts), normal gut motility, normal gut bacteria, and an intact mucosal barrier (epithelial cell renewal, epithelial cell tight junctions, selective permeability, and mucous secretions).

Complete digestion of food by intestinal and bacterial enzymes will eliminate antigenicity. Bile acids, lysozymes, lactoferrin, and interferon inactivate or destroy bacteria. Peristalsis physically eliminates food and bacteria from the gut lumen, thus preventing contact with the mucosal surface, or at least reducing exposure time. Normal intestinal microflora competitively inhibit or otherwise prevent colonization by pathogenic bacteria. The combination of mucous secretion, selective permeability, and rapid turnover rate of the epithelial cells provides an effective barrier against most food antigens and gut pathogens. Antigens that are absorbed from the gastrointestinal lumen are nonspecifically eliminated by Kupffer cells of the liver.

Approximately 2% of the dietary antigens escape digestion and the mucosal barrier and are absorbed into the lamina propria [102]. When antigens do pass the mucosal barrier, they are eliminated by gut associated lymphoid tissue (GALT), a specific immune response [100, 102]. Components of GALT include lymphoid follicles, lymphocytes of the lamina propria, intraepithelial lymphocytes and mesenteric lymph nodes. B-cells are primarily found in the germinal centers of lymphoid follicles, T-cells are found in the periphery. T-helper cells and B-cells as well as neutrophils, macrophages and mast cells are found in the lamina propria . The intraepithelial lymphocytes are primarily T-suppressor cells.

IgA is the primary antibody secreted by the GALT [102, 105]. B-cells found in the GALT originally secrete IgM when exposed to an antigen, IgM being the immediate antibody response. However, the GALT is unique as these cells rapidly switch to IgA secretion. B-cells in other lymphoid tissues routinely switch from IgM to IgG with chronic exposure or long term immunity. Lymphoblasts from the bone marrow that are predetermined to secrete IgA antibodies migrate to the GALT, and other mucosal surfaces, early in life. These lymphoblasts appear to have a predilection for the mucosa, as this is where the majority of these cells are found. The factors that influence the preferential migration of IgA lymphoblasts to the mucosal lymphoid tissues are unknown. However IgA

is particularly effective in binding and eliminating gut lumen antigens, and unlike IgM and IgG, is not readily digested by proteases [102, 106]. In addition, IgA/antigen complexes do not stimulate complement, an important factor in tolerance [100]. Another theory that may explain the predominance of IgA in the mucosal immune system is the presence of specific T-cells that preferentially switch plasma cells from IgM production to IgA production [107].

Gastrointestinal IgA is secreted by the plasma cells in the lamina propria and lymphoid follicles primarily as a dimer, and differs from glandular IgA, which is usually secreted as a monomer [102, 103, 104]. Once secreted, IgA binds, via the J chain, to the secretory component, a glycoprotein located on the basal side of the epithelial cell. The complex is taken into the epithelial cell, encapsulated in plasma membrane, carried across the cell and released into the lumen. The significance of the J chain is that it is only found on IgA and IgM antibodies, which are the primary antibodies found in the gut lumen. The combination of J chain and secretory component allows IgA to resist digestive enzymes [103, 107]. Secretory IgA then binds with the antigen in the gut lumen, preventing absorption into the lamina propria. If the antigen is able to escape elimination by intraluminal antibody, then binding with IgA occurs in the lamina propria without stimulating complement. The antigen/antibody complex then enters the portal circulation and is eliminated by the mononuclear phagocyte system of the liver, or excreted in the bile [102].

T-suppressor cell activation is one of the primary factors of tolerance, and is also part of the specific immune response [100, 101, 102]. In order to suppress antigenic stimulation by a food antigen, the T-cells inhibit B-cells from producing antibodies. T-suppressor cells secrete substances that are chemotactic for other T-suppressor cells and inhibitory to both T-helper cells and B-cells. T-suppressor cells in the lamina propria, but primarily in the lymphoid follicles, are activated by antigen that passes the mucosal barrier. These activated T-suppressor cells then migrate to the mesenteric lymph node and spleen, and inhibit IgG antibody production, thereby initiating systemic tolerance. Other factors of the specific

immune system thought to play a part in tolerance include B-cell clonal inhibition, antibody negative feedback on antibody production, and anti-antibody immunoglubulins [102]. Circulating antigen-antibody immune complexes, in particular antigen/IgA, also have a negative feedback on the immune system. Suppressor B-cells have also been identified [13].

The above information appears to be somewhat contradictory, as food antigens appear to both stimulate and suppress the immune system in order to achieve tolerance. Indeed any antigen can stimulate both T-helper cells and T-suppressor cells, the outcome depending upon which predominates [100]. However in the case of food tolerance, both inhibitory and stimulatory factors of the immune system appear to be equally activated. The recent discovery that T-helper cells preferentially stimulate IgA, and T-suppressor cells preferentially inhibit IgG, helps to explain this apparent paradox [100]. Since T-helper cells and IgA predominate in the lamina propria, and IgA secretion at this level is an important step in oral tolerance, this combination of cells, antibody and location is beneficial . Since T-suppressor activity has been identified which preferentially inhibits IgG production, and IgG is found systemically, suppression at this level is beneficial. This explains how an antigen can stimulate an immune response locally and suppress it systemically, resulting in tolerance.

Partial tolerance is mounted against gut bacteria [100]. Animals that have been raised in a germ free environment have been noted to have smaller numbers of plasma cells within the lamina propria. Once bacteria are introduced to the gut lumen, an increase in the lamina propria lymphocyte population is noted. Although the degree of increase is within the limits of normal, the fact that the lymphocyte population increases indicates a partial immune response. The factors thought to be responsible for maintaining this response are nonspecific T-suppressor cells and/or nonspefic suppressor macrophages [100].

When the immune system is working effectively, food and bacteria are tolerated in the gastrointestinal tract through a combination of nonspecific and specific immune responses. Defects in the immune system have been implicated as the underlying cause for

inflammatory bowel disease associated with food hypersensitivity [100, 107]. Hypersensitivity to a food antigen eliminates the antigen via an inflammatory response and the alternate complement pathway. In humans a genetic predisposition to food allergy has been shown [96]. The exact cause of food allergies in both humans and animals is uknown, but is thought to be associated with an incompetent immune system or with a breakdown of the mucosal barrier. Non-food antigens may cause disruption of the mucosal barrier, leading to increased permability. Increased permeability then leads to increased uptake of food antigens which results in hypersensitivity. People with IgA and T- suppressor deficiencies have an increased incidence of food allergies [96]. Defects in the tight junctions, or enteric infections that cause disruptions of the gastrointestinal mucosa (especially in infants) have been associated with food allergies, indicating the importance of an intact mucosal barrier [96]. Portosystemic shunts have also been linked to food hypersensitivity [13]. The theory behind this association is that lack of portal circulation to the liver eliminates a very important arm of tolerance, i.e. antibody/antigen processing by the mononuclear phagocyte system and elimination of antigen-antibody complexes in the bile.

Food hypersensitivity in humans has been shown to be a Type I, or immediate response, mediated through IgE [108, 109] Since IgE-producing plasma cells have not been identified in the lamina propria of normal humans or animals, plasma cells in the mesenteric lymph nodes are the most likely source of IgE [91]. Once IgE is produced, it binds to mast cells in the lamina propria. When antigen complexes with the primed mast cells, histamines, prostaglandins, leukotrienes and proteases are released. The cascade of events that follows results in inflammation. Inflammation causes mucosal permeability to increase, which results in leakage of protein and plasma into the extravascular space (i.e. edema), and increases exposure to antigens. Inflammation also affects the motility of the gut, as has been previously discussed. Clinically these changes manifest as vomiting and diarrhea. If IgE binds to mast cells in the skin, urticaria, pruritus, and erythema are the clinical signs, and

these are the most common presentations of canine food allergy [99]. Because eosinophils are part of the inflammatory reaction of Type I hypersensitivity, food allergies are suspected as being a cause of canine eosinophilic inflammatory bowel disease.

Type III and Type IV hypersensitivities are also thought to be associated with various food hypersensitivities in humans [96, 108, 109]. Type III reactions are associated with IgE and IgG accumulating in the lamina propria and stimulating the complement cascade when combined with antigen. Complement factors C3a and C5a are released, resulting in an inflammatory reaction. This is in contrast to IgA, which when complexed to an antigen does not activate complement and is removed by the liver [13]. Type III hypersensitivity can cause clinical signs immediately, resembling Type I hypersensitivity, or clinical signs may be delayed for hours. It is therefore suggested that Type III hypersensitivity does occur in food allergy, because the various manifestations of this disorder correlate with the various manifestations of food allergy. Type IV or delayed hypersensitivity involves T-lymphocytes that are sensitized to antigen. When bound to antigen, the lymphocytes release lymphokines and other mediators of inflammation. The typical Type IV graft versus host reaction in the gut results in villus atrophy and crypt hyperplasia, and is thought to occur in food-sensitive enteropathies, as similar changes are noted with both Type IV hypersenstivity and food allergy [109].

Although not documented, Type I, III and IV hypersensitivity reactions are thought to occur in canine food allergy [98, 110, 111]. Food hypersensitivity in the dog usually manifests with dermatologic signs and, as such, has not been associated with a genetic predisposition, and is not prevalent in any particular breed, age or sex group [98, 112]. Only 10-15% of dogs with food hypersensitivity show signs of gastrointestinal involvement such as vomiting and diarrhea. Food hypersensitivity has been linked to lymphocytic-plasmacytic colitis and eosinophilic gastroenteritis in dogs [80].

A technique to evaluate food hypersensitivy in the gastrointestinal tract of dogs has recently been developed. Gastroscopic food sensitivity testing (GFST) involves endoscopic topical administration of food extracts onto the mucosa of the body of the stomach [113]. A positive reaction is identified by mucosal swelling grossly and congestion and edema histologically. This technique was used to identify sensitivity to food allergens in dogs with either eosinophilic or lymphocytic/plasmacytic enteritis [114]. Diets were formulated from food antigens that did not elicit an reaction. Response to dietary therapy based on GFST was initially good, but failure was noted over time, in some cases, indicating possible allergic response to the new diets.

I. Dietary Management of Canine IBD

A review of dietary management of IBD in humans indicates the need to provide required nutrients in an easily assimilated form [115]. Human patients with inflammatory bowel disease are often malnourished because of a decreased intake, and an increased catabolic state. A highly refined carbohydrate, low fiber diet has been reported as being effective in the management of IBD. Humans with IBD have also been treated with total parenteral nutrition (TPN) in an effort to rest the gastrointestinal tract, with variable success [116]. TPN can maintain or restore the normal metabolic state and can induce remission of clinical signs, but is not feasible to maintain for long periods of time. Enteral feeding of both an elemental and polymeric diet to patients with Crohn's disease initially resulted in remission [117]. However, most of those patients had recurrence of clinical signs within a year. Although patients with Crohn's disease appear to respond to dietary management more frequently than patients with ulcerative colitis, often the response is only temporary [115].

General considerations for canine patients with IBD include an easily assimilated diet, adequate but not excessive amounts of protein, the use of rice as a carbohydrate source, diets free of gluten and milk, and low fiber, low fat diets. An easily assimulated diet is easily

digested into its primary components, amino acids, fatty acids and simple sugars, therefore being less antigenic [95]. Hypoproteinemia is often a compication of IBD of the small intestine, therefore adequate protein levels are important [70]. However excess protein is to be avoided as protein is broken down to ammonia by colonic bacteria, and excess amonia can cause colonic irritation [97]. Rice is the preferred source of carbohydrate as it has low antigenic potential, is easily digested and absorbed, and serves as a source of protein [80]. Milk and wheat gluten have been associated with food allergy, milk causing colitis in cats [98], and wheat gluten causing small intestinal disease in dogs [118, 119] therefore it is recommended that these components be eliminated from the diet. Fat is difficult to assimilate and a high fat diet stimulates colonic motility and release of bile acids [120]. Based on these recommendations, either a homemade or commercial highly digestible diet is often used for treatment of IBD in dogs. These diets are considered to be easily assimilated, low in fiber [1] and are free of milk products and gluten. Hypoallergenic diets are also recommended for dietary therapy of IBD [77, 79]. These diets offer a source of protein that the animal has not already been exposed to, are low in fiber, and usually have rice as a carbohydrate source. These diets initially may be successful in treating IBD, however, if the source of protein is not easily asimmilated, it can reserve antigenic potential. After a period of time, food allergy may develope to the "new" protein, as chronic exposure may stimulate an immune response. In fact, highly digestible diets may be more effective, or at least as effective, in management of IBD, as hypoallergenic diets [1, 93]. High fiber diets are currently recommended for treatment of certain fiber-responsive large bowel diarrhea, the role of fiber in IBD however is controversial [77, 70].

The following is a brief description of the common disorders of the colon recognized in dogs, and the effect of dietary management.

1) Lymphocytic Plasmacytic (Chronic) Colitis

There is no apparent breed, sex, or age distribution for lymphocytic-plasmacytic colitis (LPC), although a recent report of 9 dogs with LPC found the majority of the animals to be females [121]. Clinical signs are consistent with large bowel diarrhea, and the animals are usually in good health. In the retrospective study by Leib et.al., hematochezia, increased frequency of defecation, and tenesmus were the most frequently noted clinical signs [121]. Clinical signs are usually progressive, but have been reported to be cyclic [79]. Routine laboratory data are usually within normal limits.

Endoscopic findings are variable, ranging from normal, to mild hyperemia with loss of visualization of the submucosal vessels, to severe granulation and ulceration [79]. Lymphoid follicles may be prominent. There may be visual loss of submucosal vessels and increased granularity due to mucosal edema or inflammation [14]. With severe disease, the colon may be nondistensible; strictures are rarely noted [79]. In an evaluation of 9 dogs with LPC [121], hyperemia, increased granularity, and increased friability were the most commonly noted endoscopic findings; one dog had an endoscopically normal appearing colonic mucosa.

Histopathologic changes typically seen in colonic biopsies from dogs with lymphocytic plasmacytic colitis include dilation of crypts; flattening and vacuolzation of the epithelial cells; and infiltration of lymphocytes and plasma cells into the lamina propria [79, 80]. Eosinophils and neutrophils are present in the lamina propria, in varying numbers. Mild changes include an increase in lymphocytes and plasma cells within the lamina propria and mild fibrosis [122]. In advanced stages, severe fibrosis, microabscessation and colonic stricture have been noted.

Reactive hyperplasia, seen early in this disease, is characterized by increased length and tortuosity of the crypts and is due to increased epithelial reproduction [122]. However,

Spinato et. al. [123] did not find a change in crypt length, in their study of biopsies from dogs with inflammatory bowel disease . They did, however, note a significantly decreased number of epithelial cells along the crypts of dogs with inflammatory bowel disease when compared to control dogs. In addition, there was an obvious loss of goblet cells which may have been caused by depletion of mucus from these cells, since mucoid stools are often seen with most forms of chronic colitis. This finding is usually considered to be an indication of active disease in humans [116]. In severe disease, goblet cells may be absent which results in decreased mucus secretion, leading to decreased efficacy of the mucosal barrier. Eventual breakdown of the mucosal barrier and inappropriate response to surface epithelial loss can lead to ulceration [80]. The degree of inflammation determines histologic grade but does not always correlate with endoscopic findings [124]. Profuse diarrhea can be seen with mild histologic changes [80].

Studies evaluating the effects of diet on this disorder are few. One study evaluated the effect of a highly digestible diet in 13 dogs with LPC [1]. Diagnosis of LPC was confirmed by clinical signs, the elimination of other diseases as possible causes for colitis, proctoscopic examination, and colonic biopsy. The dogs were placed on a cottage cheese and rice diet until clinical signs of diarrhea resolved (4 days to 6 weeks) and then were challenged with either a commercially available hypoallergenic diet or a high performance diet. Clinical signs of large bowel diarrhea returned in two of the dogs. The dogs that did not show clinical signs on the challenge test diets were then placed on their original diet. Clinical signs returned in all but two dogs within two weeks. Clinical signs resolved when these animals were placed on one of the three test diets (i.e. the cottage cheese and rice diet, the hypoallergenic diet or the high performance diet). Although this study failed to show if the clinical response was due to either a structural or functional change (since biopsies were not obtained post-treatment), it does show that there is an association between dietary management and resolution of clinical signs in dogs with LPC.

Hypoallergenic diets are usually recommended as the first form of therapy [1, 77, 125]. In Leib's study [121], 2/3 of the dogs were placed on either restricted diets alone (hypoallergenic or highly digestible diets), or a combination of restricted diets and sulfasalazine with good to excellent response to therapy. The dogs placed on sulfasalazine alone did not have as good a response to therapy as those with dietary management.

2) Eosinophilic Colitis

Eosinophilic enterocolitis has been reported to occur more frequently in German Shepherds, Cocker Spaniels, Rottweilers, and Dobermans [86]. As with the human form of this disease, food allergy has been proposed as an underlying cause . However, unlike the human disease, dietary food trials alone are usually not sufficient to resolve clinical signs. Other possible causes include gastrointestinal parasites, bacterial toxins, or any potential gut antigen [86]. Visceral larval migrans has been implicated as causing eosinophilic colitis experimentally, but not clinically [84, 85].

Systemic eosinophilia is often noted in the hemogram of patients with eosinophilic colitis [86]. Eosinophilic granulomas (i.e. focal masses or ulcers) may be noted grossly, and ulcers appear frequently in this disease. Usually only the mucosal layer is affected, but the submucosa and muscularis have also been reported as being involved [84, 126]. Eosinophils can be found in all layers of the colon, and globule leukcocytes (the origin and function of these cells have not been determined) have also been noted [86].

Dietary management alone is often not effective in treating this disease [86, 125]. Medical therapy, usually corticosteroids, is frequently added when dietary trials fail. Dietary trials are indicated however, and are usually the first form of treatment since food hypersensitvity is considered to be one of the possible underlying causes [86]. Home-made or commercial lamb and rice diets are usually recommended, as few dogs have been exposed to lamb. A fish and potato base diet is also considered to be hypoallergenic, presumably because the animal has not been exposed to these foods [127]. Animals initally requiring

corticosteroid therapy and dietary management for complete resolution of clinical signs may eventually be maintained with dietary management alone [86]. Other animals may need corticosteroids either continually or intermittently if there is recurrence of clinical signs. However, some authors recommend aggressive therapy because of poor dietary control, and suggest corticosteroids initially, followed by sulfasalazine, if there is no response [125]. Feeding a bland diet is also part of aggressive medical therapy. Overall, the prognosis for eosinophilic colitis is good with either dietary and/or medical management [86].

3) Histiocytic Ulcerative Colitis

This disease was initially reported in Boxers [4, 82] and French Bulldogs[128]. Histiocytic colitis, granulomatous colitis, ulcerative colitis and colitis of Boxer dogs are all synonyms for this disease. The Boxer dogs are usually young, less than 2 years of age [83]. Histiocytic ulcerative colitis is idiopathic, although causes associated with other forms of IBD have been suggested. In one report, females were reported to be affected twice as often as males [82]. However, another review of cases found that both sexes were affected equally [83].

In the advanced stage of the disease, the dogs are usually severely affected with weight loss, anorexia, fever, abdominal pain, and malaise [117]. Endoscopic findings vary with severity of the disease, and range from mild mucosal edema and hyperemia to marked ulceration, granulomas and strictures [122]. Histiocytes that stain positive with periodic acid Shiff stain (PAS), similar to those seen in human patients with Whipple's disease, are the diagnostic criteria for this disease [129]. However, unlike Whipple's disease, no causative organism has been consistently identified with light or electron microscopy in dogs with histiocytic ulcerative colitis [4]. A coccoid organism, similar to *Chlamydia*, was found in some affected dogs, but was not a consistent finding, and was thought to be opportunistic instead of causative. Therapeutic measures are usually aggressive, and include diet (either hypoallergenic or highly digestible), broad spectrum antibiotics, sulfasalazine, corticosteroids or other immunosuppressive drugs [80, 117]. Diet alone will not control this

disease, and unlike other forms of IBD, histiocytic ulcerative colitis usually carries a poor to guarded prognosis.

4) Idiopathic (Fiber-Responsive) Large Bowel Diarrhea

Idiopathic or fiber responsive large bowel diarrhea is characterized by clinical signs consistent with chronic large bowel diarrhea, the inability to identify an underlying cause and normal mucosal biopsies [77, 125]. Fiber-responsive large bowel diarrhea has many characteristics similar to human irritable bowel syndrome. Irritable bowel syndrome has been identified in large-breed, high-strung dogs, and is presumably induced by stress. Dogs with fiber-responsive diarrhea, however, are of various breeds, and the condition does not seem to be associated with stressful situations [2, 125]. A recent study evaluated the effect of a highly digestible diet supplemented with soluble fiber in dogs with idiopathic large bowel diarrhea [2]. Seven of eight dogs responded favorably to the dietary change for the duration of the follow up period, which was 10 weeks to 24 months [2]. The highly digestible diet alone had not been successful in resolving clinical signs. The actual effects of dietary fiber on fiber-responsive large bowel diarrhea may take 2-6 weeks before clinical signs resolve [130].

4) Human Inflammatory Bowel Disease

Human inflammatory bowel disease is a general term used to describe two separate conditions, Crohn's disease and ulcerative colitis [131, 132]. As with canine inflammatory bowel disease, attempts have been made to associate some underlying cause with these diseases. Suggested etiologies include genetic predisposition; infectious organisms, including *Mycobacterium sp., Chlamydia,* and *Clostridium difficile;* immune mediated or immune deficiency diseases; diet; and psychological disorders. No direct correlation has been made with any of these conditions.

Crohn's disease is an idiopathic disorder that usually affects the ileum and colon, but can affect any area of the intestine [132]. It can be chronically progressive in nature, or can wax and wane. The most common clinical signs include diarrhea, abdominal pain and fever. Often, extraintestinal signs are noted including arthritis, uveitis, aphthous stomatitis, and erythema nodosum. Histologic findings reveal granulomas in the submucosa and lamina propria with edema and lymphatic dilation, and inflammatory infiltrates in the mesenteric lymph nodes. Immunocytochemistry shows increased numbers of all types of plasma cells routinely found in the lamina propria, as well as the presence of IgE producing plasma cells [90]. Ultrastructural studies show minimal changes in the epithelial cells, no increase in intraepithelial cells, an increase in lymphocytes in the lamina propria, and loss of both the basal lamina and pericryptal fibroblasts [19].

Ulcerative colitis in humans is also idiopathic, and characteristically will have periods of remission followed by periods of active disease [131]. This disease is found to affects the rectum and left colon 90-95 % of the time, but can occur in the transverse and ascending colon as well. Clinical signs include diarrhea and rectal bleeding, and, as with Crohn's disease, extraintestinal signs (arthritis, uveitis and dermatologic changes) are often noted. Histopathologic evaluation shows that this disorder primarily affects the mucosa, with crypt abscesses being the most common finding. Lymphocytes and neutrophils are found in increased numbers in the lamina propria, and erosions and ulceration of the mucosa are frequent findings. The ulcerations begin microscopically and coalesce into macroscopic lesions with areas of normal mucosa in between, referred to as psuedopolyps. Ulcerations occur as a result of epithelial cell destruction exceeding epithelia cell production [19]. Although ulcerative colitis usually is restricted to the mucosa, some lesions may extend into the submucosa. Increased numbers of IgG producing cells are identified by immunocytochemistry [131]. Ultrastructural findings include irregular, shortened, swollen microvilli; dilated ER; decreased numbers of mitochondria, which are often swollen; reduced

glycocalyx and C-bodies; and a decreased number of subepithelial blood vessels [19]. These changes are seen not only in ulcerated lesions, but in areas that are normal histologically.

Diagnosis of IBD in humans is based upon clinical signs, endoscopic findings, radiographic changes (in Crohn's disease, but not reliable in ulcerative colitis), and histopathology. Although the histopathologic lesions are distinct, they are not unique to either of these diseases. Because there is crossover of clinical, endoscopic and histiologic findings, a distinction cannot be made between ulcerative colitis and Crohn's disease based on the above criteria in approximately 20% of the cases [131]. However, recent ultrastructural evaluation of Crohn's disease has found axonal necrosis in both normal and ulcerated areas of intestine [133]. These changes suggest that axonal necrosis precedes submucosal changes, and also provides a method by which Crohn's disease can be differentiated from ulcerative colitis, when routine evaluation fails to do so. Another ultrastructural study recently found pseudopod-like extensions on denuded epithelial cells in patients with ulcerative colitis [134]. Similar changes have not been identified on ultrastructural evaluation of patients with Crohn's disease.

In comparing human and canine IBD, it appears that ulcerative colitis resembles canine IBD more closely than Crohn's disease, as it involves the mucosa primarily, and only occasionally extends into the submucosa. The marked ulceration and severity of ulcerative colitis parallels both eosinophilic colitis and histiocytic ulcerative colitis, but less so lymphocytic-plasmacytic colitis, which is usually a clinically and histologically milder disease. However, unlike canine eosinophilic colitis, eosinophils are not numerous in the lamina propria of human patients with ulcerative colitis, and unlike histiocytic ulcerative colitis, PAS positive histiocytes are not found. Canine IBD also differs from human IBD in that extraintestinal lesions are rarely found in dogs. Of particular interest are the ultrastructural changes noted in human IBD that precede the histiologic changes, as well as the ultrastructural changes that can be used to differentiate Crohn's disease from ulcerative

colitis. Perhaps ultrastructural evaluation of canine IBD can find differentiating changes that will aid both in diagnosis and treatment.

J. Morphometric Evaluation of Colonic Biopsies

Recent studies have looked at various morphometric parameters of endoscopically-obtained colonic biopsies in an effort to standardize the histopathologic evaluation of both normal and diseased animals. Most of the studies in dogs have examined samples at the light microscopy level. The following is a summary of those findings.

Canfield, *et. al.* [16] found that there was considerable variability between the number of cells within the lamina propria in normal dogs and concluded that a considerable increase in the number of cells in the lamina propria would have to occur before the diagnosis of IBD could be made. Roth et. [135] al devised a grading system to evaluate the degree of inflammation that occurred in the lamina propria of dogs with lymphocytic plasmacytic colitis. A scale from 0-5 was used at increments of 0.5. Specific criteria were listed for each increment, describing the number of inflammatory cells, epithelial cell appearance, number of goblet cells, presence of intraepithelial cells, degree of fibrosis and presence of ulcers. Normal animals as well as clinical cases were evaluated. The investigators found that a number of the clinical cases had only mild changes based on this scale. These findings correlated with results from human studies comparing histologic grade with clinical signs, i.e. the histologic grade and severity of clinical disease do not always correlate [133].

A more extensive morphologic evaluation of endoscopically obtained colonic biopsies comparing normal and diseased dogs was done by Spinato et. al. [123]. Parameters measured included: 1) gland length; 2) distance between gland and muscularis mucosa; 3) thickness of muscularis mucosa; 4) number of epithelial cells; 5) number of goblet cells; 6) goblet cell index; 7) mitotic index; 8) number of mast cells and lymphocytes; and 9) lymphocyte and mast cell indices. In addition, the crypts were evaluated in cross section.

Perimeter, area and diameter of crypt cross sections, as well as numbers of goblet and absorptive cells (and goblet cell index calculated) were reported for each cross section. The degree of inflammation in the lamina propria was not evaluated. Biopsies were obtained from multiple sites in the colons of all dogs. When the morphologic characteristics were compared, few differences were noted between sites, but a trend for shorter glands with fewer numbers of cells (in particular fewer goblet cells) was found in the cecum and at the anorectal junction. The most remarkable change noted in clinical cases of LPC was the decreased number of epithelial cells along the crypts.

Van der Gagg evaluated colonic biopsies from 355 dogs with signs of large bowel diarrhea [136]. Characteristics examined included: 1) degree of hypertrophy; 2) atrophy or ulceration of the epithelial cells; 3) number of goblet cells; 4) number and type of inflammatory cells within the lamina propria; 5) distance between base of crypt and muscularis mucosa; and 6) presence of neoplastic cells. Diseases diagnosed in this study included: 1) histocytic colitis; 2) lymphocytic-plasmacytic colitis; 3) eosinophilic colitis; 4) acute colitis; 5) granulomatous colitis; 6) epithelial origin neoplasia; and 7) mesynchymal origin neoplasia. In certain cases no distinction could be made between lymphocytic plasmacytic colitis and lymphoma. Although this study did not attempt to correlate cause or response to treatment with the histopathologic diagnosis, it was the first attempt to evaluate a large number of colonic biopsies from diseased animals in a methodical manner.

Van der Gagg, et. al. later conducted a study evaluating the histopathologic response to treatment [137] on previously reported cases . The dogs evaluated had a variety of disorders of the colon, including mild colitis, histiocytic ulcerative colitis, and lymphosarcoma. Dogs received salzosulfapyridin, mebeverin HCL or corticosteroids. Response was greatest in those dogs with mild colitis; no change was usually noted in lymphosarcoma or histiocytic ulcerative colitis patients. If dietary management was used, it

was not listed. This is the only report in the literature of a large number of clinical cases with follow-up biopsies.

Although these studies provide specific criteria by which to evaluate endoscopically obtained colonic biopsies in the normal and/or diseased dog, similar studies have not been performed using electron microscopic evaluation. Ultrastructural evaluation of histiocytic colitis in Boxer dogs [4, 138] and parvovirus in dogs [29] have previously been reported, but biopsies of the dog colon have not otherwise been critically evaluated by specific ultrastructural morphometric parameters with transmission electron microscopy.

Studies evaluating colonic adaptation to diet and disease have examined the effects of these processes on epithelial cell proliferation. Morphometric parameters that have proven useful in evaluating epithelial cell proliferation of colonic mucosa include: 1) epithelial cell height; 2) cell area; 3) height of microvilli; 4) number of microvilli; 5) thickness of basement membrane; 6) mitotic index; and 7) goblet cell index [69, 139, 140]. All these parameters can give an indication of rate of proliferation. However, change in area and height of the epithelial cell can also indicate excess destruction without compensatory proliferation [19]. Basement membrane width can reflect epithelial metabolic rate as well as rate of proliferation. Finally goblet cell index can be affected by disease and diet, independent of proliferation [19, 141].

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III. MATERIALS AND METHODS

The study of the endoscopic and histologic evaluation of diet in normal dogs was performed by Leib et. al., and results of their work are reported elsewhere [1]. This study evaluates the ultrastructural features from these same dogs. (Animal care and endoscopy were performed by Leib's group prior to my involvment in the study.)

Animal Care and Handling

Twelve mixed breed dogs, five spayed females and seven males were obtained from the pound for this study. All dogs were considered to be healthy by physical examination, complete blood count, and biochemical profile. Modified Knott's test for microfilaria was negative for all dogs. Some dogs were found to be infected with *Giardia, Isospora, Toxocara and Ancylostoma* as determined by zinc sulfate fecal flotation. All dogs were treated with quinacrine (6.6 mg/kg BID for 5 days), sulfadimethoxine (27.5 mg/kg SID for 8 days), and pyrantel pamoate (11 mg/kg SID for 1 day Q 4 weeks for the duration of the study). Two dogs with whipworm infestations were treated with fenbendazole (50 mg/kg SID for 3 days). Prior to initiation of dietary trials all dogs were diagnosed as free of gastrointestinal parasites by three negative zinc sulfate fecal floatation examinations. In addition duodenal aspirates for *Giardia* were negative as were colonoscopic examinations of the cecum for whipworms. Fecal samples were examined for parasites at four week intervals using at least one zinc sulfate flotation test and an aspirate of the duodenal contents for *Giardia.* All dogs received quinacrine (6.6 mg/kg SID for 5 days), when *Giardia* were identified in any animal during any of the examinations.

Animal housing and care and all experimental procedures were approved by the Virginia Tech Animal Care Committee. The dogs were randomly assigned to control and treatment groups of six dogs each. The six control dogs were fed a grocery store, dry maintenance food (Purina Dog Chow, Ralston Purina) throughout the study, which was

designated as the control diet. All dogs assigned to the treatment groups received the control diet for the initial four weeks of the study. Each treatment diet was then fed for a four week interval, followed by a second four week interval on the control diet. The control diet period was intended to allow adequate time for resolution of any changes that occurred while on the test diet. The test diets were randomly assigned, so that the treatment dogs did not all receive the same diet at the same time.

Dogs were individually fed once per day. Food not eaten within 30 minutes was removed. The amount of food fed was calculated to meet maintenance energy requirements (60 kcal/kg) based on estimated optimal body weight. Dogs were weighed weekly, and the amount of food fed was adjusted to maintain optimum body weight. Stool consistency and the presence of vomitus were recorded daily. Treatment diets used included:

1) Prescription intestinal diet, a highly digestible diet, (i/d®, Hills Pet Products)

2) Prescription high fiber diet (w/d®, Hills Pet Products)

3) Prescription hypoallergenic diet (d/d®, Hills Pet Products)

4) Commercial canned diet (Alpo Beef Chunks®, Alpo Pet Foods)

5) Commercial super-premium maintance diet (Iams Minichunks®, Iams Pet Foods)

6) Commercial super-premium high performance diet (Iams Eukanuba®, Iams Pet Foods)

The control diet was Purina Dog Chow®, Ralston Purina. The effects of diets 1-3 on the colonic mucosa were evaluated ultrastructurally. Table 2 shows the individual components in each of these diets.

Diet Evaluation [1]

These diets were chosen for evaluation because they are recommended therapy for various disorders of the colon. The highly digestible diet is considered to have easily assimilated nutrients that are easily and completely digested and less likely to be antigenic, and is also low in fiber. Indications for use of the highly digestible diet other than IBD include exocrine pancreatic insufficiency and pancreatitis. Contraindications include sodium retention states such as congestive heart failure and portal hypertension because of the high sodium content. The high fiber diet obviously provides fiber in excess of the normal diet. This is a low protein, low fat, moderately low salt and low energy diet. It is not recommended for growing or lactating animals. Recommendations for use in addition to colitis include prevention of obesity, and control of constipation, hyperlipemia and diabetes mellitus. The hypoallergenic diet has a minimum number and "unique" type of dietary components. It is recommended for use in dermatologic and gastrointestinal disorders resulting from food hypersensitivity or intolerance. It is contraindicated in young and reproducing animals because of a low calcium concentration.

Table 3 compares the dietary components of each of these diets based on dry matter. The highly digestible diet has the highest concentration of protein, fat, calcium, sodium, and phosphorus and the lowest percentage of fiber of all the other diets,. The high fiber diet has a greater percentage of fiber and a lower fat content than the others, although the fat content does not vary greatly from the control group. The percentage of fat in the hypoallergenic diet is relatively high and the fiber content relatively low, but otherwise this diet does not vary substantially from the control diet. The control diet has the second highest calcium and fiber content, while other components are not present in excessively high or low concentration. All percentages given are based on dry weight. Protein percentages represent nitrogen content available for energy, not total nitrogen content.

Endoscopic Evaluation

Endoscopic examination of the colon was performed at the end of each four week interval. Food was withheld for 24 hours prior to endoscopy. Dogs were prepared for endoscopy by two warm water enemas (20 ml/kg) and two doses of a gastrointestinal lavage solution (60 ml/kg Golytely®, Braintree Laboratories) via orogastric lavage. General anesthesia was induced with sodium thiamylal (15 mg/kg, IV) and maintained with halothane gas anesthetic (1.0-1.5%) via endotracheal tube. Endoscopy was performed with the dogs in left lateral recumbency using an Olympus GIF XQIO® endoscope (Olympus Corporation). Mucosal biopsies were taken from the ascending colon, the transverse colon, and the proximal, mid and distal descending colon of all dogs using endoscopic biopsy forceps. Locations of the biopsies from each animal were measured from the anus and recorded, and biopsies were taken from the same area in each subsequent endoscopic examination. Only the biopsies taken from the mid descending colon were examined ultrastructurally.

Results of the Light Microscopy Study

Biopsy samples taken from the same animals evaluated by ultrastructure in this current study were also evaluated by light microsocpy. The results of the light microsocpy study are published [2], and will be presented here as it was the prelude to the ultrastructure study. Histologic parameters evaluated included number and type of inflammatory cells within the lamina propria, evaluation of the absorptive cells, and number of goblet cells. A histologic grade of <2.0 was considered normal [3].

Abnormal endoscopic findings were reported in three animals, but the histologic grade was normal, and no clinical signs were associated with these findings. Abnormal histologic scores were reported in five different dogs and ranged from 2.0 to 2.3. Colonoscopic examination was normal for all of these five dogs, and no abnormal clinical

signs were associated with the abnormal histologic scores. No significant differences were detected among the treatment groups when comparing values from biopsies collected at the beginning of the study with those collected at the end. Other comparisons evaluated statistically included differences among the treatment groups when on the test diets; differences among the treatment groups when on the control diets; and differences among the control group during any of the 13 periods that samples were taken. Statistically significant differences where not found in any of these comparisons.

Since no significant differences were found in colonscopic evaluation or histologic grade, the authors concluded that diet did not have an effect on colonoscopic or histologic evaluation of the colon in normal dogs and. therefore, the grading system that was initially developed for histologic interpretation of colonic mucosa was valid and independent of diet studied.

Ultrastructural Examination

Tissue samples were fixed in a 5.0 % glutaraldhyde/3.0 % formaldehyde solution and post-fixed in 1.0% osmium tetroxide. Fixation was carried out at room temperature and at pH 7.2 - 7.4, with 0.1 sodium cacodylate buffer. The biopsies were washed in buffer, dehydrated in ethanol and embedded in Polybed 812. Sectioning was performed on a LK NOVA® ultramicrotome, and ultra-thin sections were cut with a diamond knife. Sections were collected on copper grids, stained with uranyl acetate and lead citrate and examined using the JOEL 100 CXII® transmission electron microscope.

Morphometric measurements were made on a graphics tablet using the Sigma Scan software® (Jandel Scientific). An attempt was made to measure cells that were located at the top of the crypts, and cells that were cut with the longitudinal axis parallel to the knife surface. The following parameters were measured on ten epithelial cells/sample:

1) Height of the cell. Cell height was measured from the apical portion of the terminal web to, but not including, the basement membrane.

2) Area of the epithelial cell. The perimeter of the cell was outlined by the cursor and a value for total area was calculated from this measurement using the Sigma Scan program.

3) Width of the basement membrane. The basement membrane was measured at 20 different sites/sample and an average width determined.

4) Height of microvilli. Multiple microvilli were measured/cell. The number of microvilli actually measured differed from sample to sample because the orientation of some of the microvilli (i.e. not parallel to the cell) made measurement of these microvilli invalid. Only those microvilli visibly cut from apex to base were measured.

5) Number of microvilli per width of cell apex. The number of microvilli per unit length of membrane was calculated based on the width of the apical surface. This took into account the vertical plane of the cell and allow for a more accurate evaluation of this parameter.

Statistics

For each group of 60 cells (10 cells per sample, 6 samples/group) mean, standard deviation, and standard error were calculated. A Student's T-test was used to identify any significant differences between groups. An independent T-test was used to compare the control group to the treatment groups. Because the different treatments were applied to the same animals, a paired T-test was used to compare treatment groups to each other. Significance was determined at $p \leq 0.01$, because of the sensitivity of the T-test used in this situation. Since significant differences were detected using the T-test, the data was further evaluated by a one way analysis of variance (ANOVA). Duncan's multiple comparison test was used to identify groups that differed significantly using the ANOVA ($p \leq 0.05$).

IV. RESULTS

Clinical response to diet and light microscopic evaluation of samples evaluated in this study were previously reported [2]. Two samples from the hypoallergenic diet group were lost, leaving 4 samples to evaluate. All other samples were present and available for electron microscopic evaluation.

Means, standard deviations, and coefficient of variation (CV) for individual animals are listed in Tables 4-7. The mean for cell height ranged from 19.9 to 38.4 microns, for cell area from 51.7 to 133.9 sq. microns. The mean microvillus height from this study ranged from 1.1 to 1.7 microns and the mean for number of microvilli/ apex width from 6.8 to 11.0 no./micron. The mean basement membrane width ranged from 0.8 to 1.7 microns. Coefficient of variation for the ten cells/animal was within the expected range for biological samples, with only one value exceeding 25%. The coefficient of variation among animals within each group was also within the expected range . Table 8 shows group means, standard deviations, and CV.

Some associations between parameters were noted. Cell height and cell area, in general, were directly related, i. e. as cell height increased, cell area increased, which is an expected finding. The mean microvillus height appeared to correlate directly with the number of microvilli/width. Since microvilli increase absorptive surface, it would be likely that height and number would vary together. The other parameters did not vary in any consistent relationship when compared.

T-test results indicated several significant differences between the control and treatment groups as well as among the treatment groups. These included:

1) Significantly greater cell height and cell area for all treatment groups when compared to the control group.

2) Significantly lower values for microvillus height and for number of microvilli/apex width for all treatment groups when compared to the control.

3) Significantly lower basement membrane width for the hypoallergenic diet group and the high fiber group when compared to the control group.

4) Significantly greater values for cell area, cell height and basement membrane width for the highly digestible diet group when compared to the hypoallergenic diet group and the high fiber diet group.

5) Significantly lower values for microvillus height and number of microvilli/apex width for the highly digestible diet group when compared to the other two treatment diet groups.

6) The values for the high fiber diet group did not vary significantly from those of the hypoallergenic diet group.

The only one of these differences found to be significantly different using ANOVA was a significantly greater cell height value for the highly digestible diet group when compared to the other groups. (Table 8)

V. DISCUSSION

The treatment diets evaluated in this study were chosen because these diets are frequently used to treat large bowel diarrhea in the dog. Although these diets are sometimes effective in relieving clinical signs, drug therapy is often required. Based on clinical signs and endoscopic biopsy results, there is no apparent reason why one dog may respond to diet alone, while others need additional therapy. There have been no clinical trials to actually prove the efficacy of these diets based on histologic findings; evaluation of efficacy is based only on clinical response. Therefore the mode of action of these diets is unknown. This study attempted to identify ultrastructural changes seen in the mucosa of normal dogs when fed various diets. If changes were noted, then an attempt could be made to identify the dietary component that may be responsible for that change.

When these samples were previously evaluated by light microscopy, no differences were found. The conclusion was that diet did not influence the endoscopic or histopathologic evaluation of colonic mucosa in normal animals.

In comparing results of this study with previously published results, reference values for microvillus height and for basement membrane width were found. Published range for microvillus height is 1-2 microns, for basement membrane width is 0.8-1.2 microns [4]. These are generic values, and are not specific for the colon. Microvillus height from this study correlate well with the published values. The values for basement membrane width, although somewhat higher that the published values, are not remarkable different, and this difference probably reflects variations in either tissue or species. No reference values were found for the other parameters evaluated in this study.

The first consideration when evaluating this study is whether four weeks was a sufficient amount of time to allow for changes related to diet to occur in the colonic ultrastructure. A study evaluating effects of diets of varying protein content on canine colonic mucosa found that ultrastructural changes were evident by three days and complete by day 10-14 [5]. This appears to be relate to the average life span of an epithelial

cell (7 days) and reflects the normal maturation and migration of the colonic epithelial cell along the crypts. It would appear then, that 4 weeks was a sufficient period of time for ultrastructural changes related to diet to occur.

The morphometric evaluation of the colonic ultrastucture from this current study showed a number of significant differences when evaluated by a Students t-test, but these differences were not apparent with an ANOVA. There are several reasons for the difference between these two tests. First, although the t-test is used to evaluate small sample sizes when statistical parameters of the population are unknown, as in this study, groups should be of equal sizes [6]. Because two samples from one group were lost, the t-test is not accurate in evaluating this group with the others. The ANOVA program in the SAS computer system can take into account the missing values, and is a more accurate evaluation of data with missing values. The t-test is generally used to compare two samples but there is an increased risk of making Type 1 errors, i.e. falsely rejecting the null hypothesis, when the t- test is used for multiple comparisons [6]. For comparison of multiple groups, ANOVA with an appropriate multiple comparisons test is more accurate in determining significant differences.. The use of the t-test in this situation was useful in identifying trends, and was easily run using the Sigma Scan® software program, but was too sensitive to determine actual significance. Therefore, the ANOVA was used to determine significance among groups, with Duncan's multiple comparison test to identify which groups differed when significance was determined. As a result, epithelial cell height in dogs fed the highly digestible diet was the only parameter in which there was a significant difference detected when compared to the control group using ANOVA.

Comparison of the different diets indicated that the highly digestible diet has the highest concentration of all components except carbohydrates and fiber. In addition, the dietary components were easily digested and assimilated by the gastrointestinal tract. Completely digested proteins are less antigenic and less likely to cause disruption of the epithelial surface [7]. Also, the highly digestible diet has a low fiber content, which may be

less irritating to the colonic mucosal cells than the higher fiber diets [8]. Increased availability and concentration of dietary components and low residue diets all may result in a decrease turnover rate of colonic epithelium. Decreased cell turnover rate would be indicated by mature columnar cells on the mucosal surface. A morphometric indicator of this change would be increased cell height. However, other indicators to support increased numbers of mature cells on the epithelial surface, such as increased cell area, and increased number and height of the microvilli should also be present, and are consistent with changes that occur along the crypt as the cells proliferate and mature [9,10]. Although the group fed the highly digestible diet had the greatest value for cell area, this difference was not significant and cannot be interpreted beyond stating that the highly digestible diet may be associated with decreased cell turnover. In addition, (although again using ANOVA the differences were not statistically significant) this group had the lowest microvillus height and lowest value for number of microvilli per apical width of all the groups, which does not suggest cell maturity. Although we found significantly increased cell height, we did not find other significant differences that would support an alteration in epithelial cell proliferation or colonic function.

Changes due to high fiber/low fat diets, such as increased cell proliferation and cytoprotective effects were not observed here [11]. A hypoallergenic diet presumably prevents alterations of the colonic mucosa caused by dietary antigens, or at least does not promote these changes [12]. Modifications in the mucosal epithelium of the colon seen in inflammatory bowel disease include destruction of the mucosal barrier, alterations in mucosal permeability, and epithelial cell loss, resulting in an increased turnover of epithelial cells and increased rate of proliferation. These changes may be either incited by or aggrevated by food antigens [13,14]. Changes related to a hypoallergenic diet might be decreased epithelial cell turnover and proliferation. No differences consistent with these alterations were noted for this diet.

The hypoallergenic and high fiber diets did not have a noticeable effect on the colonic mucosal ultrastructure, and the highly digestible diet had a minimal effect (increased cell height). Therefore, these diets did not cause any substantial variation in the mucosal ultrastructure from the mid-descending colon in normal dogs.

The descending colon appears to be a good choice for detecting change in cell proliferation or maturation, since studies indicate that, at least in rats, the descending colon has the most rapid cell renewal cycle [15]. Therefore any variation in proliferation or maturation related to diet might be noted here. Reasons for the lack of other significant differences among groups could be an inherent bias of the study. An attempt was made to measure cells located at the top of the crypt. The criterion used to determine this were based on the shape of the absorptive cell and the number of goblet cells. The absorptive cells located at the top of the crypt should be columnar, and the number of goblet cell should be minimal in this location. By selectively measuring cells that fit this criteria, we automatically added a bias to the study. A ratio of mature versus immature cells taken from each specimen may have reduced the chance of bias, however this was not done. The fact that cells that fit this criteria were found in each specimen, and each specimen was evaluated by the same criteria, would suggest that the bias between specimen should be the same. Even so, the preferential selection of mature looking cells cannot be ignored, and may explain why more significant differences were not found.

The the portion of the colon actually examined was very small, the assumption in evaluating biopsy samples taken from one site is that if the changes are diffuse, they should be readily apparent in any biopsy taken. This has been shown to be true in several studies in dogs, where multiple colonic biopsies were examined histopathologically [16, 17, 18]. However, alterations due to diet may be segmental and not affect the whole colon equally. Since short chain fatty acids are the major nutrient absorbed by the colonic epithelial cells, and bacterial fermentation of carbohydrates is the main source of short chain fatty acids, differences in bacterial population along the colon could explain

segmental changes [19, 20]. Also, it has been shown that bile acids can have significant effects on the colonic mucosa [20, 21]. Bacterial population as well as diet determine bile acid concentration, which could explain segmental effects [21, 22]. If segmental changes related to diet occurred, then a biopsy from a single site may have missed the lesion. In order to evaluate this critically, multiple biopsies must be taken from a single patient. This is done routinely when light microscopy is used to evaluate colonic biopsies. Cost and complexity probably would prohibit routine use of multiple biopsies for ultrastructure evaluation.

Another possible explanation for lack of significant findings is that this study was done in normal dogs fed balanced diets. Perhaps changes related to diet are significant in the diseased state, but not readily apparent in the normal animal. Therefore, evaluation of colonic biopsies before and after dietary therapy is needed to evaluate the effect of diet on the mucosal ultrastructure in the diseased state.

Finally, changes in the clinical outcome of canine colonic disease due to dietary therapy may be related to function and not structure. Function can be affected in many ways: increased secretion, decreased absorption, and alteration in motility can all result in large bowel diarrhea independent of structural changes. Function was not evaluated in this study, therefore the effect of diet on function and its relationship to minimal structural change cannot be evaluated. Effects of insoluble fiber on colonic motility in dogs has been studied [23, 24, 25,]. Effects of other dietary components on motility, absorption and secretion may prove useful in understanding the effects of diet on disorders of the colon.

VI. CONCLUSIONS

The effects of hypoallergenic, highly digestible and high fiber diets on the colonic mucosal ultrastructure in normal dogs appears to be minimal compared to a control diet. The only change noted was an increase in cell height in the highly digestible group which may indicate a decrease in cell turnover, but this was not supported by other ultrastructural alterations such as increased number and height of microvilli and increased cell area. No variations were noted in colonic mucosal ultrastructure that could be attributed to the hypoallergenic or high fiber diet. Lack of significant differences may be due to either failing to sample the area affected, or, more likely, due to a failure of these diets to cause change.

Since it is apparent from clinical experience that diet does benefit colonic diseases, evaluation of the colon before and after dietary treatment is necessary in order to identify these changes. This is important for several reasons. First, not all dogs respond to dietary therapy. Second, one dog with a particular disorder may respond to one diet, while another dog with the same disorder may respond to a different diet, with no apparent explanation for the different response. Finally, there are certain diseases where the histopathologic findings do not correlate with the clinical signs. Examples of this include inflammatory bowel disease, where mild changes may result in profuse diarrhea; and fiber responsive large bowel diarrhea, where there are no apparent underlying morphologic abnormalities [7, 26, 27]. Ultrastructural evaluation of these cases may be helpful not only in identifying structural changes that might explain the clinical signs, but also in identifying the morphologic alterations related to diet so that more effective dietary management can be implemented.

It is obvious from this discussion that ultrastructural evaluation of the diseased canine colon is in its infancy and needs further development if this method is to be useful. The findings from this study provide a basis to which further ultrastructural evaluations of

the canine colon can be compared. Values for epithelial cell height, cell area and number of mivrovilli/apex width have not been previously published. Values from this study for microvillus height and basement memebrane width are similar to previously reported values. Currently, ultrastructure is used to determine early lesions in humans with inflammatory bowel disease, when none are noted on histopathology, and to differentiate between those cases of ulcerative colitis and Crohn's disease that are impossible to distinguish with routine criteria [28, 29, 30, 31]. It is possible that ultrastructural evaluation may yet prove to be an equally valuable tool in elucidating some of the unknowns that surround canine inflammatory bowel disease.

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TABLE 1

REGULATORY PEPTIDES OF THE COLON

CIRCULATING HORMONE	LOCAL/ PARACRINE	NEUROTRANSMITTER
Enteroglucagon	Somatostatin	Vasoactive Intestinal Peptide
Neurotensin		Substance P
Substance P		Bombesin
		Enkephalins- Leucine and Methionine
		Somatostatin

TABLE 2

DIETARY COMPONENTS OF CONTROL AND TEST DIETS

DIETS	COMPONENT
CONTROL	Ground yellow corn, ground wheat, meat, bone meal, soybean meal, corn gluten meal, animal fat, dried brewer's yeast, L-lysine, dried whey, wheat germ, vitamins and minerals.
HIGHLY DIGESTIBLE	Whole eggs, liver, chicken, brewer's rice, ground corn, vegetable oil, animal fat, vitamins and minerals.
HYPOALLERGENIC	Mutton by-products, mutton liver, rice , vegetable oil, vitamins and minerals.
HIGH FIBER	Cellulose, rice, chicken, liver, egg, dried milk, vitamins and minerals.

From: Lewis LD, Morris ML, Hand MS. *Small Animal Clinical Nutrition*, 3rd edition, Mark Morris Associates, Topeka, Kansas, 1987; A2.1-A2.8.

TABLE 3

AVERAGE NUTRIENT CONTENT OF

DIETS USED IN THIS STUDY

(% USABLE ENERGY)

	DIETS			
NUTRIENT	PURINA DOG CHOW	HIGHLY DIGESTIBLE	HIGH FIBER	HYPO-ALLERGENIC
Protein	19	27	17	16
Fat	8	14	7	12
Carbohydrate (NFE)	47	49	54	67
Fiber	4.5	0.9	16	1.5
Calcium	1.2	1.4	0.6	0.5
Phosphorous	0.9	1.1	0.5	0.4
Sodium	0.4	0.5	0.2	0.3

TABLE 4

INDIVIDUAL MEANS, STANDARD DEVIATIONS, AND COEFFICIENTS OF VARIATION (CV) FOR THE CONTROL DIET GROUP

Animal Number	Cell Height (microns)	Cell Area (microns)	Microvillus Ht (microns)	Width of BM (microns)	No MV/Width
1	30.1	61.5	1.5	1.6	11.0
	3.1	12.8	0.2	0.4	1.8
CV	10%	21%	13%	25%	16%
2	29.1	87.8	1.6	1.7	7.6
	5.6	12.1	0.3	0.4	1.0
CV	19%	14%	8%	14%	13%
3	29.1	85.5	1.4	1.4	8.3
	3.0	12.0	0.3	0.2	1.1
CV	10%	14%	21%	14%	13%
4	26.0	86.4	1.3	2.1	8.1
	3.3	7.2	0.3	0.6	1.2
CV	13%	8%	23%	28%	15%
5	30.3	91.5	1.2	1.7	7.2
	3.9	9.6	0.2	0.2	0.9
CV	13%	10%	17%	12%	12%
6	28.7	82.7	1.7	1.0	6.9
	5.5	8.5	0.4	0.2	0.6
CV	20%	10%	24%	20%	9%

TABLE 5

INDIVIDUAL MEANS, STANDARD DEVIATIONS, AND COEFFICIENTS OF VARIATION (CV) FOR HIGH FIBER DIET GROUP

Animal Number	Cell Height (microns)	Cell Area (microns)	Microvillus Ht (microns)	Width of BM (microns)	No MV/Width
1	22.2	67.0	1.3	1.7	7.8
	3.6	10.8	0.2	0.3	0.9
CV	16%	16%	15%	18%	12%
2	35.4	93.0	1.7	0.8	7.8
	4.5	9.2	0.3	0.1	0.9
CV	13%	10%	18%	12%	12%
3	35.2	101.2	1.4	1.4	7.8
	4.6	21.2	0.2	0.3	0.6
CV	13%	21%	14%	21%	8%
4	31.9	117.1	1.4	1.5	7.9
	3.2	13.7	0.2	0.2	1.3
CV	10%	12%	14%	13%	16%
5	19.1	51.7	1.6	1.0	8.5
	1.5	7.3	0.2	0.2	0.7
CV	8%	14%	12%	20%	8%
6	33.7	93.9	1.1	1.1	7.3
	1.9	7.3	0.2	0.2	0.7
CV	6%	8%	18%	18%	10%

TABLE 6

INDIVIDUAL MEANS, STANDARD DEVIATIONS, AND COEFFICIENTS OF VARIATION (CV) FOR THE HYPOALLERGENIC DIET GROUP

Animal Number	Cell Height (microns)	Cell Area (microns)	Microvillus Ht (microns)	Width of BM (microns)	No MV/Width
1	30.6	98.1	1.6	1.0	8.1
	1.6	12.2	0.3	0.1	1.1
CV	5%	12%	19%	10%	14%
2	27.9	91.7	1.3	1.3	9.8
	7.2	18.4	0.1	0.2	1.1
CV	26%	20%	8%	15%	11%
3	34.3	107.9	1.4	1.5	7.3
	4.4	14.6	0.1	0.3	0.5
CV	13%	14%	7%	20%	7%
4	27.5	81.3	1.4	0.9	7.7
	4.3	9.3	0.2	0.2	0.9
CV	16%	11%	14%	22%	12%

TABLE 7

INDIVIDUAL MEANS, STANDARD DEVIATIONS, AND COEFFICIENTS OF VARIATION (CV) FOR THE HIGHLY DIGETIBLE DIET GROUP

Animal Number	Cell Height (microns)	Cell Area (microns)	Microvillus Ht (microns)	Width of BM (microns)	No MV/Width
1	38.4	92.6	1.3	1.4	7.2
	3.1	7.7	0.2	0.2	0.5
CV	8%	8%	15%	14%	7%
2	37.4	100.6	1.1	1.4	7.5
	4.0	12.6	0.1	0.2	0.9
CV	9%	13%	9%	14%	12%
3	36.1	133.9	1.2	1.9	7.2
	4.5	18.5	0.3	0.3	0.6
CV	11%	14%	25%	16%	8%
4	33.3	102.0	1.7	1.3	8.2
	6.3	10.4	0.1	0.3	0.5
CV	19%	10%	6%	23%	6%
5	36.3	108.3	1.6	1.6	6.8
	4.0	9.0	0.1	0.4	0.8
CV	11%	8%	6%	25%	12%
6	38.2	117.1	1.4	1.4	7.1
	3.3	11.4	0.1	0.3	1.1
CV	9%	10%	7%	21%	15%

TABLE 8

MEANS, STANDARD DEVIATIONS, AND COEFFICIENTS OF VARIATION (CV) FOR ALL GROUPS

DIET	Cell Ht (microns)	Area (sq microns)	Mv Ht (microns)	BM (microns)	NO Mv/Width (microns)
CONTROL	28.9	82.6	1.4	1.5	8.2
	1.5	10.7	0.2	0.4	1.5
CV	5%	13%	14%	26%	18%
HIGH FIBER	29.6	87.3	1.4	1.3	7.9
	7.1	23.8	0.2	0.4	0.4
CV	24%	27%	14%	30%	5%
HYPO-ALLERGENIC	30.1	94.8	1.4	1.3	8.2
	3.1	11.2	0.1	0.2	1.1
CV	10%	12%	7%	15%	13%
HIGHLY DIGESTIBLE	36.6*	109.1	1.4	1.5	7.3
	1.9	14.7	0.3	0.2	0.5
CV	5%	13%	21%	13%	7%

* indicates that the means are significantly different at $p \leq 0.05$ using ANOVA and Duncan's Multiple Comparison Test.
CELL HT - Cell height.
AREA - Area of the epithelial cell.
MV HT - Height of the microvilli.
BM - Width of basement membrane.
NO/WIDTH - Number of microvilli per width of the apical surface of the epithelial cell.

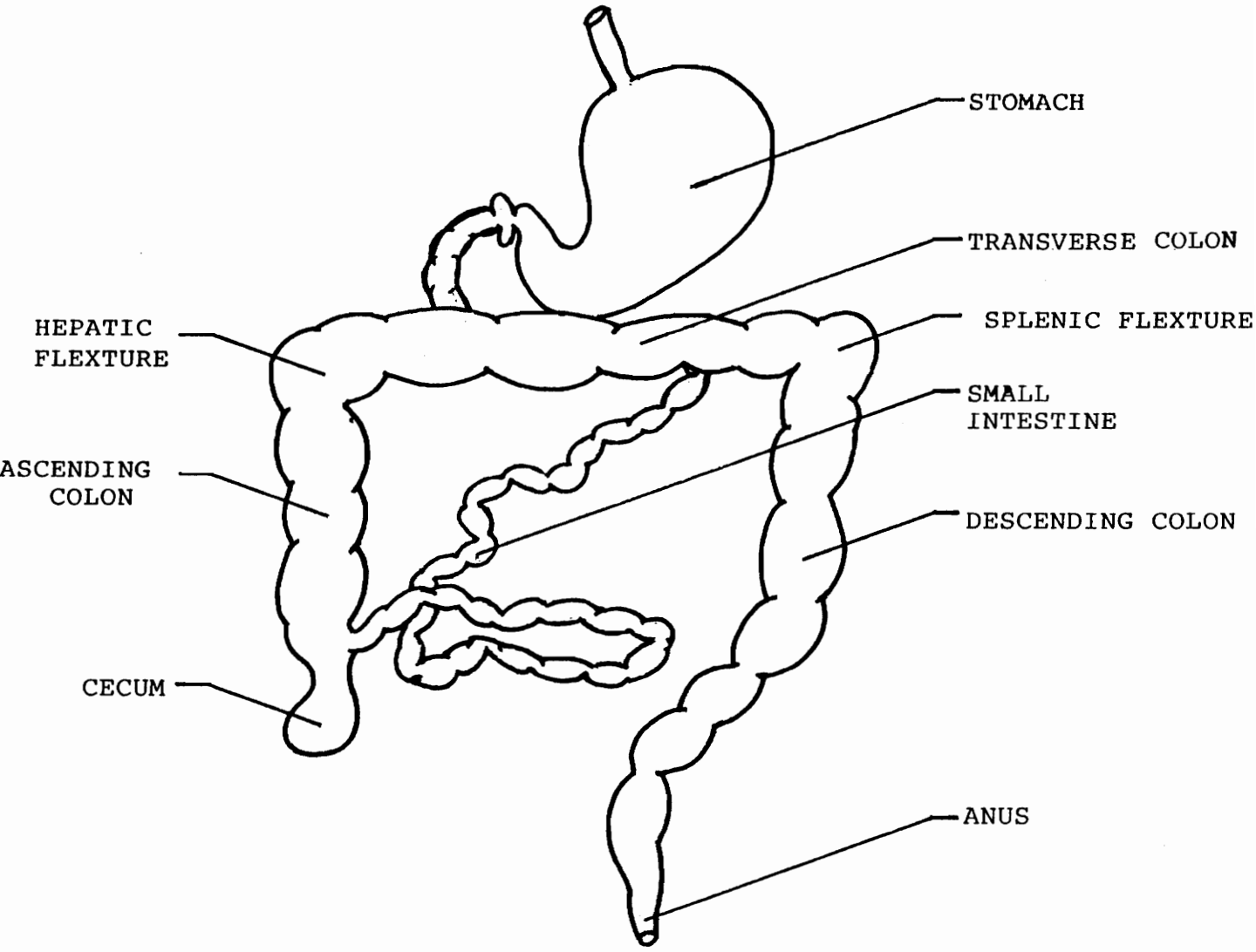

Figure 1. General anatomy of the canine colon.

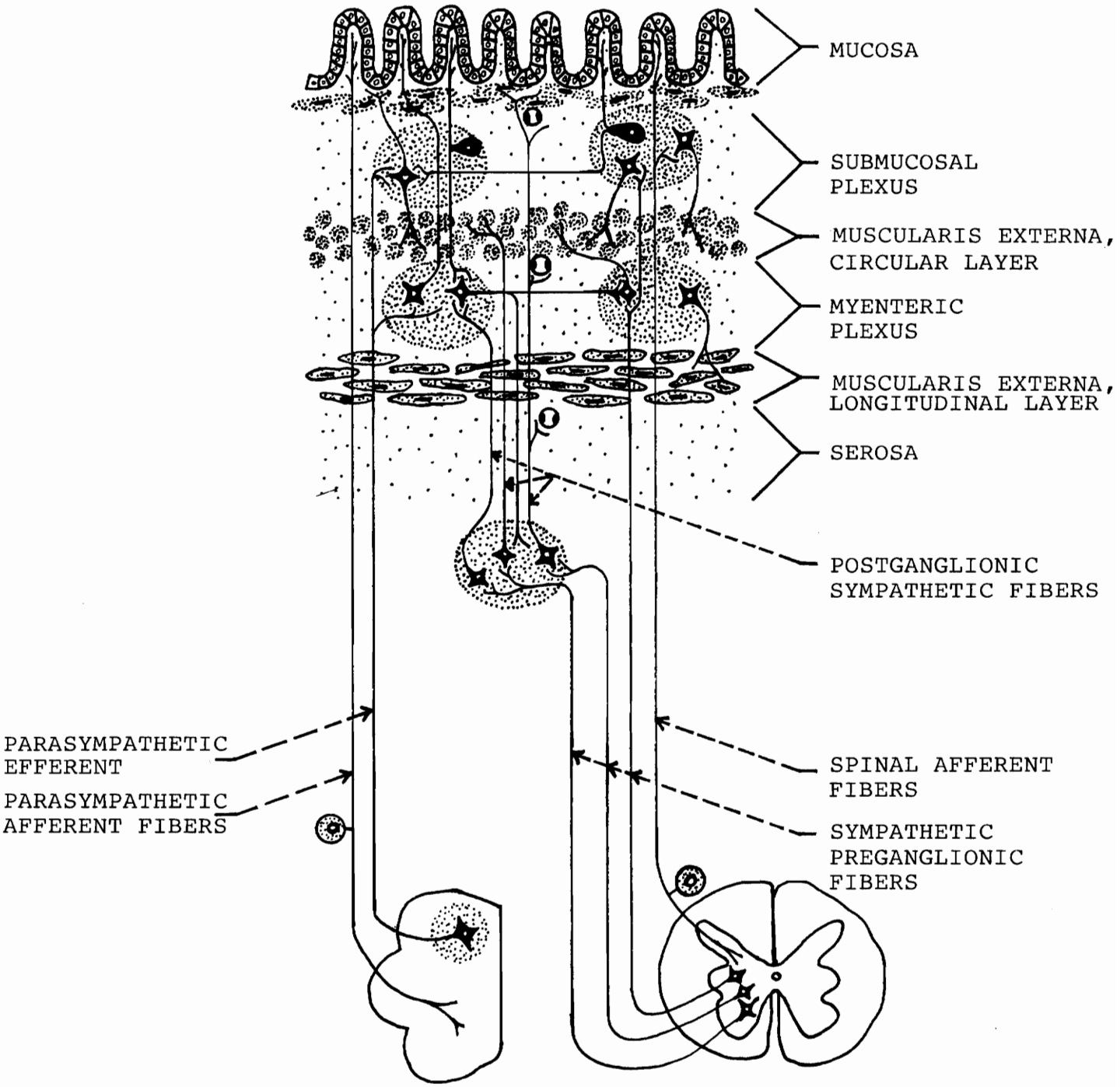

Figure 2. Extrinsic and intrinsic nervous system of the gastrointestinal tract. (Modified from: Scholfield GC, *Handbook of Physiology*, Vol IV, 1968; 1611.)

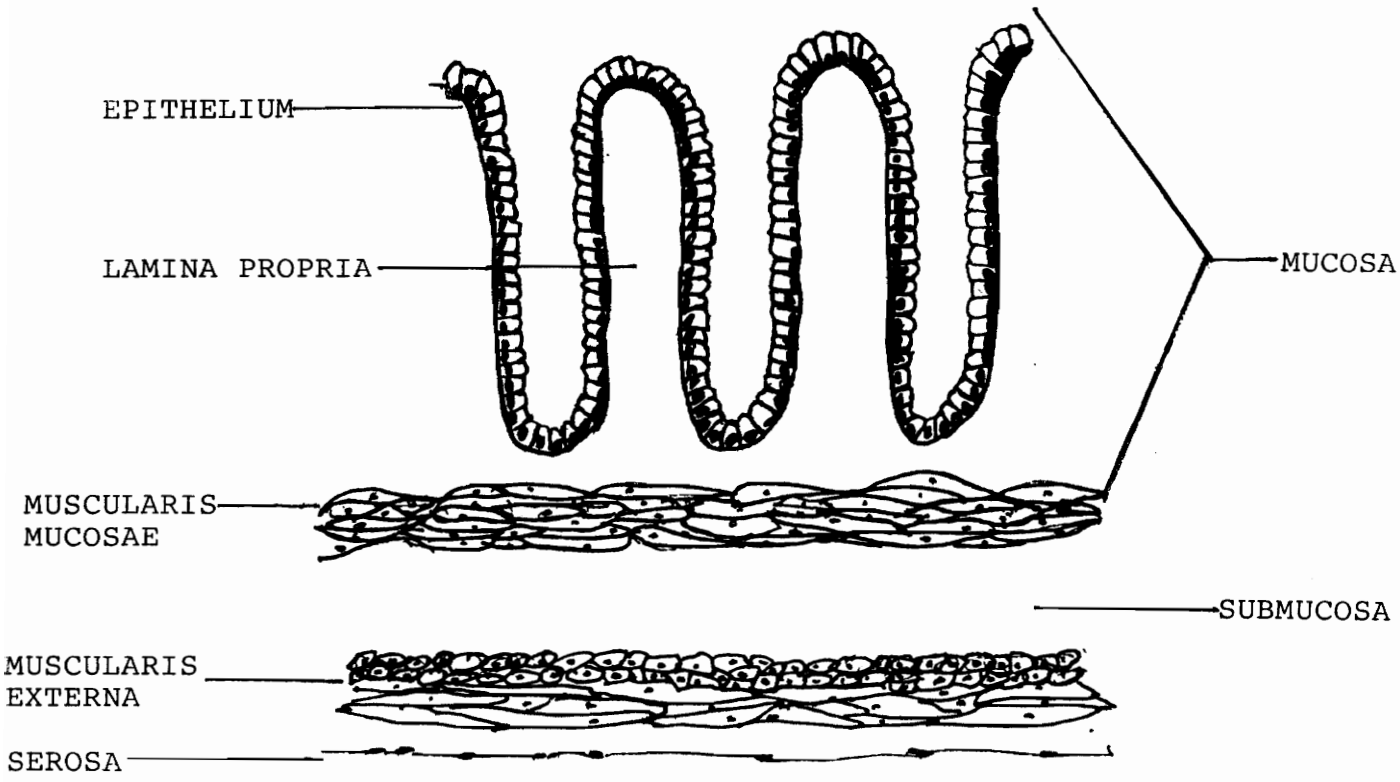

Figure 3. Layers of the colon. (From: Bustos-Fernandez L, ed. *Colon Structure and Function*, 1983; 5.)

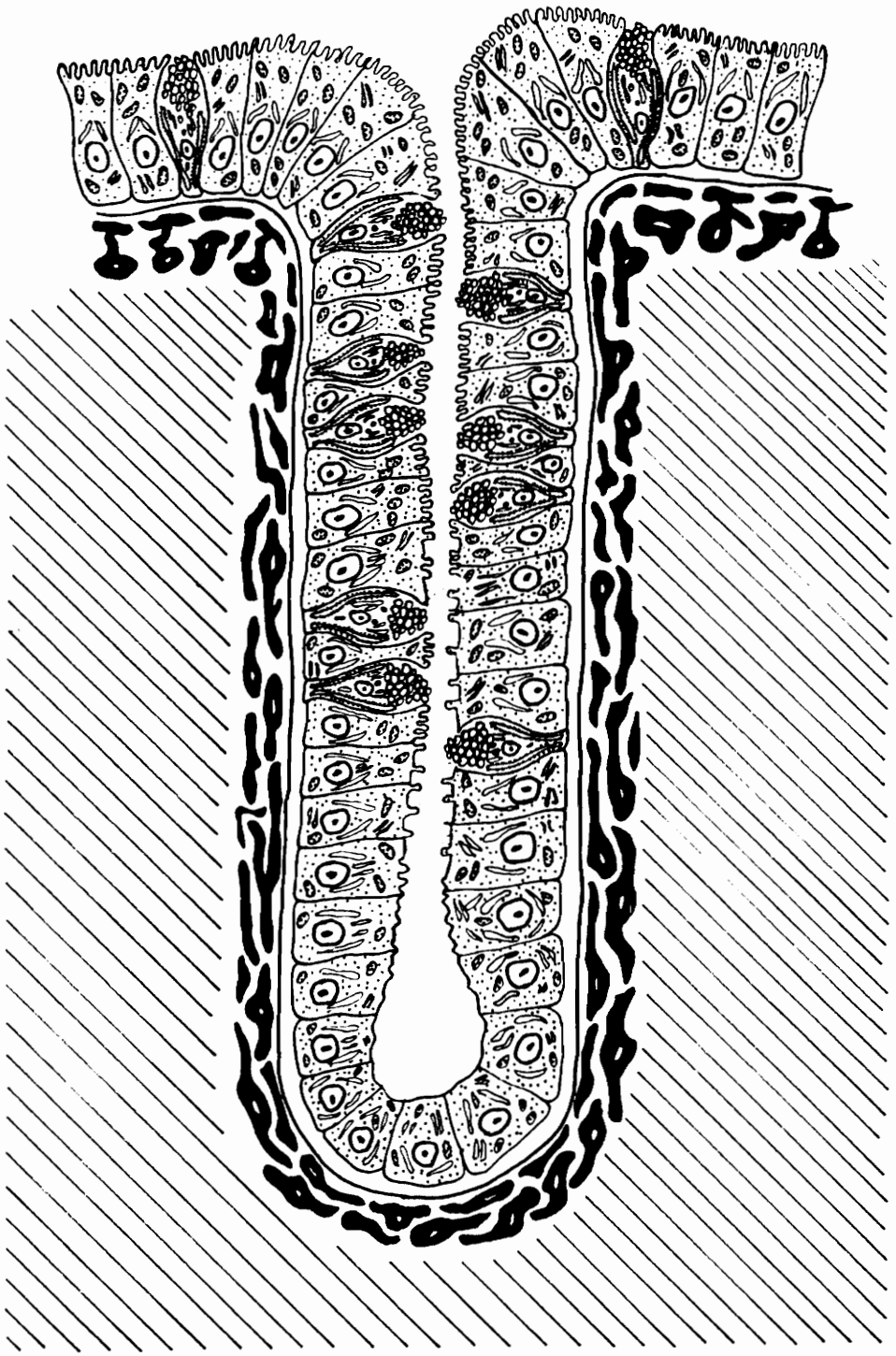

Figure 4. Schematic representation of the normal maturation and proliferation processes that occur alon the colonic crypt. See text for details. (Modified from: Bustos-Fernandez L, ed. *Colon Structure and Function*, 1983; 257.)

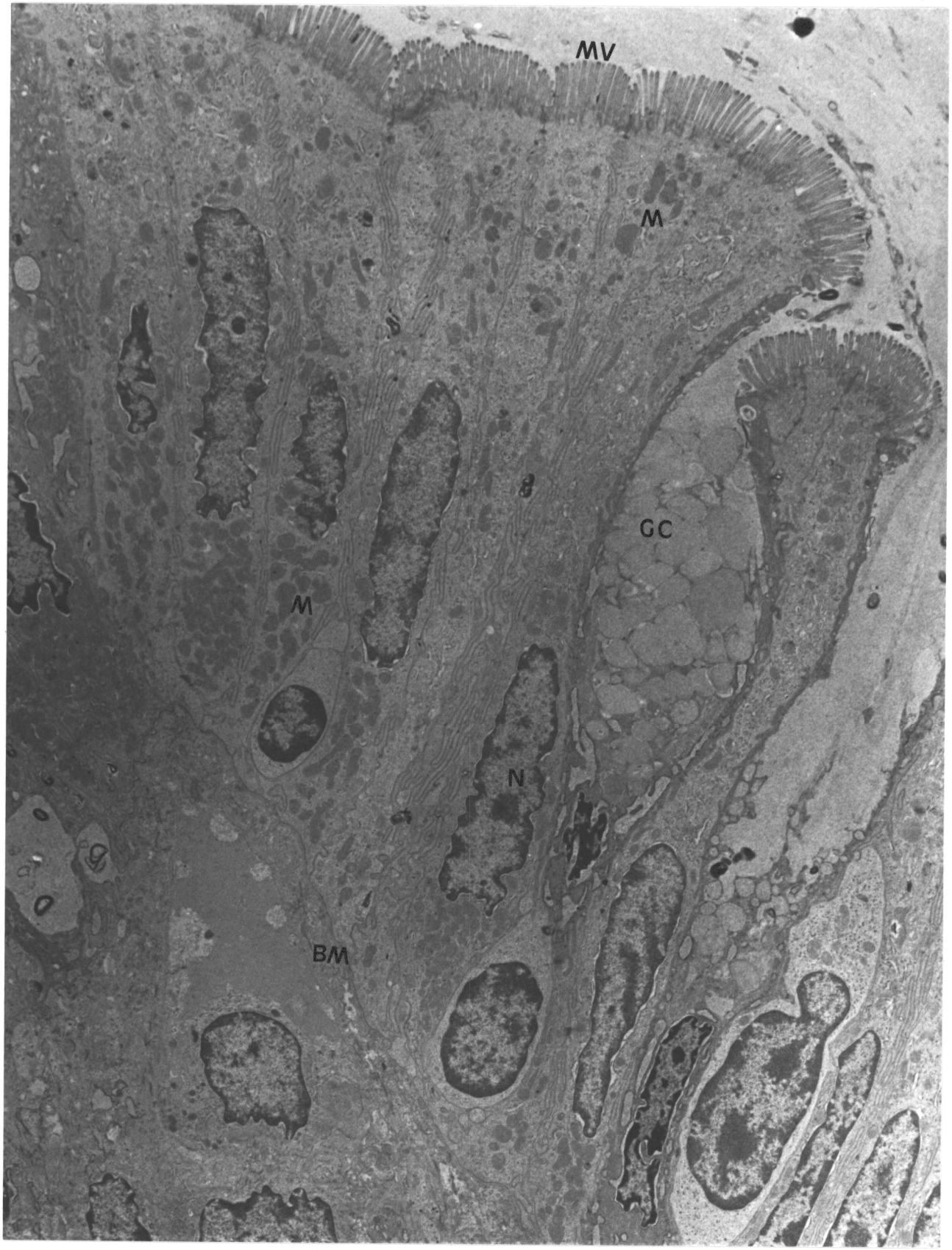

Figure 5. Photomicrograph of normal colonic epithelial cell. N, nucleus; M, mitochondria; MV microvilla; BM, basement memebrane; GC, goblet cell.

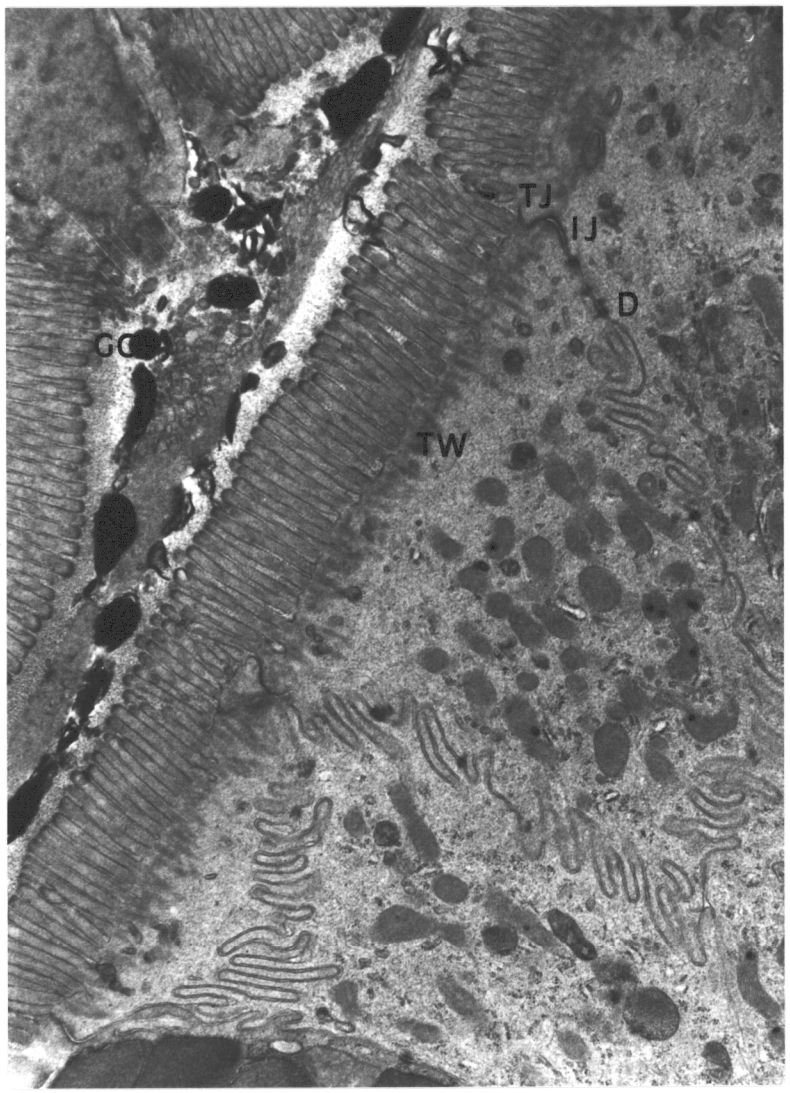

Figure 6. Junctional complex. TJ, tight junction; IJ, intermediate junction; D, desmosomes. Also showns GC, glycocalyx and TW, terminal web.

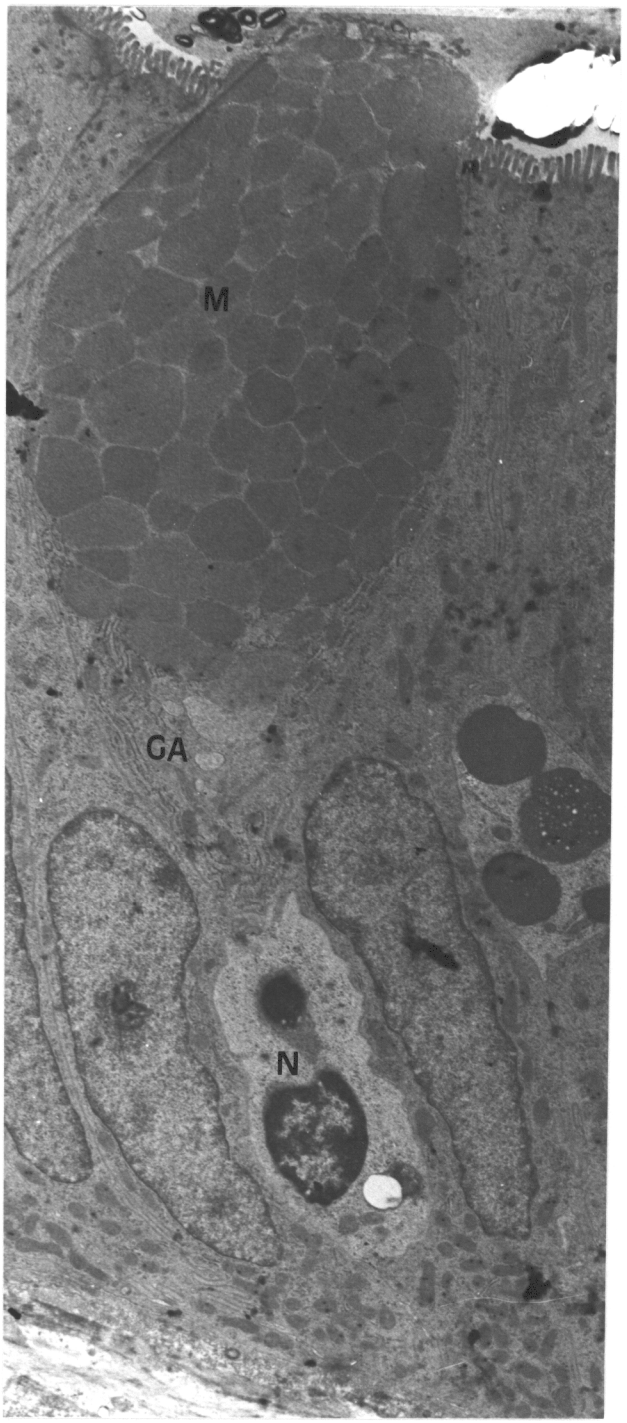

Figure 7. Goblet cell secreting mucin. Note prominent Golgi apparatus, GA; mucin, M; nucleus, N.

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