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LIGHT AND SCANNING ELECTRON MICROSCOPIC EVALUATION OF COLLECTION METHODS USED IN THE PRESERVATION OF CANINE INTESTINE

by

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To Martha who made it possible,

to Sam who made it joyful,

and to Debbie who made it meaningful.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF TABLES CONTINUED

LIST OF FIGURES

INTRODUCTION

One of the major diagnostic and research problems facing histologist, pathologist and student is distinguishing artifacts from changes due to pathological processes. What is seen with the microscope may not always be related to a pathological process or may not be normal. These changes are artifacts and this thesis deals with their presence in intestinal tissues. Artifacts may be due to poor collection methods, improper fixation or faulty processing. The purpose of this endeavor was to establish a method of preparing intestinal tissues which will minimize collection induced artifacts.

Perhaps no other tissues are as difficult to process free of artifact as those of the intestinal tract. The peculiar environment, functions and varied structures of the intestine make histological interpretation difficult. When artifactual changes are present the problem is compounded. With most tissues, standard collection, fixation and processing techniques are widely used, but the intestine is handled in a variety of ways. Each examiner prefers their own method and soon becomes familiar with the artifactual changes that are produced. The reason that no one method of collecting intestine has been widely accepted is unclear, but suggest that each has certain advantages and that no one method is superior.

This thesis is the first critical evaluation of commonly used methods of collecting intestine and documents the strengths and weaknesses of each. In providing information on artifacts produced by various collection methods, this study presents criteria for the selection of the best method of collecting intestine.

Five of the most commonly used methods of collecting intestine were examined. The fixation and laboratory processing techniques were maintained and by varying collection methods it was possible to evaluate which artifacts are produced and to what degree by each method. Statistical comparisons were made of the artifacts produced by each collection method as evaluated with light microscopy and substantiated with scanning electron microscopy. A pictorial presentation of the methods used, as well as the most commonly found artifacts, are included for reference.

LITERATURE REVIEW

Introduction

The problem of interpretation of pathological processes is dealt with daily by the histopathologist. A major obstacle to an accurate diagnosis is the presence of artifacts. Artifacts are defects or abnormalities in tissue that are caused by faulty techniques in collection, fixing or processing. Often, artifacts make distinguishing actual microscopic pathological changes in tissues difficult. In diagnostic histopathology, artifacts must be differentiated from pathological processes. Understanding the causes, locations and characteristics of artifacts would certainly make diagnosis easier.

The unique structure and function of the gastrointestinal system, as well as its internal environment, make diagnostic evaluation difficult. Additionally, intestine is prone to artifacts which commonly complicate accurate interpretation. Numerous techniques have evolved to collect and fix tissue specimens of intestine whereas other tissues, brain, eyes, liver and kidney are handled in well-accepted standardized manners. Before being able to understand the reason for the occurrence of the artifacts, or their significance and possible methods of preventing them, one must first appreciate the structure and functions of the intestinal tract.

Anatomy of the Canine Intestine

The architectural plan of the intestinal system is consistent throughout, with regional and species variation related to function. In this thesis, the dog was used and the duodenum, jejunum, ileum and colon were examined.

The duodenum begins the small intestine and is followed by the jejunum and ileum. The total length of the small intestine in the dog varies from 1.8 to 4.8 meters.[1,2,3] The function of the small intestine is to absorption of nutrients.[1,4] Beginning at the pylorus, the duodenum extends for approximately 10% of the total length of the small intestine and ends at the duodenojejunal flexure.[5] The jejunum is continuous with the duodenum, and ends with the beginning of the ileum at a rather undefined location. Most commonly, the ileum is thought to begin at the most anterior extent of the ileocecal ligament and the ileal branch of the ileocolic artery, both located on the antimensenteric surface.[6] The ileum terminates the small intestine at the ileocecal junction.

The cecum in the dog exists as a diverticulum at the junction of the ileum and colon, and designates the beginning of the large intestine. The large intestine in the dog, as opposed to the herbivors, is relatively short and unspecialized. In the dog, the large intestine functions to resorb water and store undigestable food and by-products.[5] The colon is continuous with the rectum without discernible demarcation. The rectum is arbitrarily designated to begin at the pelvic inlet and to end at the beginning of the anal canal.[6]

The microstructural anatomy of the intestinal tract is consistent, with morphological variations according to regional functions, in both the small and large portion of the tract. Even with the many structural and gross similarities in the regions of the intestine, the microscopic variations aid in identification of each portion. The wall of the intestinal tract is divided into four layers termed "tunics", the most internal being the "tunica mucosa" which functions as a barrier between the luminal environment and the other tissues of the tract, and additionally, is

involved in secretory and absorptive functions.[7] The "tunica submucosa" is interposed between the tunica mucosa and the tunica muscularis providing structural support for the mucosa, as well as containing blood vessels, lymphatics and nerves which serve the mucosa. The "tunica muscularis" provides motility for the movement of ingesta down the tract. The most distant layer from the lumen is the "tunica serosa" which prevents overextension of the intestine and aids in the movement of the intestine within the abdominal cavity.[2,7] Regional variations occur in these tissue layers depending on the function of that segment of the intestine.

The "tunica mucosa" is made up of a lining epithelium overlying the lamina propria which contains glands and a muscular layer. These layers are most pronounced in the duodenum and form the structure of the villus which are a hallmark of the small intestine.

The lumen of the intestinal canal is lines by a simple columnar epithelium of three cell types. The most prominent are cells responsible for absorptive and secretory functions and have microvillus projections on their luminal surface. Interspersed among the absorptive cells are goblet cells which produce mucin and are most abundant in the distal regions of the intestine to a point that they dominate in the colon. Occasionally, a third cell type, argentaffin cells, are also present. These cells have endocrine functions.[2] The cells of the epithelium are held together by junctional complexes and rest on a basement membrane. These act as semipermeable membranes for absorption of nutrients.[8]

The lamina propria is composed of loose connective tissue and makes up the core of the villus. Within this layer are located numerous blood vessels and aggregates of lymphocytes. Occasionally, nodules of lymphocytes are present. These lymphoid nodules increase in number in the

distal regions of the intestine. The muscularis mucosa is composed of two layers of smooth muscle and is more prominent in the dog than other species.

The villus is a specific characteristic of the small intestine and are most developed in the duodenum and anterior jejunum. Within the villus is a single lymphatic capillary, in addition to well developed longitudinally oriented smooth muscle fibers. The muscle causes the villus to shorten and lengthen, as well as provides lateral movement. These muscles are undoubtedly a major driving force for moving lymph down the central lymphatic, the "lacteal".[1,5,7] Additionally, contraction of the muscles in the villus aids in pumping blood during absorption which causes vascular congestion throughout the villus. Shortening of the villus is thought to cause the surface epithelium to fold on itself forming concentric ridges around the villus.[7,8,9,10] The length of the villi are longest in the duodenum and gradually shorten in the jejunum, and finally, in the colon are not present at all. Throughout the large intestine there is a uniform luminal surface.

The submucosal tunic is composed of bundles of collagen and elastic fibers. Within this layer are tubuloalveolar glands, Brunner's glands, which are mucous-secreting in the dog and located in the first 1.5 to 2 centimeters of the duodenum.[11] Solitary accumulation of lymphatic tissue are also present within this tunic, being most numerous in the ileum, cecum and colon. Arteries, veins and nerve plexuses are located in this layer in all regions of the intestinal tract.

The tunica muscularis consists of two layers of smooth muscle, the inner being circular and the outer being longitudinally oriented. Between these muscles are parasympathetic neronal plexuses. Contraction of these

muscles generates peristaltic movements which propel food through the intestine.[1]

The most external tunic is the tunica serosa which contains connective tissue with numerous elastic fibers. A mesothelial layer overlays the connective tissue and comprises the visceral peritoneum.

Intestinal Collection Methods

The collection and fixation techniques used in preserving most tissues have been standardized and are generally well-accepted, the only major exception is the intestinal tract. Tissue samples taken from the intestine are handled in numerous methods. These methods appear to be more a product of habit than for reasons of optimal preservation. At this time no one method for collecting and fixing intestinal tissue has emerged as superior, and no quantitative studies have been reported to answer the question as to which method is best. Unfortunately, related histopathological studies involving the intestine omit the collection and fixation techniques from their procedural descriptions.[12] Perhaps more interesting is the fact that most pathological technique monographs fail to address the question of preferred method of collection intestinal tissue samples, and if they do so, no defense of the technique suggested is given.[12-17]

Techniques for examination and removal of the intestinal tract from the cadaver vary considerably. The most consistent suggestion is the placing of ligatures around the intestine just anterior to the stomach and as distally as possible around the colon.[12,13,14] Avoiding excessive handling fo the intestine is also commonly recommended.[15,16,17] The techniques for collecting intestinal tissue samples that have been decribed or are being used in routine diagnostic pathology are: 1) opening the

intestine and pinning it flat to cork or wax sheets with the serosal surface down and floating it face down in the fixative,[18,19] 2) placing the intestine on filter paper with the serosa down and placing it into the fixative,[19] 3) ligation of a region of intestine and injecting fixative into the lumen,[13] 4) opening the intestine and rolling it around in a large gauge needle,[20] 5) simply placing the opened intestine in fixative and 6) leaving the intestine unopened.[14] All the collection techniques preserve the microstructural characteristics of the intestine, but have not been compared. Undoubtedly, all the various collection techniques can be used successfully but the question of which is supeior, if any, remains to be answered.

Histological Artifacts

The presence of artifacts in histosection is a common occurrence. Artifacts are the result of improper techniques in the collection, fixation or processing of the specimens. Additionally, certain tissues, due to their components, are predisposed to artifacts. The techniques used in the histological laboratory to process tissues are dehydration, embedding, microtomy, mounting, staining and coverslipping; all which may produce artifacts if done improperly. These procedures have been standardized and are routinely followed with all tissues. However, methods of collecting and fixing tissue samples vary according to individual preference and the tissue of interest. There is no technique which is totally artifact-free but with familiarity with the most common artifacts, one can distinguish them from normal structures or pathological changes.

In general, the intestine is prone to artifacts, but only the artifacts that occur in the epithelium have been cited to any extent in the

literature.[21-25] The epithelium is an extremely important tissue in relation to diagnostic criteria in evaluation of intestinal disease. Unfortunately, the epithelial layer is the location of numerous artifacts; the most common being shedding of cells from the underlying stroma.

It was originally found that the nitrogen content of the intestine in sheep increased after death and it was thought to be due to the shedding of the epithelial cells into the lumen of the tract.[21] The shedding of the epithelim was found to occur within 10 minutes after death in sheep,[22,23] as little as three minutes in a calf[24] and instantaneously in the rat.[24] Generally, it is believed that the shedding of cells is a simple terminal event and that by collecting intestinal samples from anesthetized animals this artifact can be avoided.[25] In the pig autolytic activity was found to follow a time schedule similar to that demonstrated in other animals.[26] In the pig the first diffuse change in the epithelium was noted at a mean time of 24 mintues after death.[27]

Other authors believed that the shedding of the intestinal epithelium is due directly or secondarily to anoxia induced muscular contractions and that perfusion-fixation would prevent the shedding.[27] Additionally, it has been speculated that heavy blood loss, major bone fractures and recent feeding will all exaggerate the loss of the epithelium.[25] A gradient has been shown along the alimentary tract in the time after death that the epithelium is lost.[25,27] With the epithelium being first lost in the duodenum, then the jejunum and rarely is loss of epithelial cells seen in the cecum, colon or rectum.[25,27] One author suggests that the loss of the epithelium is due to contraction of the villus which occurs during fixation and results in the withdrawal of the stroma from the epithelium covering the villus.[28] Most authors think that the autolysis that occurs

within the intestine is accelerated and the epithelium is lost due to the presence of digestive enzymes and bacteria.[21,22,23,25]

The microscopic features that take place during the shedding of the epithelium have been well-described.[25,27] First there is the formation of sub-epithelial spaces being most pronounced at the tip of the villus and gradually extending to effect approximately one-third of the total length of the villus. As sub-epithelial spaces develop along the sides of the villus sheets of cells are striped off.[27] Cellular individualization is noted, as well as loss of stain affinity and nuclear pyknosis. These changes are characteristic of cellular death that occurs with autolysis.[29]

Denuding of the villus, especially at the tip has been described as part of a pathological process,[30,31,32] and as an artifact of delayed fixation and autolysis even at the height of severe intestinal disease.[33] In calves with diarrhea, it is speculated that autolysis is more severe and possible secondary desquamation is more prominent.[24]

Three artifacts present in the intestine due to laboratory preparation of the tissue have been described.[34] Artifact are produced if there is inadequate infiltration of paraffin resulting in the tissue not staining well and being highly distorted. The use of a dull microtomy knife causes compression artifacts in which the cells are distorted, a venetion blind effect in which the tissue varies in thickness, and thick and thin areas where nicks or dull areas are present in the knife. The trapping of air under the coverslip results in a glassine stippling effect. An artifact not described for intestine but which occurs in other tissues is wrinkling. This may be due to the inherent nature of the tissue or improper laboratory procedures. Most commonly, wrinkles in tissues are due to the tissues being softer than the paraffin used in the embedding

step. The harder paraffin will restrict the expansion of the tissue when sectioned and cause pleating and wrinkles.[35] Also, the presence of tissues or tissue components with different hardnesses on the same slide may cause wrinkling for the same reason.

The procedures used in the collecting and processing of tissues to be examined with the scanning electron microscope (SEM) are well-described with comparable results being obtained with each technique. The intestine is no exception. Characterization of the surface structures of the intestine and the techniques used have been described by several authors.[36,37,38] Due to the lack of literature pertaining to the artifacts produced during the collection of the tissue samples, the only information found was of normal intestine collected using standardized techniques. The techniques used are either that the tissue is pinned to a ridged surface prior to fixation or placed directly into the fixative.[39,40] The fixatives used included gluatraldehyde, osmium tetraoxide and various combinations. The use of 10% buffered normal formalin (BNF) alone to fix tissues for SEM was not found in the literature.

Scanning Electron Microscopy of Intestine

Scanning electron microscopic examination of intestinal tissues have been done in the calf.[41] Surface characteristics correlated well with light microscopic findings by the same author in the calf due to autolytic changes. The changes noted using the SEM were swelling and denuding of the villus tips which were related to the development of sub-epithelial spaces as noted with the light microscope. Other authors have suggested that the presence of piled epithelium at the villue tip is a consequence of normal desquamation of the villus epithelium.[42,43,44] The presence of

horizontal folds of epithelium along the length of the villus is considered to be caused by villus contraction and may be normally present.[45,46,47]

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LIGHT AND SCANNING ELECTRON MICROSCOPIC EVALUATION OF COLLECTION METHODS USED IN THE PRESERVATION OF CANINE INTESTINE

INTRODUCTION

It is important for the histologist and pathologist to be able to differentiate changes in tissues due to processing and handling techniques from actual alteration due to disease as well as from normal structures. Artifacts often abscure or make accurate interpretation of a disease process difficult. Methods of preparing tissues for histological examination have been developed to limit artifacts, however each method has inherent artifacts for a number of reasons. The intestinal tract is prone to a number of artifacts due to its variable structure, specialized function and delicate nature.

Artifacts are common in intestinal tissue sections, some being more prevalent within a given area of the tract or in different layers of the intestinal wall than others. These artifacts may be due to various things, the most common being poor collection methods, improper fixation or incorrect processing techniques; embedding, sectioning or staining.[1] The fixation and processing methods are uniformly applied to almost all tissues, the intestine is no exception. However, intestinal collection techniques vary widely.

Numerous methods of collecting intestine are in use whereas standard techniques are used to collect other organs. This study was undertaken to determine if there is a significant difference in the histological quality of intestinal samples using various collection techniques. The areas of the digestive canal examined in this study were the duodenum, jejunum, ileum and colon. All tissues in the study were from adult dogs, but comparisons can be drawn to other species with noted exceptions. Standard fixation and processing methods were used.

MATERIALS AND METHODS

Experimental Design

Twelve adult clinically normal, foxhound-cross dogs of both sexes from two litters, weighing 19.5 to 25 kg., were used in this study. The dogs were divided into two groups. Ten of the dogs were killed with T-61[a] and intestinal samples immediately collected. The remaining two dogs were anesthetized with Surital[b], prefused intravascularly with 10% buffered neutral formalin (10% BNF) and immediately necropsied. The time between death and placement of all the tissues in 10% BNF was 15 to 22 minutes with a mean of 19 minutes. The fixative used in all cases was 10% BNF at room temperature.

Tissues were collected from the duodenum, jejunum, ileum and colon. Five different tissue samples were taken from each region of the intestine. Tissue samples were seven centimeters long when possible, but when the tissue available was not of sufficient length to collect five seven centimeter sections, the tissue was divided into equal portions. The five collection methods selected were the most commonly used by diagnostic pathologists. Each method was applied in a random order to each of the intestinal regions (Table 1). Samples were collected sequentially. The result was 20 intestinal tissue samples collected by five different techniques from four different areas of the intestinal tract. A total of 240 tissue samples were collected. Each dog was routinely examined after the intestinal samples were collected and histopathology was performed on the major organ systems.

Each intestinal tissue sample was processed routinely and histologically evaluated for the presence of artifacts. The artifacts

found were classified as to type, severity, location in the intestinal wall and region of the intestine where the sample was collected. This allowed for a rapid comparison between collection methods. Statistical analysis was performed on each of the parameters to evaluate the superiority of one collection method over another.

Scanning electron microscopic (SEM) examination was done on one perfused and one nonperfused fixed intestine. Correlations between selected artifacts noted on light microscopic examination and SEM were made to evaluate the possible use of SEM as a routine diagnostic procedure on formalin fixed tissues.

Tissue Collection Methods

After the dogs were killed, a ventral midline incision extending from pelvis to sternum was made in the abdominal wall. The esophagus was then isolated just caudal to the diaphragm and ligated. The colon was ligated as distally as possible and the entire intestinal tract removed by severing the mesenteric attachments (Appendix Fig 1-3). Tissues were collected from the duodenum, mid-jejunum, ileum and colon. After the intestinal tract had been removed and samples collected, a standard necropsy was completed and samples of major organs taken for histological examination.

Five methods of collecting tissues were randomly used in each region of the intestine (Table 1). The methods used were:

Method 1

> Longitudinal incision along the antimesenteric border and the ends of the intestine stapled to a wooden tongue depressor and placed in 10% BNF (Appendix Fig 4).

Method 2

Ends of the intestine ligated and the lumen injected with 10% BNF until slightly distended and placed in 10% BNF (Appendix Fig 6).

Method 3

Longitudinal incision along the antimesenteric border and then placed on a dry paper towel with the serosal surface down, then placed in 10% BNF (see Appendix Fig 5).

Method 4

Intestine not longitudinally incised or ends ligated before being place in 10% BNF (see Appendix Fig 19).

Method 5

Longitudinal incision along the antimesenteric border and then placed directly into 10% BNF (see Appendix Fig 20).

Tissue samples from all the collection methods were submerged in 10% BNF at room temperature and held at least 10 days before being trimmed for histological processing. The tissues were trimmed with a razor and fine thumb forceps from the center of the tissue sample (Appendix Fig 7-18). Handling of the tissues was kept to a minimum. The length of the trimmed tissue was approximately two centimeters and three millimeters wide (Appendix Fig 21).

The tissues were dehydrated through graded ethanols, cleared in xylene and embedded in paraffin in an automatic processor[c], cut at 6 microns, mounted on glass slides, stained with hematoxylin and eosin (H&E) by an automatic slide processor[d] and covered with glass coverslips.

The dogs (Numbers 11 and 12) were anesthetized with Surital[b] and heparinized. Catheters were placed in the jugular veins and warm saline perfused through a 12 guage needle into the left ventrical of the heart until saline appeared in the jugular catheters. At that time 10% BNF was perfused into the heart until noted in the jugular catethers. The dogs were then immediately necropsied with tissues collected as previously described.

Histological Examination

All tissue layers of the small and large intestine were used to evaluate the extent to which artifacts were produced by each collection method. Histological examinations were performed on all tissues that were collected without knowledge of the collection method used or area of the intestine being examined. The tissue sections were graded as to the severity of each artifact on a 0 to 3-plus system based on visual comparisons. Figures 5-60 are provided for reference. Mild artifacts were graded 1-plus, moderate artifacts were 2-plus, marked changes were graded 3-plus, and when no artifacts were present a zero score was given. The artifacts were divided into five categories: autolytic, folding separation between or within tissue layers, fractures within tissue layers, and miscellaneous. Each category was additionally divided according to location: serosa, outer or inner muscularis, submucosa and mucosa. The tissue samples were only graded in the center of the tissue some over an area the width of two low power fields (40X). On the tissue sections from collection method 5, two low power fields opposite each other were graded because these tissues were sectioned so that an intact circle, cross section of the intestine, was present on the slide.

Scanning electron microscopic (SEM) examinations were done on two dogs, one of which was perfused with fixative. Comaprison of the surface characteristics at various levels of intestine were made with the light microscopic findings. Tissues to be examined under the scanning electron microscope were collected in 10% BNF and trimmed to approximately .5 x .5 centimeters. These tissues were dehydrated through a series of ethanols to absolute ethanol and critical point dried. The tissues were then attached to cambridge stubs using silver colloidal paste and sputter coat-

ed with gold at a distance of (15 mm) for 360 seconds at a tension setting of 8.

Artifact scores were compiled according to collection method and region of the intestine. Statistical analysis was performed using a PMNZV computer program[2] in which a multiple comparison of the means were made using the Duncan multiple range procedure.[3]

Photomicrographs

All photographs were taken with an automatic 35 mm camera[f] mounted on a Leitz Orthoplan microscope.[f] Color Kodak film 2483 was used with a blue 80B filter series VII and when needed a yellow CC10Y filter.[g] The camera magnification factor used was 3.2X. The tissues prepared for SEM were examined under a Hitachi-Scanning electron microscope[h] using 20 KVP, at a working distance of 13 mm and were photographed.

Footnotes

[a]T-61, American Hoechst Corp., Somerville, New Jersey.

[b]Surital, Park-Davis and Comp., Detroit, Michigan.

[c]Autotechnicon, Technicon Corp., Chayncey, New York.

[d]Histotek, Ames Corp., Div. Miles Laboratories Inc., Elkhart, Indiana.

[e]Edwards S15DA, Edwards High Vacuum, Manor Royal, Crawley, West Sussex, England.

[f]Orthomat, Leitz Inc., Rochleigh, New Jersey.

[g]Wratten, Eastman Kodak Corp., Rochester, New Jersey.

[h]NSA Hitchi LTD. H-300 with H-3010 S.E.M., Mountain View, California.

RESULTS

Total artifact scores for each region of the intestine are summarized in Table 3 and illustrated in Figures 1 through 4 in the form of histograms. Artifact totals by collection method are presented in Tables 4 through 8 and summarized in Table 9. Comparisons of collection methods by intestinal region are made in Tables 10 through 13. Individual tissue scores for each dog by tissue and collection method are found in Tables 14 through 33. Examples of various artifacts and scores are provided in Figures 5 through 60.

The first comparison was between regions of the intestine. No significant difference was found to exist between duodenum ($\bar{x}$ = 13.0) and jejunum ($\bar{x}$ = 7.8). However, a significant difference existed between the duodenum and jejunum when compared to the ileum and colon at a confidence level of 97% for all comparisons made.

The second comparison was between collection methods. The combined artifact totals (total of artifact scores from all locations and of all types) for each collection method were analyzed. Method two ($\bar{x}$ = 6.3) was significantly superior, as measured by total artifact score, in preventing artifacts than the other collection methods at a confidence level of 98%. Collection method numbers one ($\bar{x}$ = 11.), three ($\bar{x}$ =10.2) and five ($\bar{x}$ = 9.8) were found not to be significantly different at a 98% confidence level. Additionally, collection method four ($\bar{x}$ = 14.7) had significantly higher artifact scores at the same level of confidence. Further analysis indicated that the total artifact scores of the collection methods were not significantly altered by comparisons within the various regions of the intestine.

A final statistical comparison was made to examine the data for interaction between the two variables, tissue and method (Table 1). The interaction was found to be 0.0169 and was judged not to be of sufficient magnitude to alter the conclusions of the preceding analysis.

Comparisons between the tissue artifacts that were found in tissues collected from animals that had been perfused with fixative and those which had been handled in a more routine manner showed no significant difference in total artifact scores. The SEM finding between perfused and not perfused animals were also similar.

The SEM examination of the intestinal tissues using the various collection methods were compared with the finding of previous studies.[4,5,6] No objective differences in any region of the intestine were noted between the collection methods. There was good correlation between the light microscopic findings and the surface characteristics as shown by the SEM. Horizontal fissures on the surface of the villi were noted in all areas of the duodenum and jejunum but were most prominent in the anterior most segments (Fig 61). The end of several villi were enlarged and rounded (Fig 61). In addition, many villi had lost epithelium exposing the lamina propria (Fig 62, 68). Areas of separatin were noted between epithelium and lamina propria at the margins of the denuded villus tips (Fig 64,73,74). A gradation in the frequency of villus desquamation and swelling was noted, with the duodenum being most severely affected and the jejunum only occasionally showing similar changes. The gradual decrease towards the more distal regions of the intestine was also true for the horizontal ridges around the villi.

DISCUSSION

A significant difference does exist in the commonly used methods of collecting intestinal tissues when compared by their artifact frequencies and severity. The primary question to be answered is just why is one method superior in preserving the histological architecture relatively more artifact-free than another? In order to understand possible reasons for the variations in artifact severity between collection methods, an examination of each method individually, as well as a general comparison, would be helpful. It must be remembered that a difference in the artifact severity exists between the various regions of the intestine. Possible reasons for this tissue dependent artifact frequency variation will be discussed. Finally, a comparison between the surface artifacts as seen by SEM and the light of microscope may provide an explanation as to the cause of certain artifacts.

It must be recognized that the artifacts noted in the evaluation of the tissues could have been due to incorrect processing, embedding, microtomy, mounting, staining or coverslipping. With little doubt, a portion of the changes noted were in fact produced in the histology laboratory. To account for this problem a large number of tissues were examined with all being processed by the same technician over a short period of time in the same laboratory and using the same equipment, chemicals and procedures. The tissues were processed in a random order to insure that laboratory-induced artifacts were as evenly distributed as possible. All tissues were handled in exactly the same manner except for the method of collection. Thus, any significant differences in the

distribution, severity or frequency of artifacts could be contributed to the collection methods used and not to processing techniques.

Tissues that were collected from the intestinal tract using method two were found to have significantly fewer artifacts than the other methods examined. In this collection method the tissue was first ligated at the ends of the sample and the lumen injected with fixative. The entire sample was then submerged in fixative. This collection method provides a great deal of protection to the histopathologically important mucosal surfaces as well as limits distortion and artifacts due to contraction of the muscle in the outer layers of the intestinal wall during the fixation process. After fixation, the tissue samples generally maintain their normal gross appearance. This allows for greater ease in trimming the tissue prior to processing. One distinct disadvantage to this method is that the mucosal surface can not be inspected before collection and isolated lesions may be missed.

The first, third and fifth methods of collecting intestinal tissues in the project all involved opening the sample along the antimesenteric border and either stapling the ends of the tissue to a tongue depressor, laying the sample on a dry paper towel with the serosal surface down or placing the tissue sample directly into fixative after opening. These methods all allow for visualization of the mucosa with identification of lesions, and thus, selective tissue sampling. All of these techniques were statistically equal in their frequency of artifacts. However, with the mucosal surface exposed, artifacts in this tissue layer are more common in these collection techniques than in method number two where mucosa was protected. Even though the total artifact frequency was not significantly different between these three techniques, the type of artifact did vary.

In the first collection method, artifacts in the fracture category were more common. This is likely due to contraction of smooth muscle in the tissue walls and pulling against the stationary, stapled, end of the tissue. In the third method, the tissue ends are free to move with muscular contractions and fracture artifacts are less common, but foldings and tissue layer separations were more noticeable. Additionally, in the third method more separation of the serosa was found and is thought to be due to the adherence of this tissue layer to the dry paper towel. There may be little histopathological significance to this artifact except in cases of peritonitis.

When tissue samples were placed directly into the fixative unopened, as in the fourth method, the number of total artifacts was signficantly higher than in any of the other collection methods. This method has a combination of the problems seen in the other procedures. First, it does allow for visualization of the mucosal surface but folding and separation type artifacts are more common. In addition, autolysis involving the mucosal surface was more common and most likely due to poor fixation because of inadequate fixative penetration through the constricted lumen and the presence of ingesta. A complicating feature of the technique is that there is often more difficulty in trimming the tissue and it is thus more prone to laboratory induced artifacts.[1]

A comparison of the five collection techniques used in this project shows that they all have certain advantages and disadvantages. Method number two, having a significant lower number of total artifacts, is recommended but is more time consuming, takes additional equipment and does not allow for visualization of mucosal lesions. Methods number one, three and five have about the same number of artifacts but of different

types and the use of each must be selected with the tissue layer of interest in mind. In these methods the amount of time needed to collect the sample is approximately equal and they allow for mucosal inspection. The fourth method has many of the disadvantages noted in the other methods and a significantly greater frequency of artifacts, and thus, is not recommended. The only possible advantage to method number four is that the tissue can be collected rapidly and with little manipulation.

In exploring the number of artifacts induced by the various techniques it was found that the anterior small intestine, duodenum and jejunum, had a significantly higher incidence of artifacts than the ileum and colon. The reason for this difference in the regions may be due to many factors, but two, structure and function, seem to be the most likely. The anterior small intestine plays a more active role in digestion and thus contains more enzymes than the more distal regions of the tract. In the ileum and colon little active enzyme is found.[7] In the distal intestine, water conservation is the primary function and little digestion occurs. The structure of the two regions also varies considerably with the anterior portion of the tract being more muscular and containing larger and much more distinct villi, where in the ileum and colon relatively fewer muscle fibers are present and are more elastic plus there is a smooth mucosal surface.[8]

The microscopic surface characteristics of the tissue samples correlates well with the light microscopic findings and indicates that tissues collected in 10% BNF can be used for both SEM and light microscopic examinations. The most distinctive features present on the scanning electron examination were horizontal ridges around the villi, shedding of the epithelial cells at the villus tips and the presence of

sub-epithelial spaces that were also recognized with the light microscope. The horizontal ridges are thought to normally be present[9] with the formation of sub-epithelial spaces and subsequent desquamation being a pathologic,[10] autolytic,[11] or fixational[12] process. In this study, these findings were found to be most common and more pronounced in the duodenum.

Horizontal ridging, sub-epithlial spaces and shedding of epithelium are not believed to be due to a pathological process since the tissues were collected from healthy subjects and no histological reaction was present in any of the tissues examined that suggest a disease process is present. Autolytic changes were observed in several tissue samples and were characterized by the loss of villus epithelium which, however, were not the same microscopically from regions where the epithelial cells had been lost for other reasons. In the autolytic areas, the epithelial cells were undergoing individualization, nuclear pyknosis and were generally more basophilic, whereas in the nonautolytic areas, the desquamating cells were being shed in long ribbons, were commonly not undergoing separation or showing intracellular indications of autolysis. This suggests that the formation of horizontal ridges, sub-epithelial spaces and epithelial shedding are fixation-induced artifacts and not due to autolytic activity.

During fixation, proteins are coagulated and muscular contraction occurs due to the cross-linking of proteins. Within the villus, especially those in the duodenum, are vertically oriented smooth muscle fibers. Contraction of these muscle bundles is thought to control the length and movement of the villus during life.[7] During muscle contraction the epithelium is pushed into folds due to its being relatively inelastic. During fixation, muscular contraction may exceed the ability of the epithelium to fold and still remain attached to the lamina

propria. The result can be seen both with the light and scanning electron microscope in the formation of sub-epithelial spaces and shedding of large ribbons of epithelial cells, plus possible exaggeration of the horizontal ridges. The variable tissue collection methods, or perfusion fixation, showed no recognizable effect on this process. But, the loss of epithelium, sub-epithelial spaces and horizontal ridges in the epithelium around the villi was more apparent in the duodenum than the more distal regions of the intestinal tract. This is reasonable since the villi in duodenum are longer and contain more muscle than those located in the jejunum.

SUMMARY

Analysis of the frequency of collection-induced artifacts indicates that of the commonly used methods of collecting tissues from the intestinal tract, one is signficantly superior. When intestinal tissues are collected by ligting the ends of the segment of interest and injecting the lumen with fixative prior to submersion in fixative, the number of artifacts are significantly fewer than with other collection methods. There is no significant difference in the artifact frequency between three other commonly used techniques: opening the tissue longitudinally and either stapling the ends to a tongue depressor, laying it on a paper towel or placing the tissue directly into fixative. Finally, one collection technique, placing the tissue directly into the fixative unopened results in significantly greater numbers of artifacts and thus cannot be recommended.

When different regions of the intestinal tract are compared it becomes apparent that the duodenum and jejunum have equal inherent susceptibility to collection-induced artifacts, and likewise, the ileum and colon are the same. However, the duodenum and jejunum suffer a greater frequency of artifacts than the ileum and colon. In addition, perfuse fixation does not alter the frequency or distribution of artifacts when compared to the rapid collection tissues. The use of 10% BNF is adequate for examination of intestinal tract using SEM, but additional coating time and a low KVP is necessary to reduce charging. Surface artifacts seen by SEM are most numerous in the anterior regions of the intestinal tract, especially the duodenum, and decreased in the posterior regions. There is good correlation between the artifacts seen with the light microscope and those identified with the sanning microscope.

In conclusion, the routine use of collection method number two, ligations and injecting the lumen, appears to be the best in preserving the intestine artifact-free. This method can best be used when the changes of interest are diffuse or can be localized without first opening the intestine. Collection methods one, three and five are approximately equal in their ability to prevent artifacts. These methods are suggested when a localized lesion is present that cannot be anticipated or when the mucosal surface needs to be grossly inspected. Collection method four, placing the tissue directly into fixative, should not be used for it both created more artifacts than any of the other methods and also does not allow for inspection of the mucosal surface.

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TABLE 1

Random Tissue Collection Method Sequence

	Dog Number											
	1	2	3	4	5	6	7	8	9	10	11	12
Collection Methods*	1	2	3	4	5	1	2	3	4	5	1	2
	3	4	5	1	2	3	4	5	1	2	3	4
	5	1	2	3	4	5	1	2	3	4	5	1
	2	3	4	5	1	2	3	4	5	1	2	3
	4	5	1	2	3	4	5	1	2	3	4	5

* The columns of numbers under each dog represent the collection method sequence used to collect tissues from each region of the intestine.

TABLE 2

Analysis of Variance of Total Artifact Scores by Tissue, Collection Method and Tissue vs Collection Method

	Sum of Squares	Degrees of Freedom	Mean Square	F	Tail Probability
Total Artifact					
Mean	26,041.66	1	26,041.66	699.13	0.000
Error	409.73	11	409.73		
Tissue					
Mean	980.70	3	326.90	14.47	0.000
Error	745.50	33	22.59		
Collection Method					
Mean	1,754.45	4	438.61	17.42	0.000
Error	1,108.14	44	25.18		
Tissue X Collection Method					
Mean	735.50	12	61.29	2.16	0.016
Error	3,738.29	132	28.32		

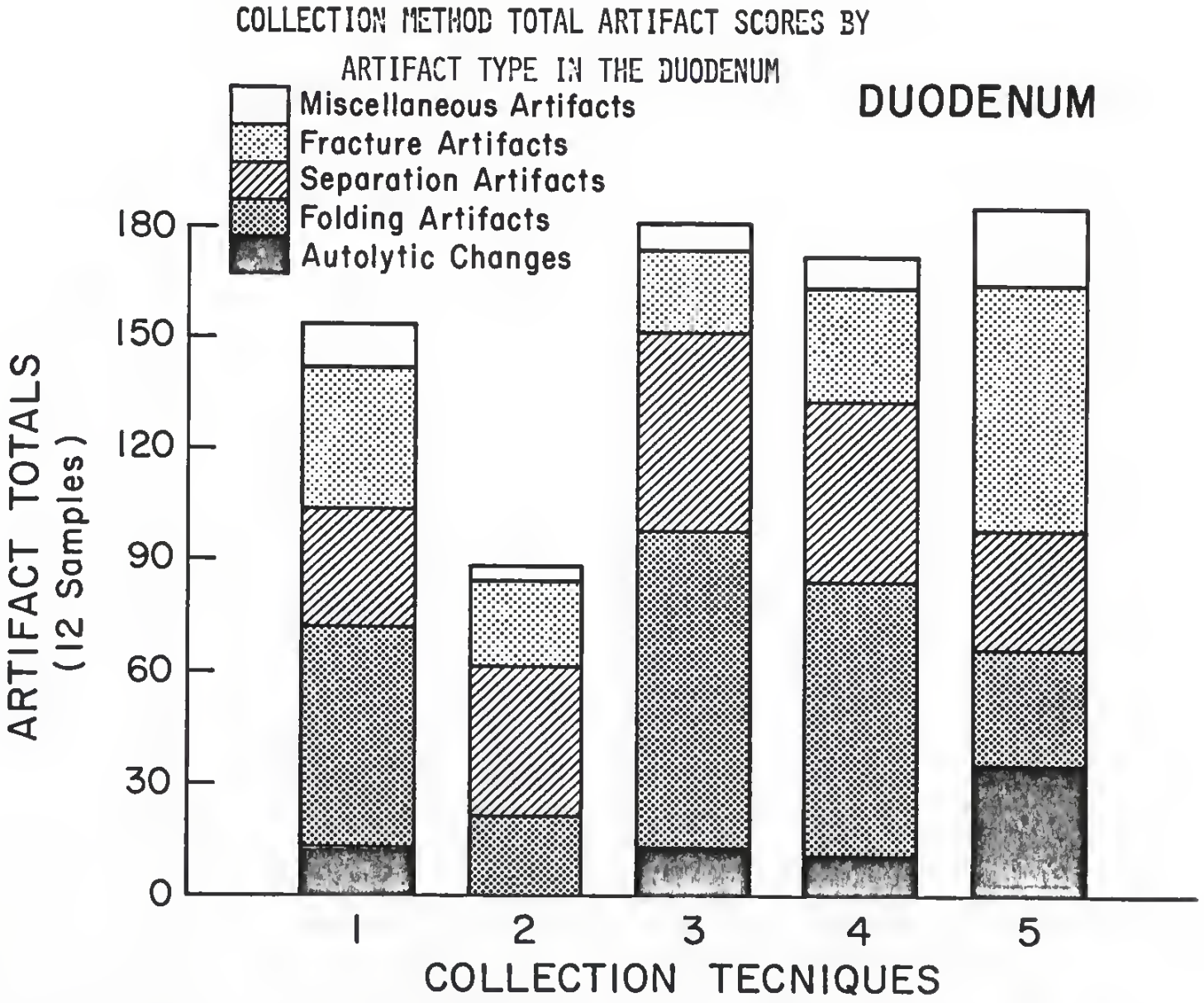
Mean = Mean artifact scores of all collection methods in all location in all dogs
Error = Standard error of the Mean

TABLE 3

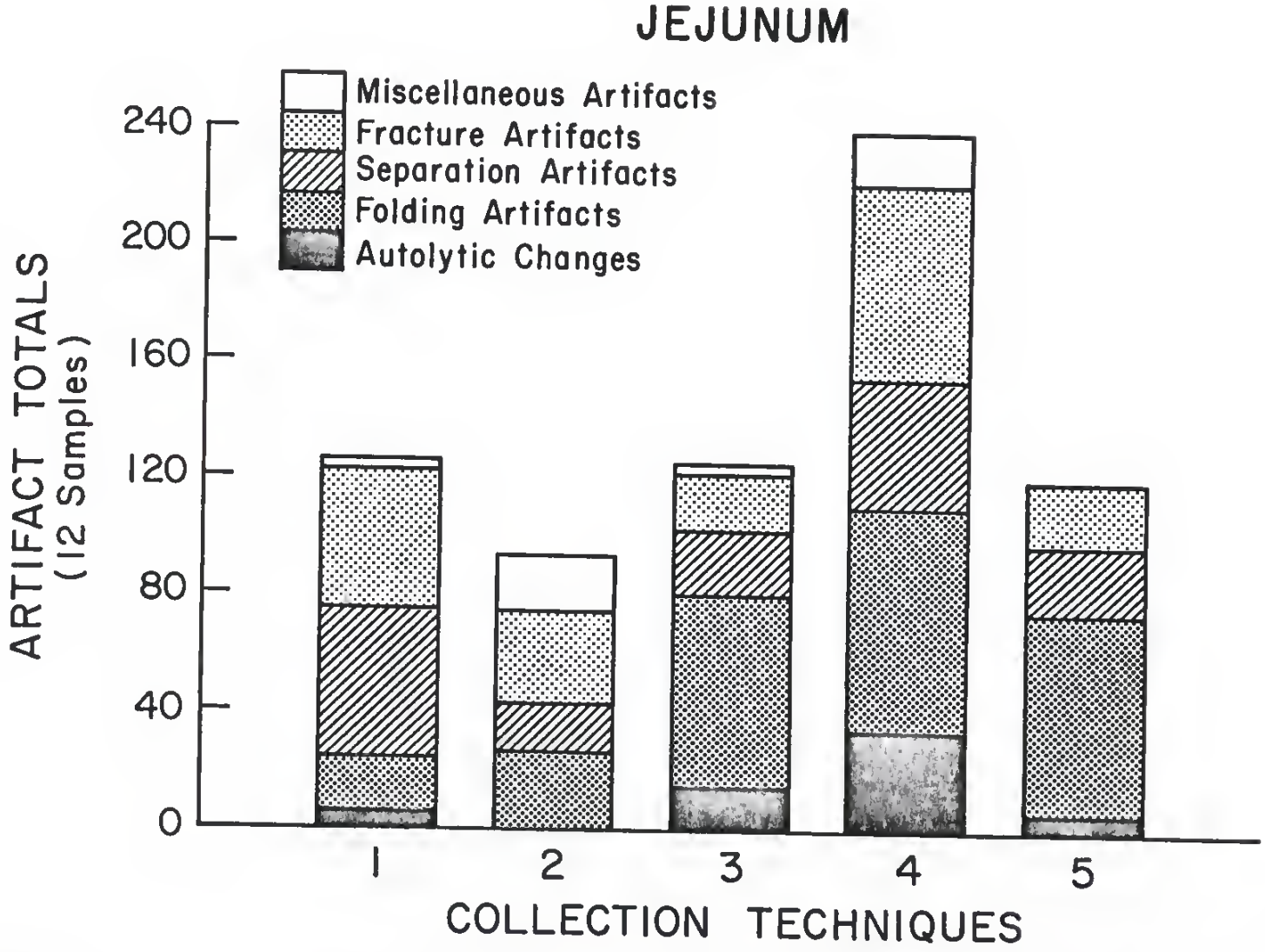
Tissue Location Comparisons of Collection Method Artifact Score Totals

Collection Methods	Tissue			
	Duodenum	Jejunum	Ileum	Colon
Method One				
Sum of Totals	152	125	141	111
Mean of Totals	12.66	10.41	11.75	9.25
Standard Deviation of Totals	7.25	3.87	6.67	5.49
Method Two				
Sum of Totals	88	93	51	70
Mean of Totals	7.33	7.75	4.25	5.83
Standard Deviation of Totals	2.46	3.59	2.70	3.37
Method Three				
Sum of Totals	181	124	105	79
Mean of Totals	15.08	10.33	8.75	6.58
Standard Deviation of Totals	3.14	6.58	4.04	5.93
Method Four				
Sum of Totals	173	238	164	133
Mean of Totals	14.41	19.83	13.66	1.00
Standard Deviation of Totals	6.08	6.26	5.69	7.41
Method Five				
Sum of Totals	186	119	89	78
Mean of Totals	15.50	9.91	7.41	6.50
Standard Deviation of Totals	5.64	4.42	5.45	3.98

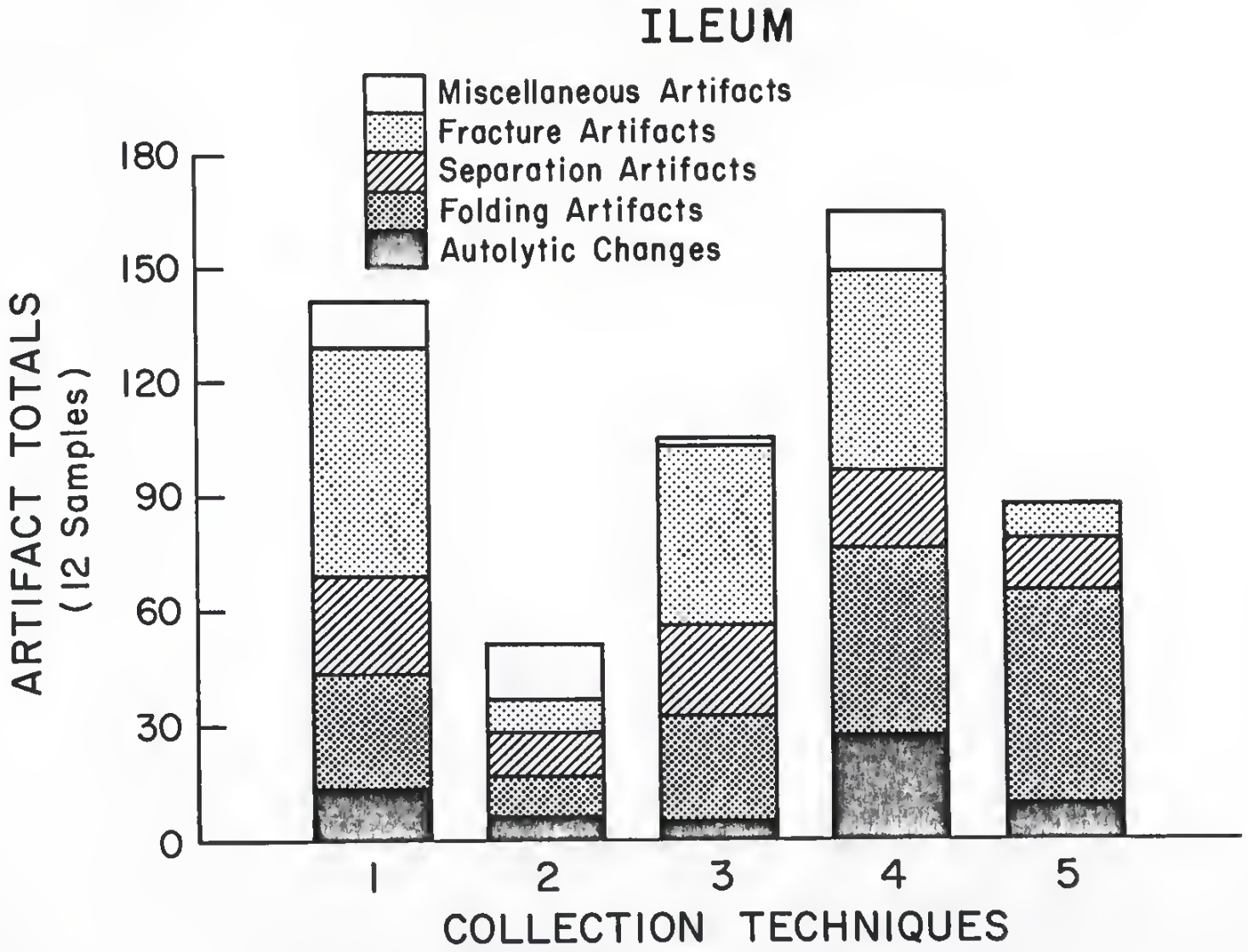
Sum of Totals = Sum of all artifact scores, all types and location, from all dogs
Mean of Totals = Sum of Totals divided by number of dogs (Average total artifact score per dog)
Standard Deviation of Totals = Standard deviation of Mean of Totals


COLLECTION METHOD TOTAL ARTIFACT SCORES BY
ARTIFACT TYPE IN THE DUODENUM
DUODENUM
Miscellaneous Artifacts
Fracture Artifacts
Separation Artifacts
Folding Artifacts
Autolytic Changes
ARTIFACT TOTALS
(12 Samples)
180
150
120
90
60
30
0
1
2
3
4
5
COLLECTION TECNIQUES

COLLECTION METHOD TOTAL ARTIFACT SCORES BY
ARTIFACT TYPE IN THE JEJUNUM



COLLECTION METHOD TOTAL ARTIFACT SCORES BY
ARTIFACT TYPE IN THE ILEUM



COLLECTION METHOD TOTAL ARTIFACT SCORES BY ARTIFACT TYPE IN THE COLON

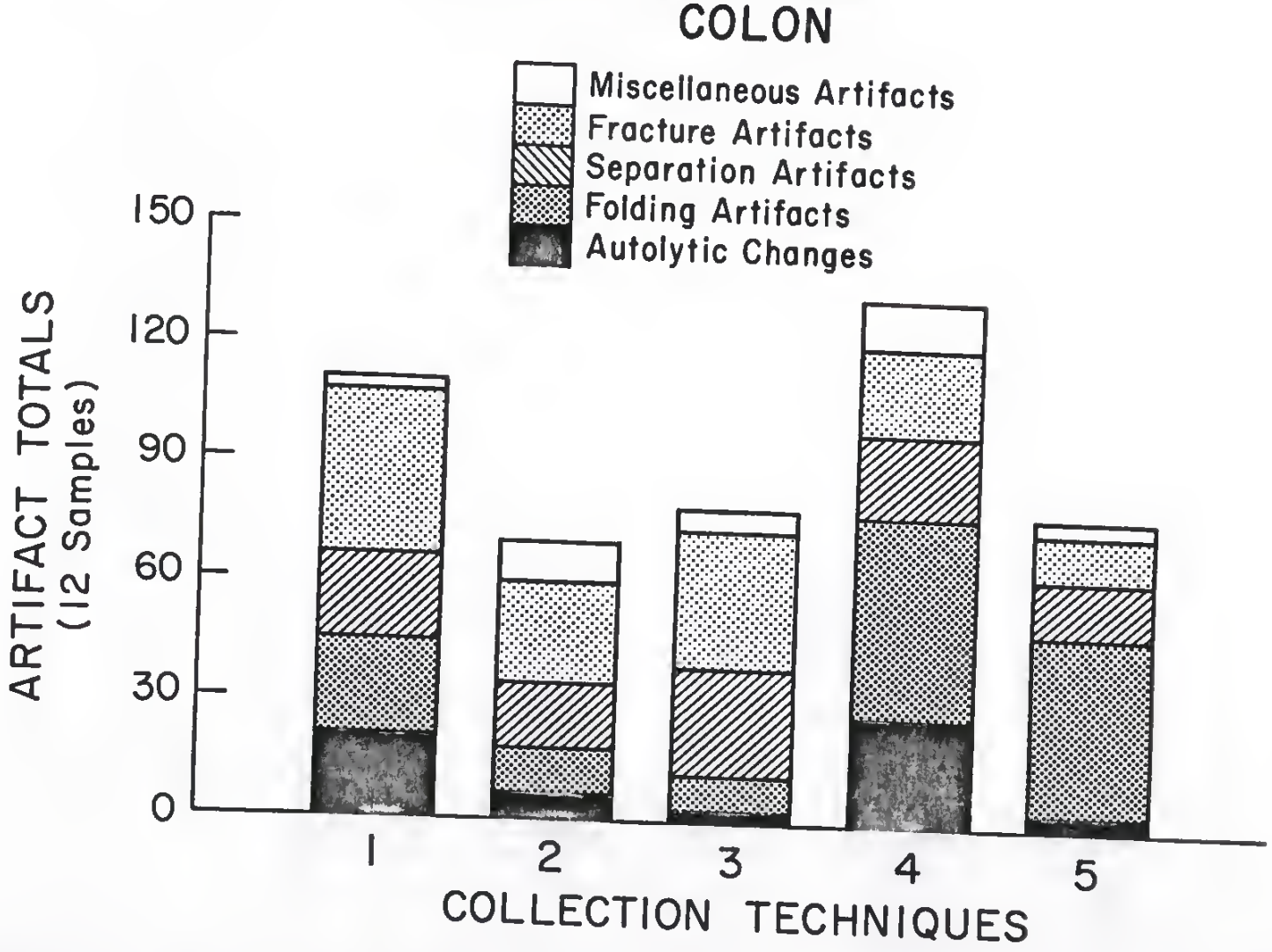


TABLE 4

Collection Method One Artifact Totals by Type and Location in Duodenum, Jejunum, Ileum and Colon

Artifact Totals*	Tissue			
	Duodenum	Jejunum	Ileum	Colon
Autolysis				
Serosa	12	6	5	11
Muscularis	0	0	0	0
Submucosa	0	0	4	3
Mucosa	1	0	5	7
Folding				
Serosa	16	0	8	2
Muscularis, outer	15	3	6	9
Muscularis, inner	12	7	6	7
Submucosa	4	6	3	2
Lymphoid nodules**	4/5	0/1	6/10	3/7
Mucosa	12	2	6	4
Separation				
Serosa-muscularis	0	11	13	11
Muscularis outer-inner	13	20	10	7
Muscularis-submucosa	2	3	0	4
Submucosa-mucosa	17	16	3	0
Fractures				
Serosa	7	1	3	2
Muscularis, outer	6	16	12	8
Muscularis, inner	5	17	15	14
Submucosa	13	12	15	14
Lymphoid nodules**	2/5	0/1	14/10	9/7
Mucosa	7	3	15	4
Miscellaneous				
Stain precipitate	2	0	4	0
Variable tissue thickness	10	2	8	2

*Total artifact scores from all dogs.
**Total artifact score over the number of lymphoid nodules examined.

TABLE 5

Collection Method One Artifact Totals by Type and Location in Ouodenum, Jejunum, Ileum and Colon

Artifact Totals*	Tissue			
	Ouodenum	Jejunum	Ileum	Colon
Autolysis				
Serosa	0	0	0	0
Muscularis	0	0	0	0
Submucosa	0	0	3	3
Mucosa	0	0	3	4
Folding				
Serosa	2	4	0	0
Muscularis, outer	6	5	3	3
Muscularis, inner	4	6	3	6
Submucosa	3	4	3	2
Lymphoid nodules**	0/3	0/0	3/9	0/4
Mucosa	6	8	2	0
Separation				
Serosa-muscularis	5	1	0	0
Muscularis outer-inner	13	6	9	8
Muscularis-submucosa	2	1	2	2
Submucosa-mucosa	20	8	0	6
Fractures				
Serosa	3	0	0	0
Muscularis, outer	3	10	3	4
Muscularis, inner	3	14	4	10
Submucosa	12	5	2	12
Lymphoid nodules**	0/3	0/0	4/9	2/4
Mucosa	3	2	0	0
Miscellaneous				
Stain precipitate	0	1	1	0
Variable tissue thickness	3	18	13	10

*Total artifact scores from all dogs.
**Total artifact score over the number of lymphoid nodules examined.

TABLE 6

Collection Method Three Artifact Totals by Type and Location in Duodenum, Jejunum, Ileum and Colon

Artifact Totals*	Tissue			
	Duodenum	Jejunum	Ileum	Colon
Autolysis				
Serosa	11	0	0	0
Muscularis	0	0	0	0
Submucosa	0	8	2	1
Mucosa	2	7	3	2
Folding				
Serosa	9	11	1	0
Muscularis, outer	18	14	8	1
Muscularis, inner	25	14	8	1
Submucosa	18	16	1	4
Lymphoid nodules**	0/1	0/0	14/11	5/6
Mucosa	15	10	9	3
Separation				
Serosa-muscularis	22	12	15	15
Muscularis outer-inner	5	4	3	7
Muscularis-submucosa	4	2	2	2
Submucosa-mucosa	23	7	3	3
Fractures				
Serosa	3	1	0	8
Muscularis, outer	8	4	7	12
Muscularis, inner	6	8	16	9
Submucosa	2	2	19	3
Lymphoid nodules**	1/1	0/0	8/11	0/6
Mucosa	3	2	6	3
Miscellaneous				
Stain precipitate	1	0	0	0
Variable tissue thickness	6	2	2	5

*Total artifact scores from all dogs.
**Total artifact score over the number of lymphoid nodules examined.

TABLE 7

Collection Method Four Artifact Totals by Type and Location in Ouodenum, Jejunum, Ileum and Colon

Artifact Totals*	Tissue			
	Ouodenum	Jejunum	Ileum	Colon
Autolysis				
Serosa	1	0	0	0
Muscularis	0	0	0	0
Submucosa	5	16	10	13
Mucosa	5	16	17	14
Folding				
Serosa	11	9	3	10
Muscularis, outer	17	21	4	13
Muscularis, inner	22	21	14	16
Submucosa	13	17	17	7
Lymphoid nodules**	0/0	0/0	0/0	0/0
Mucosa	11	11	12	5
Separation				
Serosa-muscularis	14	6	0	3
Muscularis outer-inner	11	20	13	16
Muscularis-submucosa	1	16	6	2
Submucosa-mucosa	22	2	0	0
Fractures				
Serosa	0	0	0	0
Muscularis, outer	2	21	14	9
Muscularis, inner	4	23	10	9
Submucosa	22	18	18	4
Lymphoid nodules**	0/0	0/0	9/10	1/2
Mucosa	3	5	10	0
Miscellaneous				
Stain precipitate	1	3	0	0
Variable tissue thickness	8	14	16	2

*Total artifact scores from all dogs.
**Total artifact score over the number of lymphoid nodules examined.

TABLE 8

Collection Method Five Artifact Totals by Type and Location in Ouodenum, Jejunum, Ileum and Colon

Artifact Totals*	Tissue			
	Ouodenum	Jejunum	Ileum	Colon
Autolysis				
Serosa	1	0	0	0
Muscularis	0	0	0	0
Submucosa	16	3	3	2
Mucosa	18	4	6	2
Folding				
Serosa	1	5	9	5
Muscularis, outer	7	14	16	14
Muscularis, inner	12	18	12	9
Submucosa	8	20	10	7
Lymphoid nodules**	3/4	1/4	16/11	0/2
Mucosa	3	10	10	10
Separation				
Serosa-muscularis	2	1	0	7
Muscularis outer-inner	4	14	7	6
Muscularis-submucosa	5	8	5	1
Submucosa-mucosa	21	2	2	0
Fractures				
Serosa	0	0	0	0
Muscularis, outer	17	6	4	2
Muscularis, inner	19	11	3	5
Submucosa	22	3	2	3
Lymphoid nodules**	2/4	3/4	3/11	0/2
Mucosa	9	0	0	1
Miscellaneous				
Stain precipitate	3	0	0	0
Variable tissue thickness	18	0	0	4

*Total artifact scores from all dogs.
**Total artifact score over the number of lymphoid nodules examined.

TABLE 9

Summary of Collection Method Artifact Total Scores by Type and Location*

Artifact Type and Location	Collection Methods 1	2	3	4	5
Autolysis					
Duodenum	13	0	13	11	35
Jejunum	6	0	15	32	7
Ileum	14	6	5	27	9
Colon	21	7	3	27	4
Folding					
Duodenum	59	21	85	74	31
Jejunum	18	27	65	79	67
Ileum	29	11	27	50	55
Colon	24	11	9	51	45
Separations					
Duodenum	32	40	54	48	32
Jejunum	50	16	25	44	25
Ileum	26	11	23	19	14
Colon	22	16	27	21	14
Fractures					
Duodenum	38	24	22	31	67
Jejunum	49	31	17	67	20
Ileum	60	9	48	52	6
Colon	42	26	35	22	11
Miscellaneous					
Duodenum	12	3	7	9	21
Jejunum	2	19	2	17	0
Ileum	12	14	2	16	0
Colon	2	10	5	12	4

* Total artifact scores from all samples from one location of a single artifact type.

TABLE 10

Comparison of Collection Methods in the Duodenum by Total Artifact Type and Location Scores*

Artifact Type and Location	Collection Method 1	2	3	4	5
Autolysis					
Serosa	12	0	11	1	1
Muscularis	0	0	0	0	0
Submucosa	0	0	0	5	16
Mucosa	1	0	2	5	18
Folding					
Serosa	16	2	9	11	1
Muscularis, outer	15	6	18	17	7
Muscularis, inner	12	4	25	22	12
Submucosa	4	3	18	13	8
Lymphoid nodules**	4/5	0/2	0/1	0/0	3/4
Mucosa	12	6	15	11	3
Separation					
Serosa-muscularis	0	5	22	14	2
Muscularis outer-inner	13	13	5	11	4
Muscularis-submucosa	2	2	4	1	5
Submucosa-mucosa	17	20	23	22	21
Fracture					
Serosa	7	3	3	0	0
Muscularis, outer	6	3	8	2	17
Muscularis, inner	5	3	6	4	19
Submucosa	13	12	2	22	22
Lymphoid nodules**	2/5	0/3	1/1	0/0	2/4
Mucosa	7	3	3	3	9
Miscellaneous					
Stain precipitate	2	0	1	1	3
Variable tissue thickness	10	3	6	8	18

*Total artifact scores from all dogs.
**Total artifact score over the number of lymphoid nodules examined

TABLE 11

Comparison of Collection Methods in the Jejunum by Total Artifact Type and Location Scores*

Artifact Type and Location	Collection Method 1	2	3	4	5
Autolysis					
Serosa	6	0	0	0	0
Muscularis	0	0	0	0	0
Submucosa	0	0	8	16	3
Mucosa	0	0	7	16	4
Folding					
Serosa	0	4	11	9	5
Muscularis, outer	3	5	14	21	14
Muscularis, inner	7	6	14	21	18
Submucosa	6	4	16	17	20
Lymphoid nodules**	0/1	0/0	0/0	0/0	1/4
Mucosa	2	8	10	11	10
Separation					
Serosa-muscularis	11	1	12	6	1
Muscularis outer-inner	20	6	4	20	14
Muscularis-submucosa	3	1	2	16	8
Submucosa-mucosa	16	8	7	2	2
Fracture					
Serosa	1	0	1	0	0
Muscularis, outer	16	10	3	21	6
Muscularis, inner	17	14	8	23	11
Submucosa	12	5	2	18	3
Lumphoid nodules**	0/1	0/0	0/0	0/0	3/4
Mucosa	10	5	2	9	3
Miscellaneous					
Stain precipitate	0	1	0	3	0
Variable tissue thickness	2	18	2	14	0

*Total artifact scores from all dogs.
**Total artifact score over the number of lymphoid nodules examined.

TABLE 12

Comparison of Collection Methods in the Duodenum by Total Artifact Type and Location Scores*

Artifact Type and Location	Collection Method 1	2	3	4	5
Autolysis					
Serosa	5	0	0	0	0
Muscularis	0	0	0	0	0
Submucosa	4	3	2	10	3
Mucosa	5	3	3	17	6
Folding					
Serosa	8	0	1	3	9
Muscularis, outer	6	3	8	4	16
Muscularis, inner	6	3	8	14	16
Submucosa	3	3	1	17	10
Lymphoid nodules**	6/10	3/9	14/11	6/10	16/17
Mucosa	6	2	9	12	10
Separations					
Serosa-muscularis	13	0	15	0	0
Muscularis outer-inner	10	9	3	13	7
Muscularis-submucosa	0	2	2	6	5
Submucosa-mucosa	3	0	3	0	2
Fractures					
Serosa	3	0	0	0	0
Muscularis, outer	12	3	7	14	4
Muscularis, inner	15	4	16	10	3
Submucosa	15	2	19	18	2
Lymphoid nodules**	14/10	4/9	8/11	9/10	3/11
Mucosa	15	0	6	10	0
Miscellaneous					
Stain precipitate	4	1	0	0	0
Variable tissue thickness	8	13	2	16	0

*Total artifact scores from all dogs.
**Total artifact score over the number of lymphoid nodules examined.

TABLE 13

Comparison of Collection Methods in the Colon by Total Artifact Type and Location Scores*

Artifact Type and Location	Collection Method 1	2	3	4	5
Autolysis					
Serosa	11	0	0	0	0
Muscularis	0	0	0	0	0
Submucosa	3	3	1	13	2
Mucosa	7	4	2	14	2
Folding					
Serosa	2	0	0	10	5
Muscularis, outer	9	3	1	13	14
Muscularis, inner	7	6	1	13	14
Submucosa	2	2	4	7	7
Lymphoid nodules**	3/7	0/4	5/6	1/2	0/2
Mucosa	4	0	3	5	10
Separations					
Serosa-muscularis	11	0	15	3	7
Muscularis outer-inner	7	8	7	16	6
Muscularis-submucosa	4	2	2	2	1
Submucosa-mucosa	0	6	3	0	0
Fractures					
Serosa	2	0	8	0	0
Muscularis, outer	8	4	12	9	2
Muscularis, inner	14	10	9	9	5
Submucosa	14	12	3	4	3
Lymphoid nodules**	9/7	2/4	0/6	1/2	0/2
Mucosa	4	0	3	0	1
Miscellaneous					
Stain precipitate	0	0	0	0	0
Variable tissue thickness	2	10	5	12	4

*Total artifact scores from all dogs.
**Total artifact score over the number of lymphoid nodules examined.

TABLE 14

Duodenal Artifact Scores by Type and Location
Using Collection Method One

Artifact Type and Location	Dog Number 1	2	3	4	5	6	7	8	9	10	11	12
Autolysis												
Serosa	0	3	0	3	1	1	0	0	1	2	0	0
Muscularis	0	0	0	0	0	0	0	0	0	0	0	0
Submucosa	0	0	0	0	0	0	0	0	0	0	0	0
Mucosa	0	0	0	0	0	0	0	0	0	0	0	0
Folding												
Serosa	1	2	2	1	3	0	2	1	0	0	1	3
Muscularis, outer	1	2	2	1	2	1	2	1	0	0	1	2
Muscularis, inner	1	2	1	2	2	1	2	1	0	0	0	0
Submucosa	0	0	0	0	0	1	2	1	0	0	0	0
Lymphoid nodules*	-	-	-	-	-	1	2	1	0	0	-	-
Mucosa	1	2	0	2	2	1	2	1	0	0	0	1
Separation												
Serosa-muscularis	0	0	0	0	0	0	0	0	0	0	0	0
Muscularis outer-inner	2	1	2	0	0	1	2	1	0	3	0	1
Muscularis-submucosa	1	0	0	0	0	0	0	0	0	1	0	0
Submucosa-mucosa	1	0	1	3	2	0	3	1	2	3	0	1
Fracture												
Serosa	1	1	1	2	1	0	0	0	0	1	0	0
Muscularis, outer	1	1	1	3	0	0	0	0	0	0	0	0
Muscularis, inner	0	0	0	3	0	0	1	1	0	0	0	0
Submucosa	3	3	1	3	1	1	0	1	0	0	0	0
Lymphoid nodules*	-	-	-	-	-	1	0	1	0	0	-	-
Mucosa	0	1	1	3	0	1	0	1	0	0	0	0
Miscellaneous												
Stain precipitate	0	0	0	1	0	0	0	0	0	0	1	0
Variables thicknesses	0	2	0	3	1	0	0	2	0	0	2	0
Totals	12	21	12	30	14	9	16	12	3	10	5	8

Sum of totals 152
Mean of totals 12.66
Standard deviation 7.25

0 = No artifact
1 = Mild artifact
2 = Moderate artifact
3 = Marked artifact

* Not included in totals
\- Not present

Sum of totals = Sum of all animals artifact total scores
Mean of totals = Sum of totals divided by number of dogs
Standard deviation = Standard deviation of individual dog total artifact scores

TABLE 15

Duodenal Artifact Scores by Type and Location
Using Collection Method Two

Artifact Type and Location	Dog Number 1	2	3	4	5	6	7	8	9	10	11	12
Autolysis												
Serosa	0	0	0	0	0	0	0	0	0	0	0	0
Muscularis	0	0	0	0	0	0	0	0	0	0	0	0
Submucosa	0	0	0	0	0	0	0	0	0	0	0	0
Mucosa	0	0	0	0	0	0	0	0	0	0	0	0
Folding												
Serosa	0	0	0	0	0	0	1	0	0	0	1	0
Muscularis, outer	0	0	2	1	0	0	1	0	2	0	0	0
Muscularis, inner	0	1	0	0	1	0	0	0	0	0	2	0
Submucosa	2	1	0	0	0	1	0	0	0	1	0	0
Lymphoid nocdules*	0	-	-	-	-	-	0	-	-	-	-	0
Mucosa	0	0	0	0	2	2	0	0	0	0	2	0
Separation												
Serosa-muscularis	0	0	0	0	1	1	0	2	0	1	0	0
Muscularis outer-inner	2	2	3	1	0	1	0	2	0	1	1	0
Muscularis-submucosa	0	0	0	0	0	0	0	2	0	0	0	0
Submucosa-mucosa	1	2	1	1	3	2	3	1	2	2	1	1
Fracture												
Serosa	0	0	0	0	0	0	1	0	0	1	1	0
Muscularis outer	0	1	0	1	0	0	0	0	0	0	1	0
Muscularis, inner	0	0	0	0	0	0	0	0	2	0	0	0
Submucosa	1	2	1	0	1	1	0	1	1	3	1	0
Lymphoid nodules*	0	-	-	-	-	-	0	-	-	-	-	0
Mucosa	0	0	0	0	0	0	0	0	0	2	1	0
Miscellaneous												
Stain precipitate	0	0	0	0	0	0	0	0	0	0	0	0
Variables thicknesses	1	1	0	0	0	0	1	0	0	0	0	0
Totals	7	9	7	6	8	7	7	8	7	11	10	1

Sum of totals 88
Mean of totals 7.33
Standard deviation 2.46

0 = No artifact
1 = Mild artifact
2 = Moderate artifact
3 = Marked artifact

* Not included in totals
\- Not present

Sum of totals = Sum of all animals artifact total scores
Mean of totals = Sum of totals divided by number of dogs
Standard deviation = Standardard deviation of individual dog total artifact scores

TABLE 16

Ouodenal Artifact Scores by Type and Location
Using Collection Method Three

Artifact and Location	Dog Number 1	2	3	4	5	6	7	8	9	10	11	12
Autolysis												
Serosa	0	0	2	1	0	2	0	0	3	1	2	0
Muscularis	0	0	0	0	0	0	0	0	0	0	0	0
Submucosa	0	0	0	0	0	0	0	0	0	0	0	0
Mucosa	1	0	0	0	0	1	0	0	0	0	0	0
Folding												
Serosa	1	0	2	2	0	0	2	0	1	1	0	0
Muscularis, outer	3	0	0	2	0	2	2	0	3	3	3	0
Muscularis, inner	3	1	3	2	3	2	0	2	2	2	3	2
Submucosa	2	0	1	0	2	2	2	3	2	2	0	2
Lymphoid nodules*	-	-	-	-	0	-	-	-	-	-	-	-
Mucosa	2	3	1	3	2	1	2	3	2	1	1	2
Separation												
Serosa-muscularis	2	0	2	3	0	3	0	3	3	2	1	3
Muscularis outer-inner	2	2	0	0	0	0	0	1	0	0	0	0
Muscularis-submucosa	0	1	0	0	0	0	1	0	0	0	2	0
Submucosa-mucosa	2	3	1	3	2	1	2	3	2	1	1	2
Fracture												
Serosa	0	1	0	1	0	1	0	0	0	0	0	0
Muscularis, outer	1	0	0	2	0	2	0	1	0	0	1	1
Muscularis, inner	1	0	1	0	0	0	0	1	0	1	0	2
Submucosa	0	0	0	0	0	0	0	0	0	0	2	0
Lymphoid nodules*	-	-	-	-	1	-	-	-	-	-	-	-
Mucosa	0	0	0	0	1	0	2	0	0	0	0	0
Miscellaneous												
Stain precipitate	0	0	0	1	0	0	0	0	0	0	0	0
Variables thicknesses	0	2	2	0	0	1	0	1	0	0	0	0
Totals	20	10	16	18	12	19	12	17	16	14	15	12

Sum of totals 181
Mean of totals 15.08
Standard deviation 3.14

0 = No artifact
1 = Mild artifact
2 = Moderate artifact
3 = Marked artifact

* Not included in totals
- Not present

Sum of totals = Sum of all animals artifact total scores
Mean of totals = Sum of totals divided by number of dogs
Standard deviation = Standard deviation of individual dog total artifact scores

TABLE 17

Duodenal Artifact Scores by Type and Location
Using Collection Method Four

Artifact Type and Location	Dog Number 1	2	3	4	5	6	7	8	9	10	11	12
Autolysis												
Serosa	0	0	0	0	1	0	0	0	0	0	0	0
Muscularis	0	0	0	0	0	0	0	0	0	0	0	0
Submucosa	0	2	0	0	3	0	0	0	0	0	0	0
Mucosa	0	2	0	0	3	0	0	0	0	0	0	0
Folding												
Serosa	3	2	2	0	0	1	1	0	2	0	0	0
Muscularis, outer	3	3	2	0	0	0	3	0	3	0	3	0
Muscularis, inner	2	3	2	1	3	3	3	1	2	0	2	0
Submucosa	0	3	2	0	3	2	0	0	0	0	3	0
Lymphoid nodules*	-	-	-	-	-	-	-	-	-	-	-	-
Mucosa	0	0	1	0	2	0	1	0	3	0	3	1
Separation												
Serosa-muscularis	0	0	0	1	0	2	2	3	2	3	0	1
Muscularis outer-inner	0	3	0	2	0	1	2	2	0	1	0	0
Muscularis-submucosa	0	0	0	0	0	0	1	0	0	0	0	0
Submucosa-mucosa	3	1	0	3	1	2	1	2	3	3	0	3
Fracture												
Serosa	0	0	0	0	0	0	0	0	0	0	0	0
Muscularis, outer	0	0	1	0	0	0	0	0	1	0	0	0
Muscularis, inner	2	0	1	0	0	0	0	0	1	0	0	0
Submucosa	3	2	1	0	3	2	3	0	3	2	0	3
Lymphoid nodules*	-	-	-	-	-	-	-	-	-	-	-	-
Mucosa	0	3	0	0	0	0	0	0	0	0	0	0
Miscellaneous												
Stain precipitate	0	0	0	0	0	0	1	0	0	0	0	0
Variables thicknesses	3	0	0	0	2	0	0	0	2	0	1	0
Totals	19	24	12	7	21	13	18	8	22	9	12	8

Sum of totals 173
Mean of totals 14.41
Standard deviation 6.08

0 = No artifact
1 = Mild artifact
2 = Moderate artifact
3 = Marked artifact

* Not included in totals
\- Not present

Sum of totals = Sum of all animals artifact total scores
Mean of totals = Sum of totals divided by number of dogs
Standard deviation = Standard deviation of individual dog total artifact scores

TABLE 18

Ouodenal Artifact Scores by Type and Location
Using Collection Method Five

Artifact and Location	Oog Number 1	2	3	4	5	6	7	8	9	10	11	12
Autolysis												
Serosa	1	0	0	0	0	0	0	0	0	0	0	0
Muscularis	0	0	0	0	0	0	0	0	0	0	0	0
Submucosa	0	3	0	2	2	0	2	0	2	0	2	3
Mucosa	0	2	0	3	1	1	2	0	2	1	3	3
Folding												
Serosa	0	0	0	0	0	0	0	0	1	0	0	0
Muscularis, outer	0	0	0	0	1	0	1	1	1	0	0	3
Muscularis, inner	2	0	1	0	2	1	0	2	1	0	0	3
Submucosa	0	3	0	0	2	0	0	0	0	0	0	3
Lymphoid nodules*	1	-	0	-	2	0	-	-	-	-	-	-
Mucosa	0	0	0	0	0	0	0	0	0	0	0	3
Separation												
Serosa-muscularis	0	0	0	1	0	0	0	1	0	0	0	0
Muscularis outer-inner	0	0	0	0	0	1	2	1	0	0	0	0
Muscularis-submucosa	0	0	0	0	0	0	0	1	2	1	0	1
Submucosa-mucosa	1	3	3	0	0	3	0	2	3	3	2	1
Fracture												
Serosa	0	0	0	0	0	0	0	0	0	0	0	0
Muscularis, outer	0	3	2	3	3	0	1	1	3	0	0	1
Muscularis, inner	3	3	2	1	2	0	1	3	3	0	0	1
Submucosa	3	3	0	2	3	3	0	0	1	3	2	2
Lymphoid nodules*	1	-	0	-	1	1	-	-	-	-	-	-
Mucosa	1	0	0	3	3	0	0	0	0	1	0	1
Miscellaneous												
Stain precipitate	0	0	1	0	0	1	0	0	0	0	0	1
Variables thicknesses	0	3	2	1	2	3	0	2	1	1	3	0
Totals	11	23	11	16	21	13	9	14	20	10	12	26

Sum of totals 186
Mean of totals 15.50
Standard deviation 5.64

0 = No artifact
1 = Mild artifact
2 = Moderate artifact
3 = Marked artifact

* Not included in totals
- Not present

Sum of totals = Sum of all animals artifact total scores
Mean of totals = Sum of totals divided by number of dogs
Standard deviation = Standard deviation of individual dog total artifact scores

TABLE 19

Jejunal Artifact Scores by Type and Location
Using Collection Method One

Artifact Type and Location	Dog Number 1	2	3	4	5	6	7	8	9	10	11	12
Autolysis												
Serosa	1	0	1	1	0	0	0	0	1	2	0	0
Muscularis	0	0	0	0	0	0	0	0	0	0	0	0
Submucosa	0	0	0	0	0	0	0	0	0	0	0	0
Mucosa	0	0	0	0	0	0	0	0	0	0	0	0
Folding												
Serosa	0	0	0	0	0	0	0	0	0	0	0	0
Muscularis, outer	0	0	0	0	0	2	0	0	1	0	0	0
Muscularis, inner	1	0	0	1	0	0	1	0	1	0	3	0
Submucosa	0	3	0	1	0	0	0	0	0	0	2	0
Lymphoid nodules*	-	-	-	0	-	-	-	-	-	-	-	-
Mucosa	0	0	1	0	1	0	0	0	0	0	0	0
Separation												
Serosa-muscularis	0	0	0	1	1	1	1	0	2	1	3	1
Muscularis outer-inner	3	2	3	1	1	1	3	0	0	3	2	1
Muscularis-submucosa	0	0	0	1	1	0	1	0	0	0	0	0
Submucosa-mucosa	2	1	1	0	3	3	0	1	0	3	2	0
Fracture												
Serosa	0	0	0	1	0	0	0	0	0	0	0	0
Muscularis, outer	0	0	0	3	0	2	0	3	3	1	3	1
Muscularis, inner	3	2	3	0	0	2	3	1	0	0	3	0
Submucosa	2	0	0	3	1	1	3	0	2	0	0	0
Lymphoid nodules*	-	-	-	0	-	-	-	-	-	-	-	-
Mucosa	0	0	1	1	0	0	0	1	0	0	0	0
Miscellaneous												
Stain precipitate	0	0	0	0	0	0	0	0	0	0	0	0
Variables thicknesses	0	0	0	0	0	0	0	0	1	1	0	0
Totals	12	8	10	14	8	12	12	6	11	11	18	3

Sum of totals 125
Mean of totals 10.41
Standard deviation 3.87

0 = No artifact
1 = Mild artifact
2 = Moderate artifact
3 = Marked artifact

* Not included in totals
- Not present

Sum of totals = Sum of all animals artifact total scores
Mean of totals = Sum of totals divided by number of dogs
Standard deviation = Standard deviation of individual dog total artifact scores

TABLE 20

Jejunal Artifact Scores by Type and Location
Using Collection Method Two

Artifact Type and Location	Dog Number 1	2	3	4	5	6	7	8	9	10	11	12
Autolysis												
Serosa	0	0	0	0	0	0	0	0	0	0	0	0
Muscularis	0	0	0	0	0	0	0	0	0	0	0	0
Submucosa	0	0	0	0	0	0	0	0	0	0	0	0
Mucosa	0	0	0	0	0	0	0	0	0	0	0	0
Folding												
Serosa	1	0	0	0	0	1	0	0	2	0	1	0
Muscularis, outer	2	0	0	1	0	2	0	0	0	0	0	0
Muscularis, inner	0	1	0	1	0	0	0	3	0	1	0	0
Submucosa	0	0	0	1	0	0	0	3	0	0	0	0
Lymphoid nodules*	-	-	-	-	-	-	-	-	-	-	-	-
Mucosa	0	0	0	2	0	0	0	0	1	3	2	0
Separation												
Serosa-muscularis	0	0	0	0	0	1	0	0	0	0	0	0
Muscularis outer-inner	1	0	0	2	0	0	1	0	0	1	1	0
Muscularis-submucosa	0	0	0	0	0	0	0	0	1	0	0	0
Submucosa-mucosa	0	0	0	0	1	0	0	1	0	0	0	0
Fracture												
Serosa	0	0	0	0	0	0	0	0	0	0	0	0
Muscularis, outer	0	2	0	0	3	1	0	1	0	2	1	0
Muscularis, inner	3	0	2	0	3	0	0	3	0	0	3	0
Submucosa	0	0	0	0	0	0	1	1	2	0	1	0
Lymphoid nodules*	-	-	-	-	-	-	-	-	-	-	-	-
Mucosa	0	0	0	0	1	0	0	1	0	0	0	0
Miscellaneous												
Stain precipitate	0	0	0	1	0	0	0	0	0	0	0	0
Variables thicknesses	0	3	2	0	1	3	0	2	2	1	1	3
Totals	7	7	4	8	10	9	2	15	8	9	11	3

Sum of totals 93
Mean of totals 7.75
Standard deviation 3.59

0 = No artifact
1 = Mild artifact
2 = Moderate artifact
3 = Marked artifact

* Not included in totals
\- Not present

Sum of totals = Sum of all animals artifact total scores
Mean of totals = Sum of totals divided by number of dogs
Standard deviation = Standard deviation of individual dog total artifact scores

TABLE 21

Jejunal Artifact Scores by Type and Location
Using Collection Method Three

Artifact Type and Location	Dog Number 1	2	3	4	5	6	7	8	9	10	11	12
Autolysis	0	0	0	0	0	0	0	0	0	0	0	0
Serosa	0	0	0	0	0	0	0	0	0	0	0	0
Muscularis	0	0	0	0	0	3	0	0	0	2	0	3
Submucosa	0	0	0	0	0	3	0	0	0	1	0	3
Mucosa												
Folding												
Serosa	3	0	3	0	0	3	0	0	0	2	0	0
Muscularis, outer	2	0	1	0	2	3	0	2	0	3	0	1
Muscularis, inner	0	1	2	1	3	1	0	2	1	1	3	0
Submucosa	0	1	2	0	3	3	1	2	1	0	3	0
Lymphoid nodules*	-	-	-	-	-	-	-	-	-	-	-	-
Mucosa	0	0	2	0	1	3	0	1	0	1	2	0
Separation												
Serosa-muscularis	1	0	2	0	0	3	0	1	0	2	3	0
Muscularis outer-inner	1	0	1	1	0	0	0	1	0	0	0	0
Muscularis-submucosa	0	0	0	0	0	1	0	0	0	1	0	0
Submucosa-mucosa	0	3	0	0	1	0	1	0	1	1	0	0
Fractures												
Serosa	0	0	0	0	1	0	0	0	0	0	0	0
Muscularis, outer	0	0	0	1	0	0	1	0	1	1	0	0
Muscularis, inner	0	1	0	0	2	1	1	2	0	0	1	0
Submucosa	0	0	0	0	0	0	0	0	0	2	0	0
Lymphoid nodules*	-	-	-	-	-	-	-	-	-	-	-	-
Mucosa	0	0	0	0	0	0	1	0	0	0	0	1
Miscellaneous												
Stain precipitate	0	0	0	0	0	0	0	0	0	0	0	0
Variables thicknesses	1	1	0	0	0	0	1	0	0	0	0	0
Totals	8	7	13	2	11	27	6	11	4	16	12	7

Sum of totals 124
Mean of totals 10.33
Standard deviation 6.58

0 = No artifact
1 = Mild artifact
2 = Moderate artifact
3 = Marked artifact

* Not included in totals
\- Not present

Sum of totals = Sum of all animals artifact total scores
Mean of totals = Sum of totals divided by number of dogs
Standard deviation = Standard deviation of individual dog total artifact scores

TABLE 22

Jejunal Artifact Scores by Type and Location
Using Collection Method Four

Artifact Type and Location	Dog Number 1	2	3	4	5	6	7	8	9	10	11	12
Autolysis												
Serosa	0	0	0	0	0	0	0	0	0	0	0	0
Muscularis	0	0	0	0	0	0	0	0	0	0	0	0
Submucosa	0	2	0	1	2	3	0	3	1	1	0	3
Mucosa	0	3	0	1	2	3	0	2	1	1	0	3
Folding												
Serosa	0	2	0	1	0	1	2	1	2	0	0	0
Muscularis, outer	1	3	0	2	3	0	2	2	3	2	3	1
Muscularis, inner	1	3	3	0	0	2	3	2	1	3	2	1
Submucosa	1	3	1	1	3	1	0	2	1	1	2	1
Lymphoid nodules*	-	-	-	-	-	-	-	-	-	-	-	-
Mucosa	1	3	0	0	1	1	0	2	1	1	1	0
Separation												
Serosa-muscularis	0	0	0	3	0	0	0	1	0	0	0	2
Muscularis outer-inner	1	3	1	3	1	1	0	3	3	0	2	2
Muscularis-submucosa	1	3	0	3	2	0	2	0	2	1	1	1
Submucosa-mucosa	0	0	0	0	0	0	1	1	0	0	0	0
Fracture												
Serosa	0	0	0	0	0	0	0	0	0	0	0	0
Muscularis, outer	1	3	3	1	3	0	2	2	3	3	0	0
Muscularis, inner	2	3	2	1	2	3	0	3	2	2	3	0
Submucosa	1	2	1	3	0	3	0	3	0	3	1	1
Lymphoid nodules*	-	-	-	-	-	-	-	-	-	-	-	-
Mucosa	0	0	0	0	0	2	0	1	0	2	0	0
Miscellaneous												
Stain precipitate	1	0	0	0	0	0	1	0	1	0	0	0
Variables thicknesses	2	0	3	2	1	0	2	1	1	0	1	1
Totals	11	33	14	22	20	20	15	29	22	20	16	16

Sum of totals 238
Mean of totals 19.83
Standard deviation 6.26

0 = No artifact
1 = Mild artifact
2 = Moderate artifact
3 = Marked artifact

* Not included in totals
\- Not present

Sum of totals = Sum of all animals artifact total scores
Mean of totals = Sum of totals divided by number of dogs
Standard deviation = Standard deviation of individual dog total artifact scores

TABLE 23

Jejunal Artifact Scores by Type and Location
Using Collection Method Five

Artifact Type and Location	Dog Number 1	2	3	4	5	6	7	8	9	10	11	12
Autolysis												
Serosa	0	0	0	0	0	0	0	0	0	0	0	0
Muscularis	0	0	0	0	0	0	0	0	0	0	0	0
Submucosa	0	0	0	0	0	0	0	0	0	0	1	2
Mucosa	0	0	1	0	0	0	0	0	0	0	1	2
Folding												
Serosa	1	0	0	1	1	0	0	0	1	0	1	0
Muscularis, outer	3	0	1	2	2	1	0	1	1	0	3	0
Muscularis, inner	3	0	0	3	3	1	3	2	3	0	0	0
Submucosa	1	3	0	2	2	3	3	2	3	1	0	1
Lymphoid nodules*	-	-	-	-	-	-	-	-	0	0	0	1
Separation												
Serosa-muscularis	1	0	0	0	0	0	0	0	0	0	0	0
Muscularis outer-inner	3	2	1	0	1	2	0	3	1	1	0	0
Muscularis-submucosa	1	3	0	1	0	0	3	0	0	0	0	0
Submucosa-mucosa	1	0	0	0	0	0	1	0	0	0	0	0
Fractures												
Serosa	0	0	0	0	0	0	0	0	0	0	0	0
Muscularis, outer	0	3	0	1	1	0	0	1	0	0	0	0
Muscularis, inner	0	3	0	1	1	2	1	0	0	2	1	0
Submucosa	0	3	0	0	0	0	0	0	0	0	0	0
Lymphoid nodules*	-	-	-	-	-	-	-	-	1	2	0	0
Mucosa	0	0	0	0	0	0	0	0	0	0	0	0
Miscellaneous												
Stain precipitate	0	0	0	0	0	0	0	0	0	0	0	0
Variables thicknesses	0	0	0	0	0	0	0	0	0	0	0	0
Totals	14	18	5	13	13	9	13	10	9	4	7	4

Sum of totals 119
Mean of totals 9.91
Standard deviation 4.42

0 = No artifact
1 = Mild artifact
2 = Moderate artifact
3 = Marked artifact

* Not included in totals
\- Not present

Sum of totals = Sum of all animals artifact total scores
Mean of totals = Sum of totals divided by number of dogs
Standard deviation = Standard deviation of individual dog total artifact scores

TABLE 24

Ileal Artifact Scores by Type and Location
Using Collection Method One

Artifact and Location	Dog Number 1	2	3	4	5	6	7	8	9	10	11	12
Autolysis												
Serosa	1	0	0	0	2	0	0	1	1	0	0	0
Muscularis	0	0	0	0	0	0	0	0	0	0	0	0
Submucosa	0	0	0	0	0	0	0	0	0	1	0	3
Mucosa	0	0	0	0	0	0	0	0	0	2	0	3
Folding												
Serosa	1	0	0	0	3	0	0	2	2	0	0	0
Muscularis, outer	0	0	0	0	0	0	0	0	3	0	0	3
Muscularis, inner	0	1	0	0	0	2	0	0	1	0	2	0
Submucosa	0	1	0	0	0	1	0	0	1	0	0	0
Lymphoid nodules*	1	0	2	-	1	-	0	0	0	0	1	1
Mucosa	1	0	1	0	1	0	0	0	1	0	1	1
Separation												
Serosa-muscularis	0	3	2	1	0	0	3	0	2	1	1	0
Muscularis outer-inner	2	3	0	0	0	0	3	0	0	0	0	2
Muscularis-submucosa	0	0	0	0	0	0	0	0	0	0	0	0
Submucosa-mucosa	1	0	0	0	1	1	0	0	0	0	0	0
Fracture												
Serosa	0	0	0	0	0	0	0	0	1	0	1	1
Muscularis, outer	3	0	0	0	3	3	0	0	2	0	1	0
Muscularis, inner	3	3	0	0	3	3	1	1	1	0	0	0
Submucosa	3	3	0	0	3	3	1	1	1	0	0	0
Lymphoid nodules*	1	2	1	-	1	-	0	3	1	2	0	3
Mucosa	3	3	1	0	0	0	1	3	0	1	0	3
Miscellaneous												
Stain precipitate	3	0	0	0	0	0	0	0	0	0	0	1
Variables thicknesses	3	0	3	0	0	2	0	0	0	0	0	0
Totals	24	17	7	1	16	15	9	8	16	5	6	17

Sum of totals 141
Mean of totals 11.75
Standard deviation 6.67

0 = No artifact
1 = Mild artifact
2 = Moderate artifact
3 = Marked artifact

* Not included in totals
\- Not present

Sum of totals = Sum of all animals artifact total scores
Mean of totals = Sum of totals divided by number of dogs
Standard deviation = Standard deviation of individual dog total artifact scores

TABLE 25

Ileal Artifact Scores by Type and Location
Using Collection Method Two

Artifact and Location	Dog Number 1	2	3	4	5	6	7	8	9	10	11	12
Autolysis												
Serosa	0	0	0	0	0	0	0	0	0	0	0	0
Muscularis	0	0	0	0	0	0	0	0	0	0	0	0
Submucosa	0	0	0	0	0	0	0	0	0	0	0	3
Mucosa	0	0	0	0	0	0	0	0	1	0	0	2
Folding												
Serosa	0	0	0	0	0	0	0	0	0	0	0	0
Muscularis, outer	0	0	0	0	0	1	0	2	0	0	0	0
Muscularis, inner	0	0	1	0	0	0	0	1	0	0	1	0
Submucosa	0	0	1	0	0	0	0	1	0	0	1	0
Lymphoid nodules*	0	0	0	0	0	1	-	-	2	0	0	-
Mucosa	0	0	1	0	0	0	0	1	0	0	0	0
Separation												
Serosa-muscularis	0	0	0	0	0	0	0	0	0	0	0	0
Muscularis outer-inner	1	0	1	0	1	0	1	2	3	0	0	0
Muscularis-submucosa	0	0	0	2	0	0	0	0	0	0	0	0
Submucosa-mucosa	0	0	0	0	0	0	0	0	0	0	0	0
Fracture												
Serosa	0	0	0	0	0	0	0	0	0	0	0	0
Muscularis, outer	1	0	0	0	0	1	0	1	0	0	0	0
Muscularis, inner	1	0	0	1	0	0	1	1	0	0	0	0
Submucosa	0	0	0	0	0	1	0	1	0	0	0	0
Lymphoid nodules*	0	0	1	0	0	1	-	-	2	0	0	-
Mucosa	0	0	0	0	0	0	0	0	0	0	0	0
Miscellaneous												
Stain precipitate	0	0	0	0	1	0	0	0	0	0	0	0
Variables thicknesses	3	2	0	1	3	0	0	1	1	1	0	1
Totals	6	2	4	4	5	3	2	11	5	1	2	6

Sum of totals	51
Mean of totals	4.25
Standard deviation	2.70

0 = No artifact
1 = Mild artifact
2 = Moderate artifact
3 = Marked artifact

* Not included in totals
\- Not present

Sum of totals = Sum of all animals artifact total scores
Mean of totals = Sum of totals divided by number of dogs
Standard deviation = Standard deviation of individual dog total artifact scores

TABLE 26

Ileal Artifact Scores by Type and Location
Using Collection Method Three

Artifact and Location	Dog Number 1	2	3	4	5	6	7	8	9	10	11	12
Autolysis												
Serosa	0	0	0	0	0	0	0	0	0	0	0	0
Muscularis	0	0	0	0	0	0	0	0	0	0	0	0
Submucosa	0	0	0	0	0	0	1	0	0	0	0	1
Mucosa	0	0	0	0	0	0	1	0	0	0	0	2
Folding												
Serosa	0	1	0	0	0	0	0	0	0	0	0	0
Muscularis, outer	1	1	0	3	0	0	2	0	0	1	0	0
Muscularis, inner	0	1	0	3	0	0	2	0	0	1	1	0
Submucosa	0	0	0	0	0	0	0	0	0	0	1	0
Lymphoid nodules*	1	1	1	0	0	2	0	3	3	1	2	-
Mucosa	0	1	1	0	0	2	0	1	1	1	1	1
Separation												
Serosa-muscularis	2	0	3	1	2	0	2	1	1	0	3	0
Muscularis outer-inner	1	0	0	1	0	1	0	0	0	0	0	0
Muscularis-submucosa	0	0	1	1	0	0	0	0	0	0	0	0
Submucosa-mucosa	0	0	0	0	0	1	0	0	0	0	0	2
Fracture												
Serosa	0	0	0	0	0	0	0	0	0	0	0	0
Muscularis, outer	0	0	0	1	0	3	0	2	0	0	0	1
Muscularis, inner	1	3	2	1	0	3	0	1	1	3	0	1
Submucosa	0	3	2	2	0	3	0	1	3	3	1	1
Lymphoid nodules*	0	0	0	0	0	3	1	1	2	1	0	-
Mucosa	0	0	0	2	0	3	0	1	0	0	0	0
Miscellaneous												
Stain precipitate	0	0	0	0	0	0	0	0	0	0	0	0
Variables thicknesses	0	0	0	0	0	1	0	1	0	0	0	0
Totals	5	10	9	15	2	17	8	8	6	9	7	9

Sum of totals 105
Mean of totals 8.75
Standard deviation 4.04

0 = No artifact
1 = Mild artifact
2 = Moderate artifact
3 = Marked artifact

* Not included in totals
- Not present

Sum of totals = Sum of all animals artifact total scores
Mean of totals = Sum of totals divided by number of dogs
Standard deviation = Standard deviation of individual dog total artifact scores

TABLE 27

Ileal Artifact Scores by Type and Location
Using Collection Method Four

Artifact Type and Location	Dog Number 1	2	3	4	5	6	7	8	9	10	11	12
Autolysis												
Serosa	0	0	0	0	0	0	0	0	0	0	0	0
Muscularis	0	0	0	0	0	0	0	0	0	0	0	0
Submucosa	1	1	1	0	3	0	1	0	0	0	0	3
Mucosa	1	2	2	0	3	0	2	1	3	0	0	3
Folding												
Serosa	0	0	0	0	0	0	0	0	0	3	0	0
Muscularis, outer	0	0	1	0	0	0	0	0	0	3	0	0
Muscularis, inner	0	0	1	0	3	0	1	2	1	3	2	1
Submucosa	3	0	2	0	3	0	1	1	1	3	2	1
Lymphoid nodules*	1	1	2	1	0	0	0	0	1	0	-	-
Mucosa	3	0	0	0	3	0	1	1	1	3	0	0
Separation												
Serosa-muscularis	0	0	0	0	0	0	0	0	0	0	0	0
Muscularis outer-inner	3	2	1	1	1	2	1	0	0	0	1	1
Muscularis-submucosa	1	1	2	0	0	0	0	1	0	0	1	0
Submucosa-mucosa	0	0	0	0	0	0	0	0	0	0	0	0
Fracture												
Serosa	0	0	0	0	0	0	0	0	0	0	0	0
Muscularis, outer	0	0	0	3	3	0	2	2	1	0	3	0
Muscularis, inner	3	0	0	0	0	0	2	1	1	0	3	0
Submucosa	2	0	0	3	0	2	3	3	1	1	3	0
Lymphoid nodules*	2	2	1	0	0	0	1	0	0	3	-	-
Mucosa	2	0	1	0	0	0	1	0	0	3	3	0
Miscellaneous												
Stain precipitate	0	0	0	0	0	0	0	0	0	0	0	0
Variables thicknesses	3	0	2	3	0	1	1	0	2	0	3	1
Totals	22	6	13	10	19	5	16	12	11	19	21	10

Sum of totals 164
Mean of totals 13.66
Standard deviation 5.69

0 = No artifact
1 = Mild artifact
2 = Moderate artifact
3 = Marked artifact

* Not included in totals
\- Not present

Sum of totals = Sum of all animals artifact total scores
Mean of totals = Sum of totals divided by number of dogs
Standard deviation = Standard deviation of individual dog total artifact scores

TABLE 28

Ileal Artifact Scores by Type and Location
Using Collection Method Five

Artifact and Location	Dog Number											
	1	2	3	4	5	6	7	8	9	10	11	12
Autolysis												
Serosa	0	0	0	0	0	0	0	0	0	0	0	0
Muscularis	0	0	0	0	0	0	0	0	0	0	0	0
Submucosa	0	0	0	0	0	0	0	0	0	1	0	2
Mucosa	0	0	0	1	0	0	0	0	0	2	0	3
Folding												
Serosa	0	3	1	0	0	0	0	3	0	0	2	0
Muscularis, outer	0	3	1	0	3	0	0	3	1	2	3	0
Muscularis, inner	0	3	1	1	3	0	0	2	0	1	1	0
Submucosa	0	3	1	0	2	1	0	2	0	0	0	1
Lymphoid nodules*	-	0	2	0	2	3	0	1	2	0	3	3
Mucosa	0	3	0	0	0	2	0	2	0	0	1	2
Separation												
Serosa-muscularis	0	0	0	0	0	0	0	0	0	0	0	0
Muscularis outer-inner	0	2	2	0	0	0	1	2	0	0	0	0
Muscularis-submucosa	2	0	0	1	0	0	0	1	1	0	0	0
Submucosa-mucosa	0	0	0	1	0	0	0	1	0	0	0	0
Fracture												
Serosa	0	0	0	0	0	0	0	0	0	0	0	0
Muscularis, outer	0	0	1	0	1	0	0	0	0	2	0	0
Muscularis, inner	0	0	1	0	1	0	0	0	0	1	0	0
Submucosa	0	0	1	0	0	0	0	1	0	0	0	0
Lymphoid nodules*	-	0	2	0	0	0	0	1	0	0	0	0
Mucosal	0	0	0	0	0	0	0	0	0	0	0	0
Miscellaneous												
Stain precipitate	0	0	0	0	0	0	0	0	0	0	0	0
Variables thicknesses	0	0	0	0	0	0	0	0	0	0	0	0
Totals	2	17	9	4	10	3	1	17	2	9	7	8

Sum of totals 89
Mean of totals 7.41
Standard deviation 5.45

0 = No artifact
1 = Mild artifact
2 = Moderate artifact
3 = Marked artifact

* Not included in totals
\- Not present

Sum of totals = Sum of all animals artifact total scores
Mean of totals = Sum of totals divided by number of dogs
Standard deviation = Standard deviation of individual dog total artifact scores

TABLE 29

Colonic Artifact Scores by Type and Location
Using Collection Method One

Artifact Type and Location	Dog Number											
	1	2	3	4	5	6	7	8	9	10	11	12
Autolysis												
Serosa	3	0	2	0	1	1	0	1	2	0	0	1
Muscularis	0	0	0	0	0	0	0	0	0	0	0	0
Submucosa	1	0	0	0	0	0	0	0	0	0	0	2
Mucosa	2	0	0	0	1	0	0	1	0	0	0	3
Folding												
Serosa	0	0	0	1	0	0	0	1	0	0	0	0
Muscularis, outer	3	0	0	3	0	1	0	2	0	0	0	0
Muscularis, inner	3	0	1	0	0	1	0	0	0	2	0	0
Submucosa	0	0	0	0	0	0	0	0	0	2	0	0
Lymphoid nodules*	0	0	0	-	-	-	0	-	-	3	0	0
Mucosa	0	0	0	0	1	0	0	0	0	3	0	0
Separation												
Serosa-muscularis	3	0	0	2	2	1	1	0	2	0	0	0
Muscularis outer-inner	2	1	0	0	0	0	0	0	0	2	1	1
Muscularis-submucosa	2	0	1	1	0	0	0	0	0	0	0	0
Submucosa-mucosa	0	0	0	0	0	0	0	0	0	0	0	0
Fracture												
Serosa	0	0	0	0	1	1	0	0	0	0	0	0
Muscularis, outer	1	3	0	0	0	0	0	2	0	2	0	0
Muscularis, inner	1	3	0	3	0	0	3	0	2	1	0	1
Submucosa	1	3	0	3	0	0	3	0	1	1	0	1
Lymphoid nodules*	1	1	2	-	-	-	0	-	-	0	3	2
Mucosa	1	2	0	0	0	0	0	0	0	0	1	0
Miscellaneous												
Stain precipitate	0	0	0	0	0	0	0	0	0	0	0	0
Variables thicknesses	0	0	0	0	0	0	1	0	0	0	1	0
Totals	23	12	4	13	6	5	8	7	7	13	3	10

Sum of totals 111
Mean of totals 9.25
Standard deviation 5.49

0 = No artifact
1 = Mild artifact
2 = Moderate artifact
3 = Marked artifact

* Not included in totals
\- Not present

Sum of totals = Sum of all animals artifact total scores
Mean of totals = Sum of totals divided by number of dogs
Standard deviation = Standard deviation of individual dog total artifact scores

TABLE 30

Colonic Artifact Scores by Type and Location
Using Collection Method Two

Artifact Type and Location	Dog Number											
	1	2	3	4	5	6	7	8	9	10	11	12
Autolysis												
Serosa	0	0	0	0	0	0	0	0	0	0	0	0
Muscularis	0	0	0	0	0	0	0	0	0	0	0	0
Submucosa	0	0	0	0	0	0	0	0	0	0	0	3
Mucosa	0	0	0	0	1	0	0	0	0	0	0	3
Folding												
Serosa	0	0	0	0	0	0	0	0	0	0	0	0
Muscularis, outer	0	0	0	1	0	0	0	0	2	0	0	0
Muscularis, inner	0	0	0	1	0	0	2	0	2	0	1	0
Submucosa	0	0	0	0	0	0	1	0	1	0	0	0
Lymphoid nodules*	-	-	0	-	-	-	0	0	-	0	-	-
Mucosa	0	0	0	0	0	0	0	0	0	0	0	0
Separation												
Serosa-muscularis	0	0	0	0	0	0	0	0	0	0	0	0
Muscularis outer-inner	0	2	0	0	0	0	1	0	1	3	1	0
Muscularis-submucosa	0	0	0	0	0	1	1	0	0	0	0	0
Submucosa-mucosa	0	3	0	0	1	2	0	0	0	0	0	0
Fracture												
Serosa	0	0	0	0	0	0	0	0	0	0	0	0
Muscularis, outer	0	0	0	0	1	0	0	0	3	0	0	0
Muscularis, inner	0	3	1	0	3	0	0	2	0	0	1	0
Submucosa	0	2	1	0	3	0	0	2	0	3	1	0
Lymphoid nodules*	-	-	0	-	-	-	0	1	-	1	-	-
Mucosa	0	0	0	0	0	0	0	0	0	0	0	0
Miscellaneous												
Stain precipitate	0	0	0	0	0	0	0	0	0	0	0	0
Variables thicknesses	0	2	1	3	0	0	2	0	1	1	0	0
Totals	0	12	3	5	9	3	7	5	10	7	4	6

Sum of totals 70
Mean of totals 5.8
Standard deviation 3.37

0 = No artifact
1 = Mild artifact
2 = Moderate artifact
3 = Marked artifact

* Not included in totals
\- Not present

Sum of totals = Sum of all animals artifact total scores
Mean of totals = Sum of totals divided by number of dogs
Standard deviation = Standard deviation of individual dog total artifact scores

TABLE 31

Colonic Artifact Scores by Type and Location
Using Collection Method Three

Artifact and Location	Dog Number 1	2	3	4	5	6	7	8	9	10	11	12
Autolysis												
Serosa	0	0	0	0	0	0	0	0	0	0	0	0
Muscularis	0	0	0	0	0	0	0	0	0	0	0	0
Submucosa	0	0	0	0	0	0	0	0	0	0	0	1
Mucosa	0	0	0	0	0	0	0	0	0	0	0	2
Folding												
Serosa	0	0	0	0	0	0	0	0	0	0	0	0
Muscularis, outer	0	0	0	0	0	0	0	1	0	0	0	0
Muscularis, inner	0	0	0	0	0	0	0	1	0	0	0	0
Submucosa	0	0	0	0	0	0	0	1	2	0	0	1
Lymphoid nodules*	-	1	0	-	-	0	-	1	1	-	-	2
Mucosa	0	1	0	0	0	0	0	1	1	0	0	0
Separation												
Serosa-muscularis	1	0	1	3	2	0	0	3	0	3	0	2
Muscularis outer-inner	1	0	0	3	0	0	0	1	2	0	0	0
Muscularis-submucosa	0	0	0	0	0	0	0	2	0	0	0	0
Submucosa-mucosa	0	0	0	3	0	0	0	0	0	0	0	0
Fracture												
Serosa	1	0	0	0	2	0	0	3	0	0	0	2
Muscularis, outer	3	0	0	2	0	0	1	2	0	3	0	1
Muscularis, inner	3	0	0	1	0	0	1	1	0	2	0	1
Submucosa	1	0	0	0	0	0	0	2	0	0	0	0
Lymphoid nodules*	-	0	0	-	-	0	-	0	0	-	-	0
Mucosa	0	0	0	3	0	0	0	0	0	0	0	0
Miscellaneous												
Stain precipitate	0	0	0	0	0	0	0	0	0	0	0	0
Variables thicknesses	1	0	0	0	2	1	1	0	0	0	0	0
Totals	11	1	1	15	6	1	3	18	5	8	0	10

Sum of totals 79
Mean of totals 6.58
Standard deviation 5.93

0 = No artifact
1 = Mild artifact
2 = Moderate artifact
3 = Marked artifact

* Not included in totals
- Not present

Sum of totals = Sum of all animals artifact total scores
Mean of totals = Sum of totals divided by number of dogs
Standard deviation = Standard deviation of individual dog total artifact scores

TABLE 32

TABLE 32

Colonic Artifact Scores by Type and Location
Using Collection Method Four

Artifact and Location	Dog Number 1	2	3	4	5	6	7	8	9	10	11	12
Autolysis												
Serosa	0	0	0	0	0	0	0	0	0	0	0	0
Muscularis	0	0	0	0	0	0	0	0	0	0	0	0
Submucosa	0	0	0	3	0	2	2	2	2	0	0	2
Mucosa	0	0	0	3	0	2	1	1	2	3	0	2
Folding												
Serosa	0	1	0	3	0	1	0	2	0	2	0	1
Muscularis, outer	0	1	0	3	0	3	1	2	0	2	0	1
Muscularis, inner	0	2	0	3	0	1	3	2	0	3	0	2
Submucosa	0	0	0	3	0	0	1	2	0	1	0	0
Lymphoid nodules*	-	-	-	0	-	-	-	-	1	-	-	-
Mucosa	0	0	0	3	0	0	0	2	0	0	0	0
Separation												
Serosa-muscularis	1	0	0	0	0	1	1	0	0	0	0	0
Muscularis outer-inner	3	0	0	2	0	3	3	2	1	1	1	0
Muscularis-submucosa	0	0	0	0	0	1	1	0	0	0	0	0
Submucosa-mucosa	0	0	0	0	0	0	0	0	0	0	0	0
Fracture												
Serosa	0	0	0	0	0	0	0	0	0	0	0	0
Muscularis, outer	0	0	3	0	2	0	1	2	0	0	1	0
Muscularis, inner	0	0	2	0	2	0	1	2	0	1	1	0
Submucosa	0	0	1	0	1	0	0	1	0	1	0	0
Lymphoid nodules*	-	-	-	1	-	-	-	-	0	-	-	-
Mucosa	0	0	0	0	0	0	0	0	0	0	0	0
Miscellaneous												
Stain precipitate	0	0	0	0	0	0	0	0	0	0	0	0
Variables thicknesses	3	0	1	1	0	2	1	3	0	1	0	0
Totals	7	4	7	24	5	16	16	23	5	15	3	8

Sum of totals 133
Mean of totals 11.00
Standard deviation 7.41

0 = No artifact
1 = Mild artifact
2 = Moderate artifact
3 = Marked artifact

* Not included in totals
\- Not present

Sum of totals = Sum of all animals artifact total scores
Mean of totals = Sum of totals divided by number of dogs
Standard deviation = Standard deviation of individual dog total artifact scores

TABLE 33

Colonic Artifact Scores by Type and Location
Using Collection Method Five

Artifact and Location	Dog Number 1	2	3	4	5	6	7	8	9	10	11	12
Autolysis												
Serosa	0	0	0	0	0	0	0	0	0	0	0	0
Muscularis	0	0	0	0	0	0	0	0	0	0	0	0
Submucosa	0	0	0	0	0	0	0	0	0	0	0	2
Mucosa	0	0	0	0	0	0	0	0	0	0	0	2
Folding												
Serosa	0	0	0	1	0	0	0	0	3	1	0	0
Muscularis, outer	1	3	0	1	0	2	3	0	3	0	1	0
Muscularis, inner	1	2	0	1	0	1	0	0	3	0	1	0
Submucosa	0	0	0	2	2	0	0	0	3	0	0	0
Lymphoid nodules*	-	-	-	-	-	-	0	-	-	0	-	-
Mucosa	0	0	0	2	3	0	0	0	3	0	2	0
Separation												
Serosa-muscularis	0	0	2	0	0	0	3	1	1	0	0	0
Muscularis outer-inner	0	0	3	0	1	0	0	1	0	0	0	1
Muscularis-submucosa	0	0	0	0	0	0	0	0	0	0	0	1
Submucosa-mucosa	0	0	0	0	0	0	0	0	0	0	0	0
Fracture												
Serosa	0	0	0	0	0	0	0	0	0	0	0	0
Muscularis, outer	0	0	0	0	1	0	0	1	0	0	0	0
Muscularis, inner	0	1	1	0	0	0	0	1	0	2	0	0
Submucosa	0	0	0	0	2	0	0	1	0	0	0	0
Lymphoid nodules*	-	-	-	-	-	-	0	-	-	0	-	-
Mucosa	0	1	0	0	0	0	0	0	0	0	0	0
Miscellaneous												
Stain precipitate	0	0	0	0	0	0	0	0	0	0	0	0
Variables thicknesses	0	1	0	0	0	0	2	0	1	0	0	0
Totals	2	8	6	7	9	8	8	5	17	3	4	6

Sum of totals	78
Mean of totals	6.50
Standard deviation	3.98

0 = No artifact
1 = Mild artifact
2 = Moderate artifact
3 = Marked artifact

* Not included in totals
\- Not present

Sum of totals = Sum of all animals artifact total scores
Mean of totals = Sum of totals divided by number of dogs
Standard deviation = Standard deviation of individual dog total artifact scores

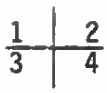


Fig 1. Ouodenum of dog 4, collection method 3. Separation of the tunica serosa from the underlying muscularis, graded marked. (120X)

Fig 2. Jejunum of dog 6, collection method 1. Tissue folding of the outer muscularis layer, graded moderate. Outer muscularis fiber separation and disruption, graded moderate. (100X).

Fig 3. Ouodenum of dog 1, collection method 3. Separation of the tunica serosa from the muscularis layer, graded moderate. (300X)

Fig 4. Colon of dog 8, collection method 3. Tissue folding in outer muscularis, graded mild. Oisruption of the serosal epithelium with localized separation from muscularis, graded marked. (100X)

Figure 1

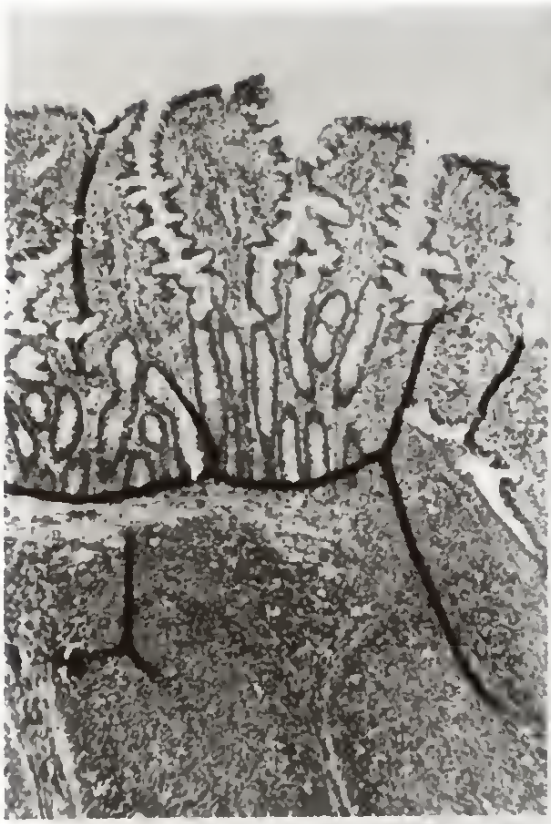


Figure 2

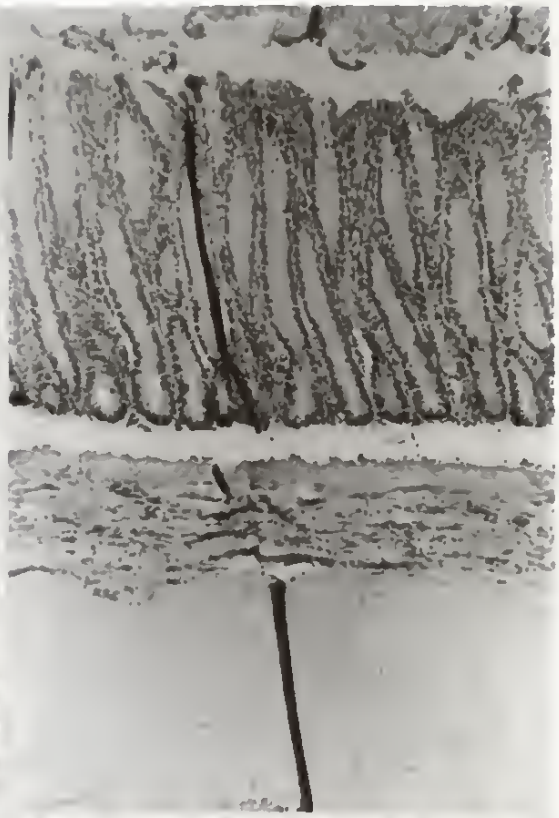


Figure 3

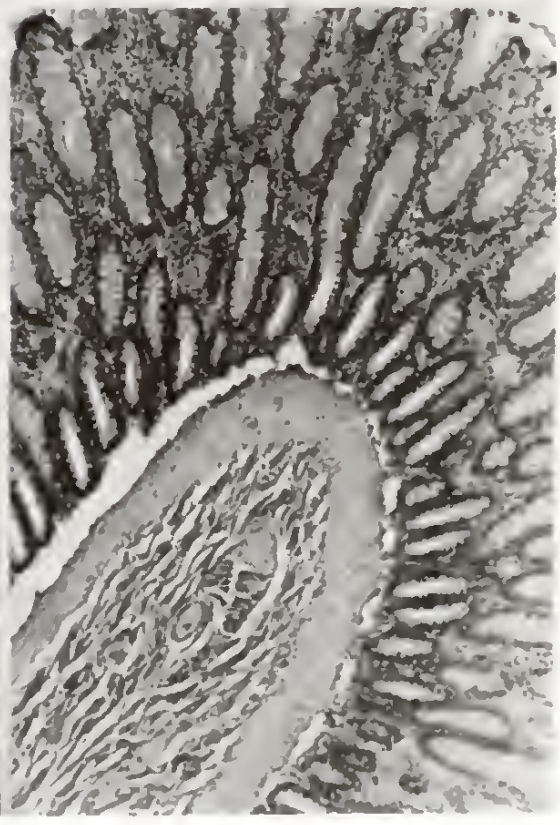


Figure 4

Fig 5. Ouodenum of dog 4, collection method 3. Separation of the tunica serosa from underlying muscularis, graded marked (120X).

Fig 6. Jejunum of dog 6, collection method 1. Tissue folding in the outer muscularis, graded moderate. Outer muscularis fiber separation and disruption, graded moderate (100X).

Fig 7. Ouodenum of dog 1, collection method 3. Separation of the tunica serosa from the inner muscularis, graded moderate (300X).

Fig 8. Colon of dog 8, collection method 3. Folding in the outer muscularis, graded mild. Localized separation of the serosa from the outer muscularis, graded marked (100X).

Figure 5

Figure 6

Figure 7

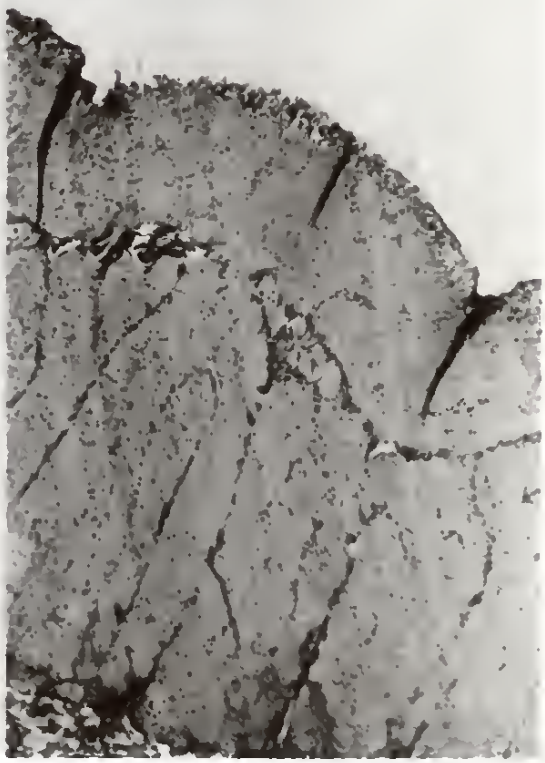


Figure 8

9	10
11	12

Fig 9. Ileum of dog 10, collection method 4. Folding artifact extending from serosa to mucosa, graded marked (120X).

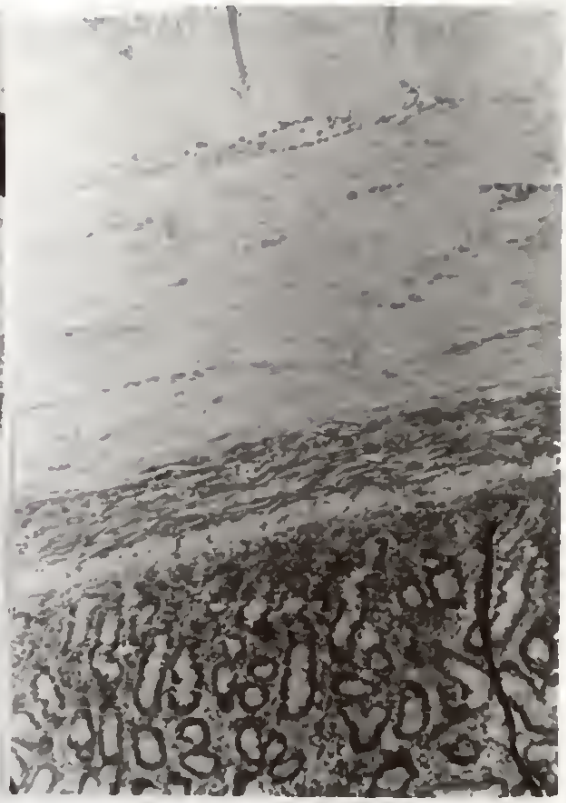
Fig 10. Jejunum of dog 4, collection method 2. Folding artifact in the outer muscularis, graded mild. Folding in the mucosa was graded moderate (120X).

Fig 11. Duodenum of dog 9, collection method 4. Folding of the outer muscularis, graded mild. Other tissues are free of artifacts (120X).

Fig 12. Ileum of dog 10, collection method 5. Outer muscularis with folding artifacts, graded moderate (120X).

Figure 9

Figure 10



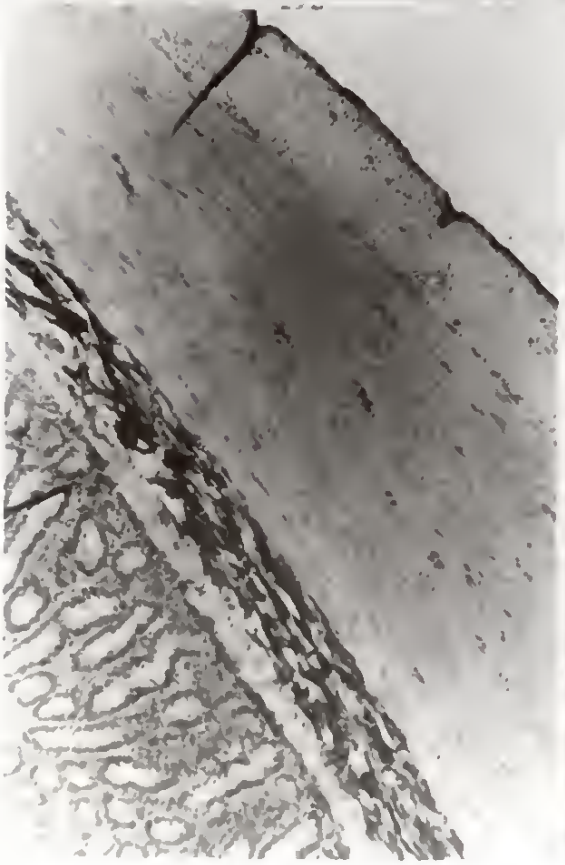


Figure 11

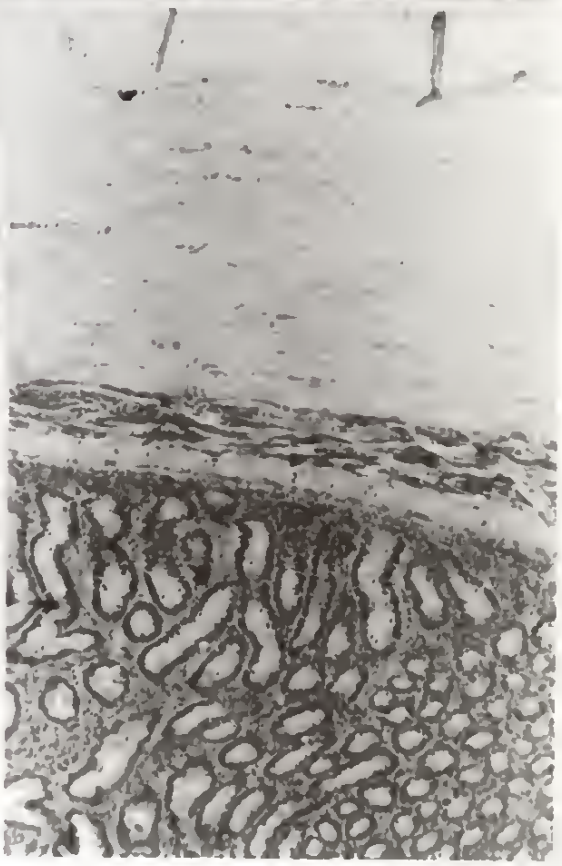


Figure 12

13	14
15	16

Fig 13. Duodenum of dog 2, collection method 1. Folding artifacts extending through the tunica muscularis but into the submucosa, graded moderate (100X).

Fig 14. Ileum of dog 1, collection method 4. Random folding artifacts throughout the mucosa and submucosa, graded marked (120X).

Fig 15. Duodenum of dog 6, collection method 4. Variable tissue thickness in the muscularis, graded marked. Separations in the submucosa, graded marked (300X).

Fig 16. Ileum of dog 1, collection method 5. Folding artifacts extending through all tissue layers, graded marked (300X).

Figure 13

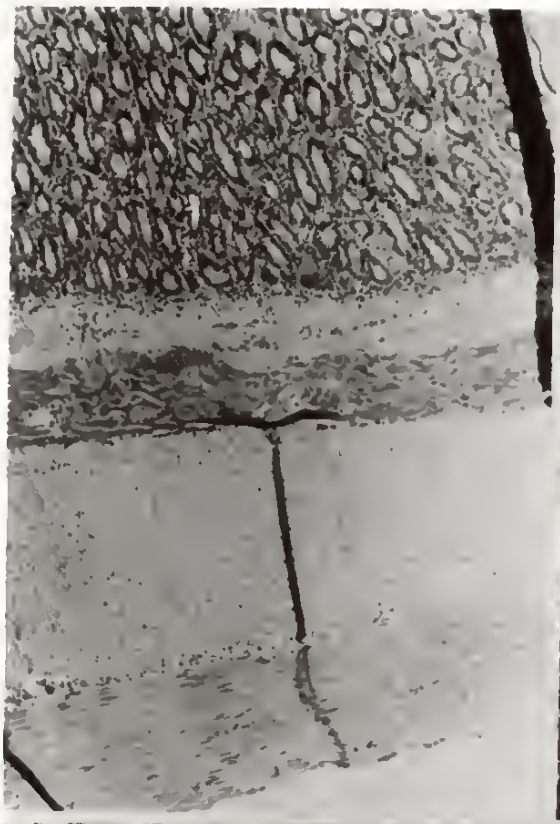


Figure 14

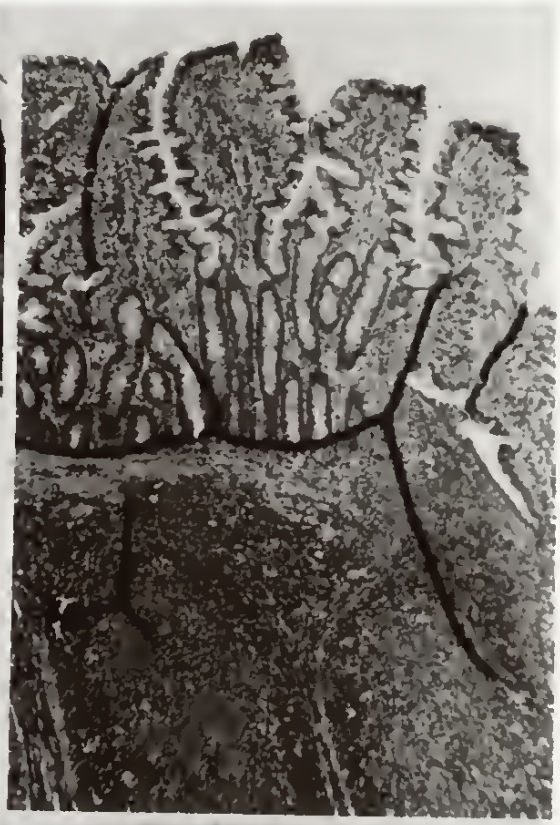


Figure 15

Figure 16

17	18
19	20

Fig 17. Jejunum of dog 9, collection method 4. Separation of muscle bundles in the tunica muscularis that are most severe in the inner layer, grade marked (300X).

Fig 18. Duodenum of dog 4, collection method 1. Fracture artifacts in the tunica muscularis, graded marked. Separations in the submucosa, graded marked (120X).

Fig 19. Jejunum of dog 2, collection method 5. Fracture artifacts in the tunica muscularis, graded marked. Separations in the submucosa, graded moderate (120X).

Fig 20. Ileum of dog 1, collection method 1. Fracture artifacts and variable tissue thickness in the muscularis, graded marked. Stain precipitate present within the fractured area, graded marked (120X).

Figure 17

Figure 18

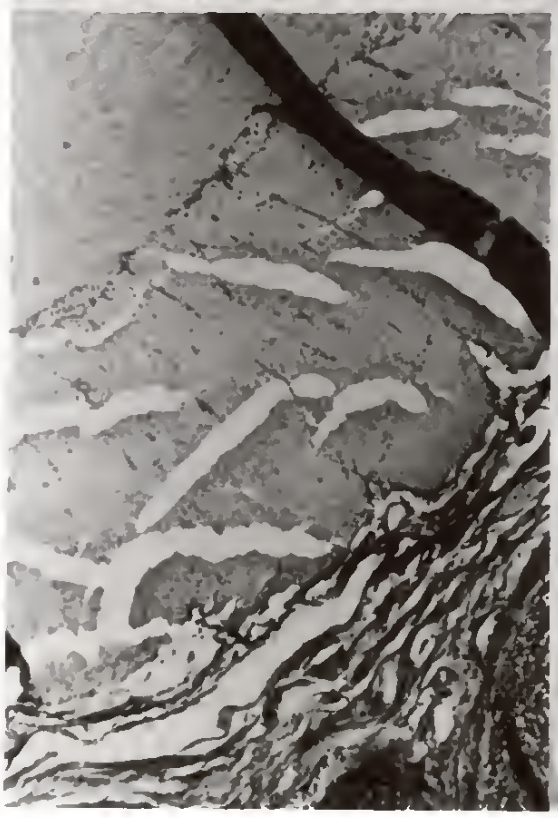


Figure 19

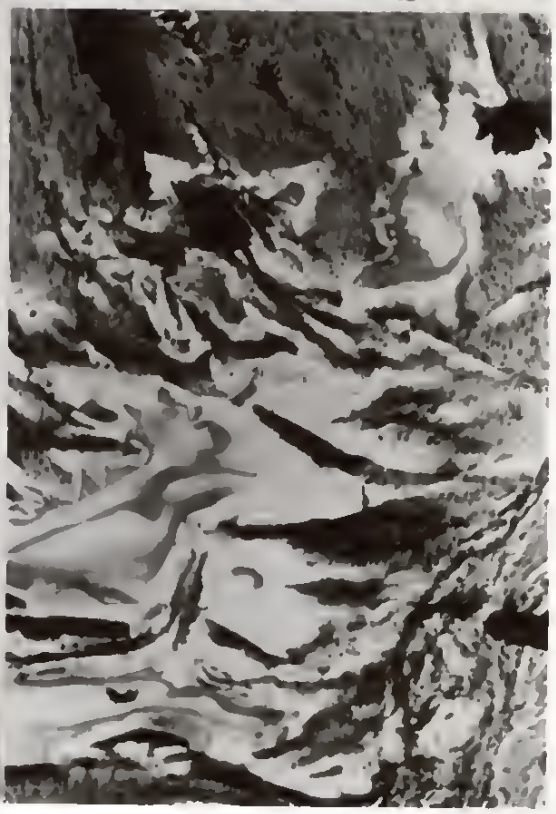


Figure 20

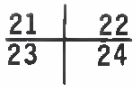


Fig 21. Ileum of dog 6, collection method 3. Fracture artifacts within lymphoid nodules, graded marked (120X).

Fig 22. Colon of dog 6, collection method 2. Variable tissue thickness, graded moderate (120X).

Fig 23. Ileum of dog 10, collection method 4. Random fracture artifacts in lymphoid nodules, graded marked (120X).

Fig 24. Ileum of dog 3, collection method 5. Folding artifacts in lymphoid nodules, graded moderate (120X).

Figure 21

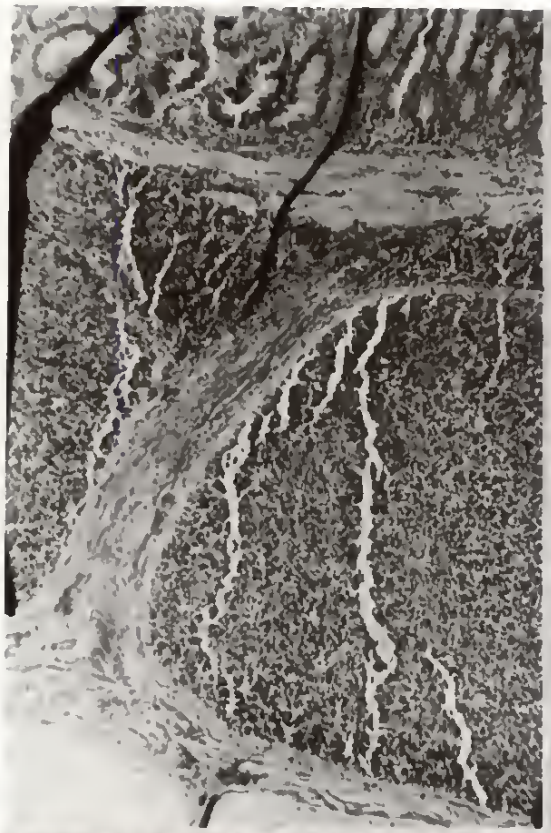
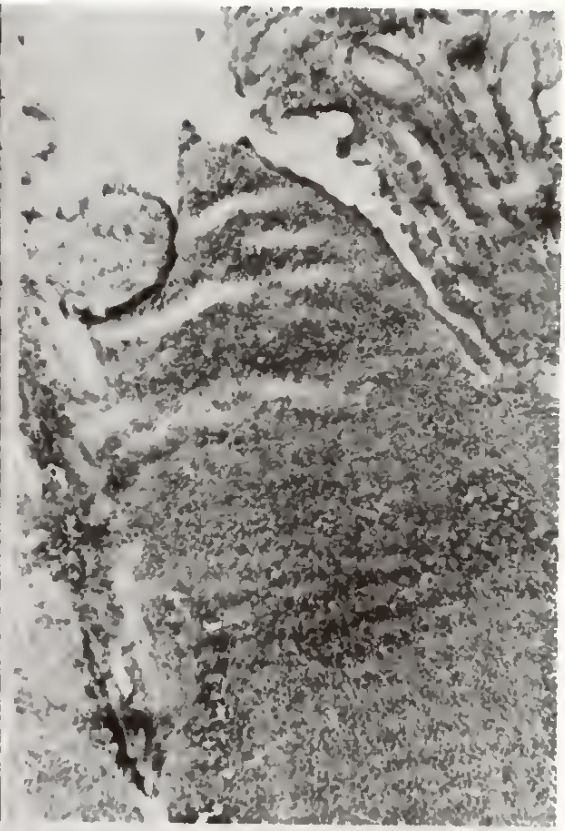
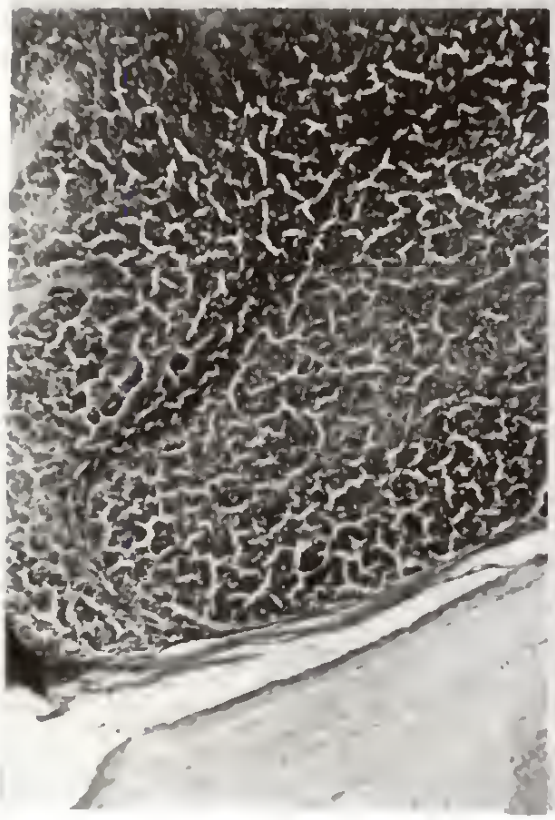


Figure 22





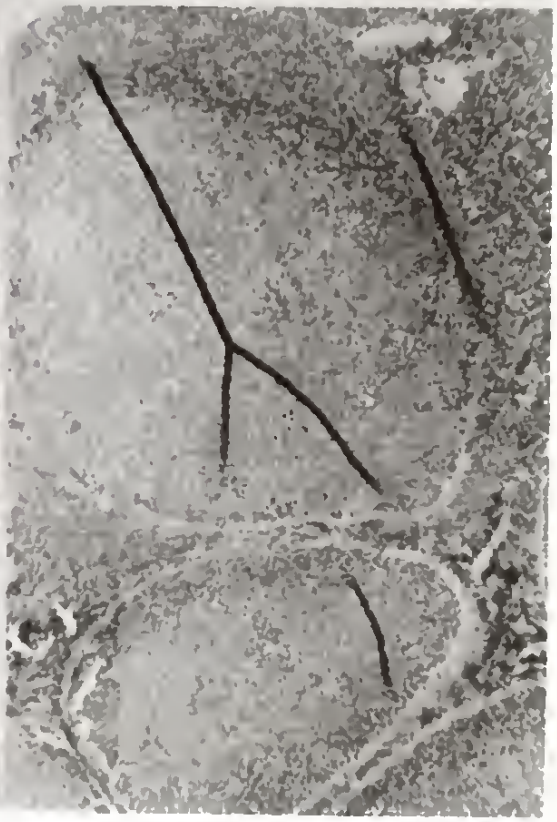


Figure 23

Figure 24

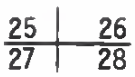


Fig 25. Ouodenum of dog 7, collection method 2. Folding artifacts in the outer muscularis, graded mild. Other tissues are free of artifacts (120X).

Fig 26. Jejunum of dog 8, collectin method 4. Separation in the tunica muscularis, graded marked. Separation at the base of the tunica mucosa, graded mild (100X).

Fig 27. Ouodenum of dog 12, collection method 5. Separation within the submucosa, graded marked (220X).

Fig 28. Colon of dog 4, collection method 3. Separation of the mucosa from the submucosa, graded marked (160X).

Figure 25

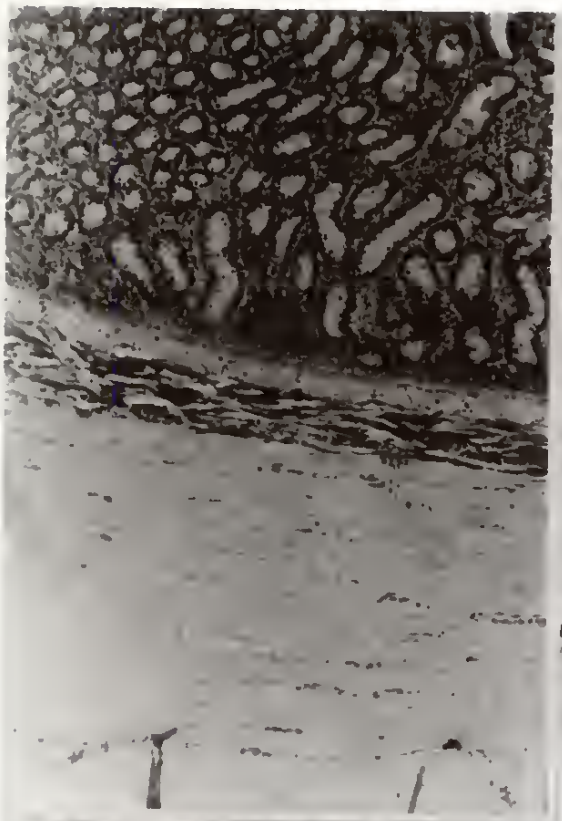
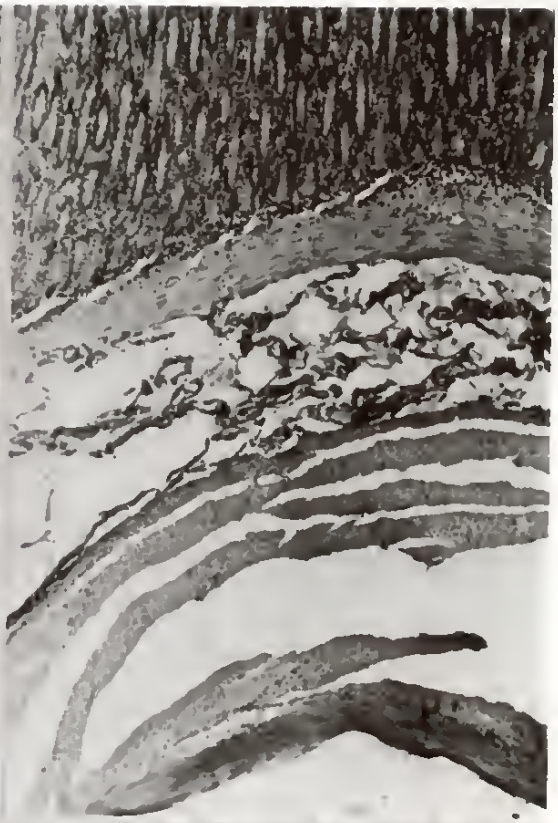
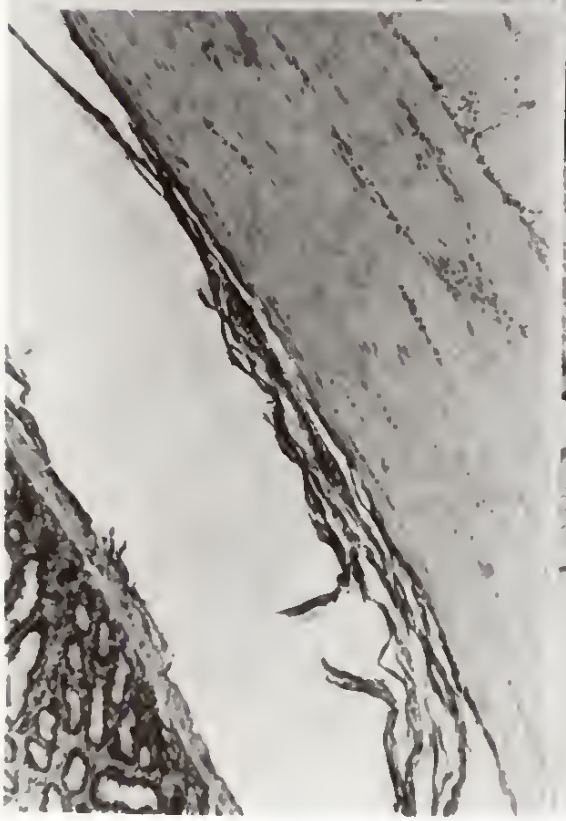


Figure 26





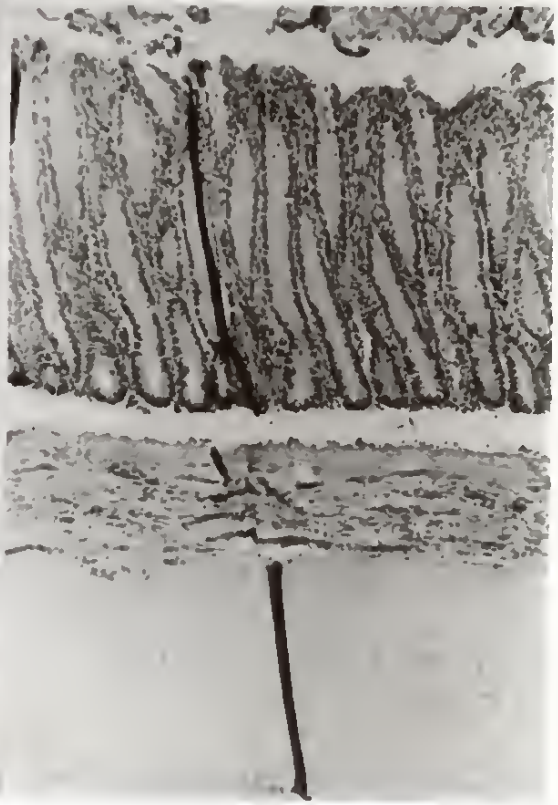


Figure 27

Figure 28

Fig 29. Colon of dog 8, collection method 3. Separation within the submucosa, graded moderate (120X).

Fig 30. Colon of dog 1, collection method 1. Folding artifacts in the muscularis, graded marked (300X).

Fig 31. Ileum of dog 5, collection method 5. Separation artifacts in the submucosa, graded moderate (120X).

Fig 32. Ouodenum of dog 3, collection method 4. Fracture artifacts in the inner muscularis, graded marked (120X).

Figure 29

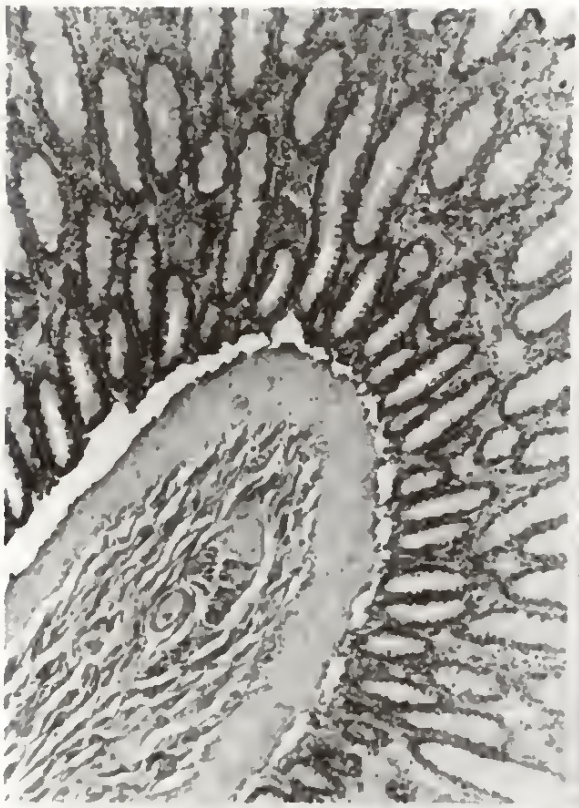
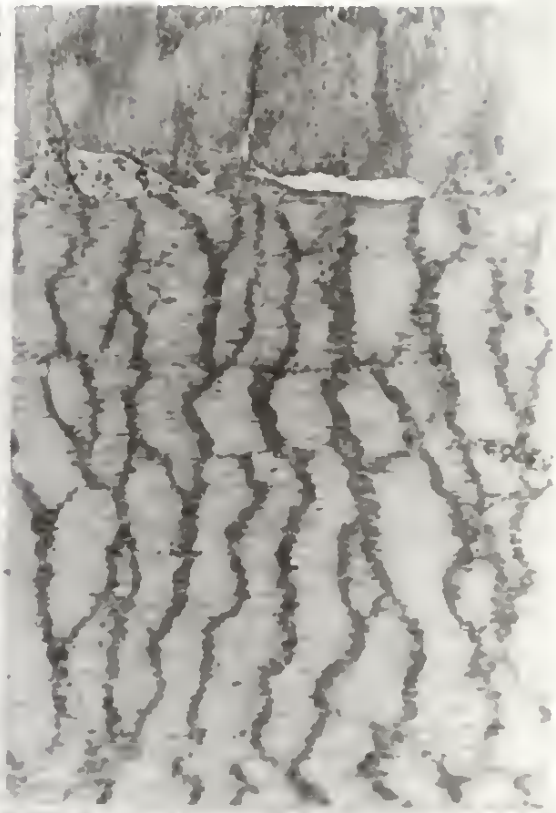


Figure 30



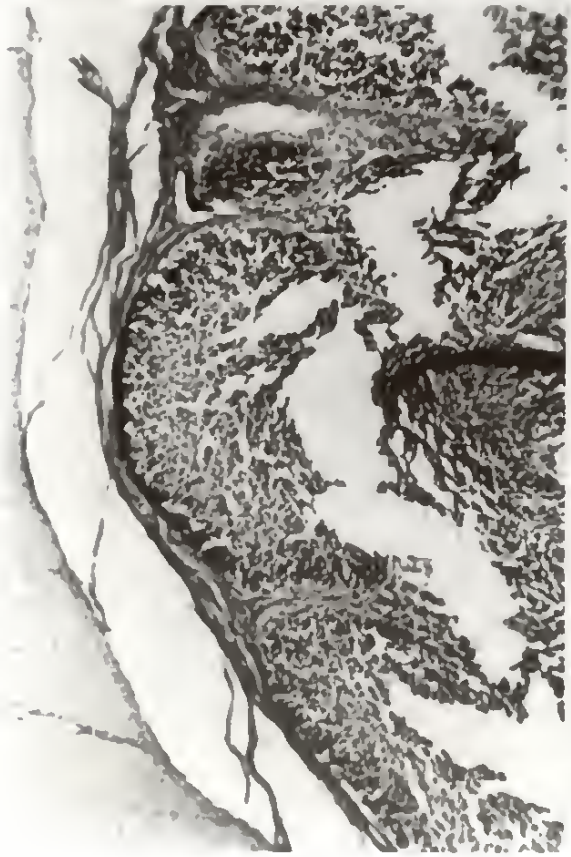


Figure 31

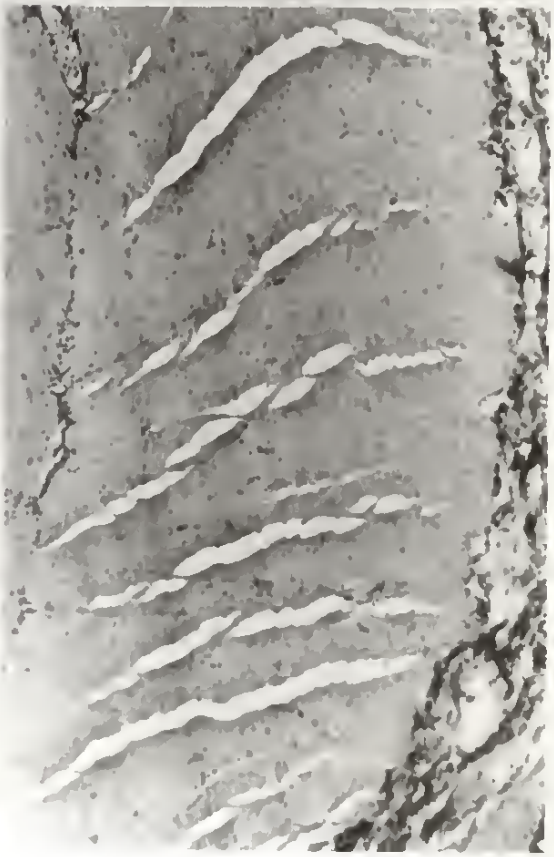


Figure 32

Fig 33. Jejunum of dog 6, collection method 1. Epithelium separated from the basement membrane, graded marked (120X).

Fig 34. Duodenum of dog 7, collection method 3. Fracture artifacts in the mucosa, graded moderate (100X).

Fig 35. Ileum of dog 11, collection method 4. Fracture artifacts in the mucosa, graded marked. Variable tissue thickness, graded marked (120X).

Fig 36. Colon of dog 2, collection method 2. Variable tissue thickness, graded moderate. Separation in the lamina propria, graded marked (120X).

Figure 33

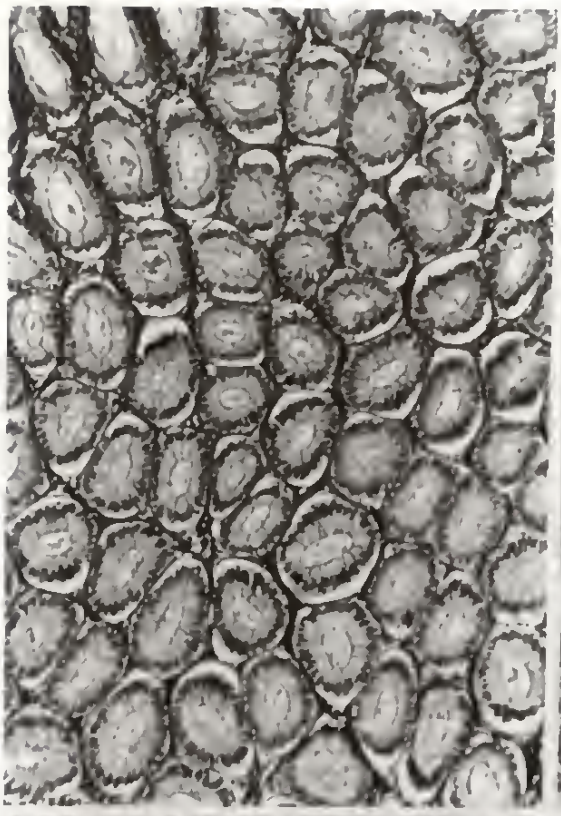


Figure 34

Figure 35

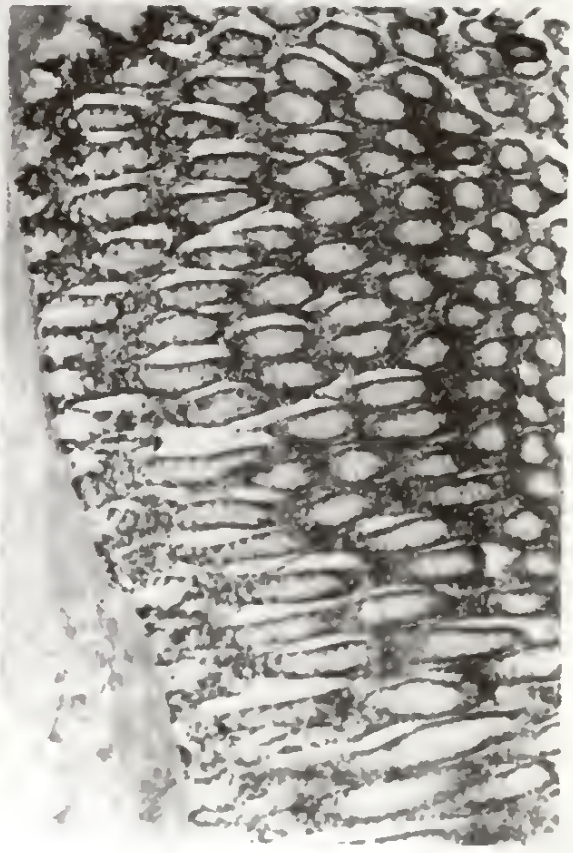


Figure 36

Fig 37. Ouodenum of dog 5, collection method 5. Separation of the submucosal glands from the surrounding submucosa, graded marked. Variable tissue thickness, graded moderate (120X).

Fig 38. Ouodenum of dog 2, collection method 2. Separation of the submucosal glands from the surrounding submucosa, graded mild. Fracture artifacts in the submucosa, graded moderate. Variable tissue thickness, graded mild (120X).

Fig 39. Ouodenum of dog 8, collection method 1. Variable tissue thickness, graded moderate. Submucosal gland separation from the surrounding tissue, graded mild (120X).

Fig 40. Ouodenum of dog 12, collection method 4. Fracture artifacts in the submucosal glands, graded moderate. Folding artifacts in the mucosa and submucosa, graded marked (120X).

Figure 37

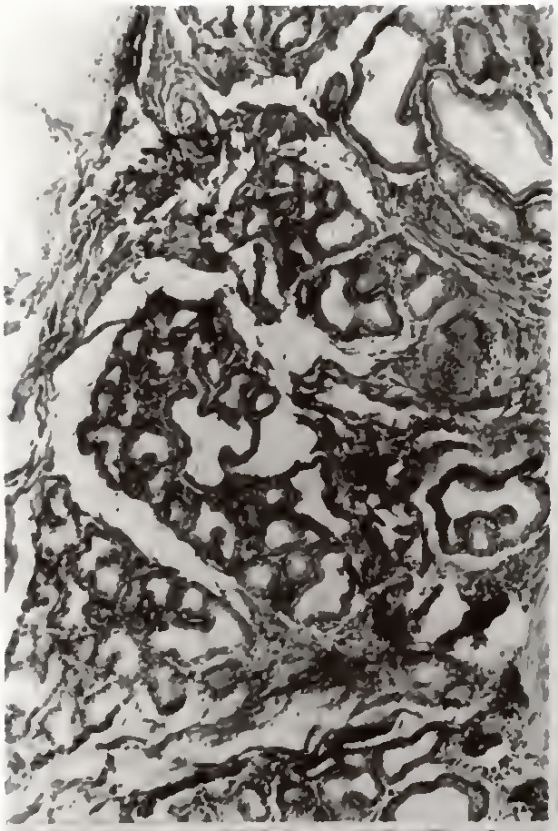
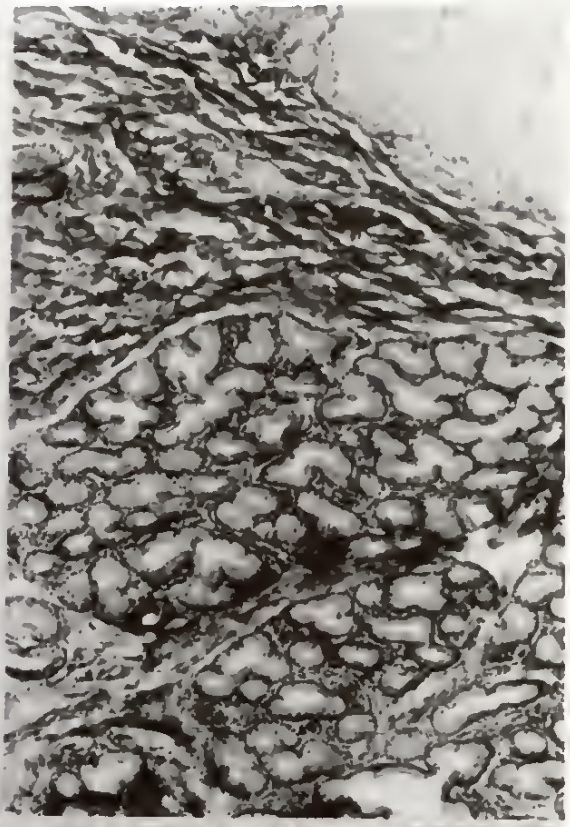


Figure 38



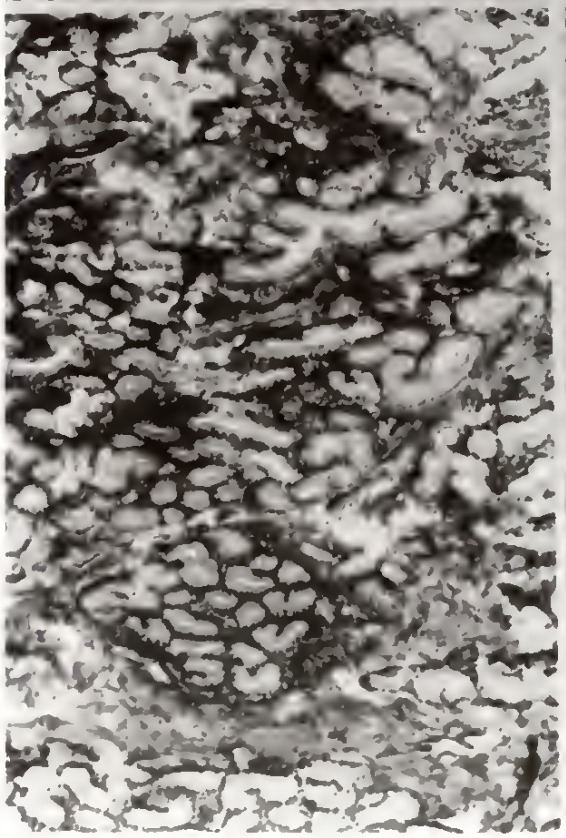


Figure 39

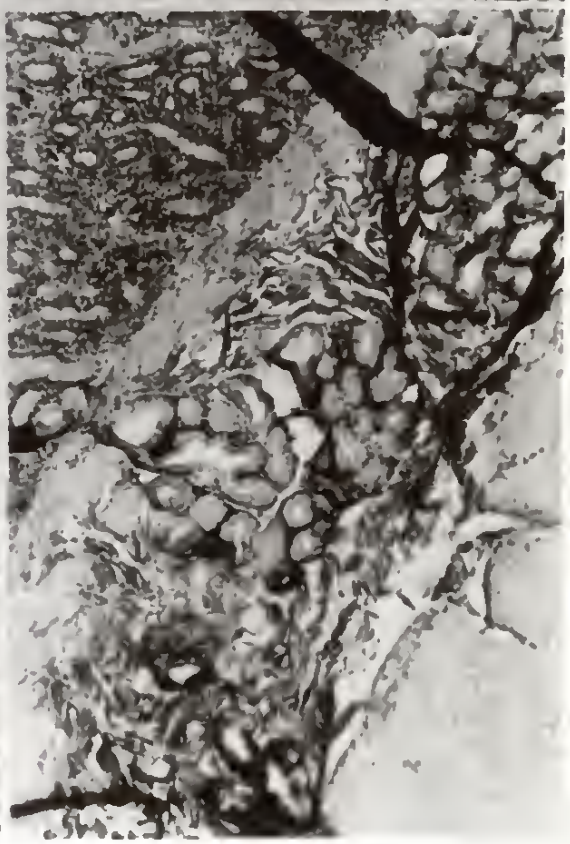


Figure 40

Fig 41. Ouodenum of dog 4, collection method 5. Separation of epithelium from lamina propria at the tip of the villus, graded marked (200X).

Fig 42. Ouodenum of dog 3, collection method 4. Epithelial separation from lamina propria along the sides of the villus, graded (250X).

Fig 43. Ouodenum of dog 7, collection method 1. Epithelial separation from the lamina propria at the top of the villus, graded marked (200X).

Fig 44. Jejunum of dog 2, collection method 3. Epithelial separation with accumulation of serum in the formed space, graded marked (250X).

Figure 41

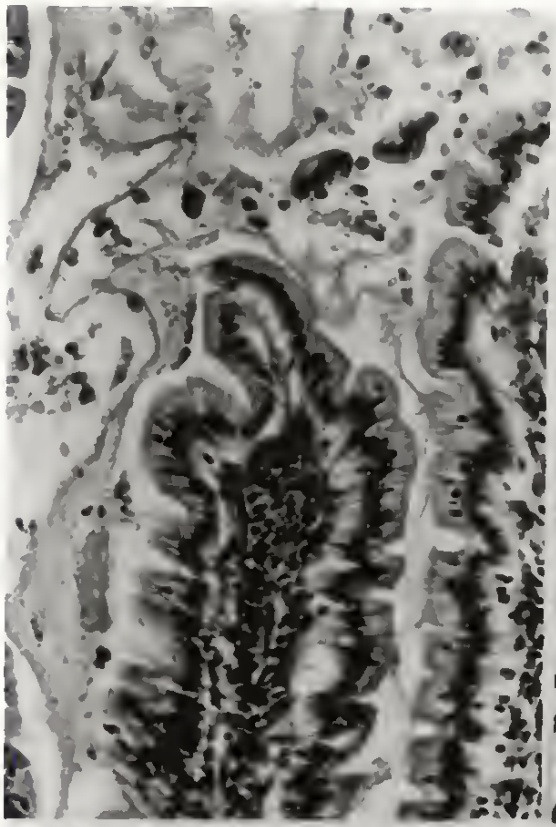


Figure 42

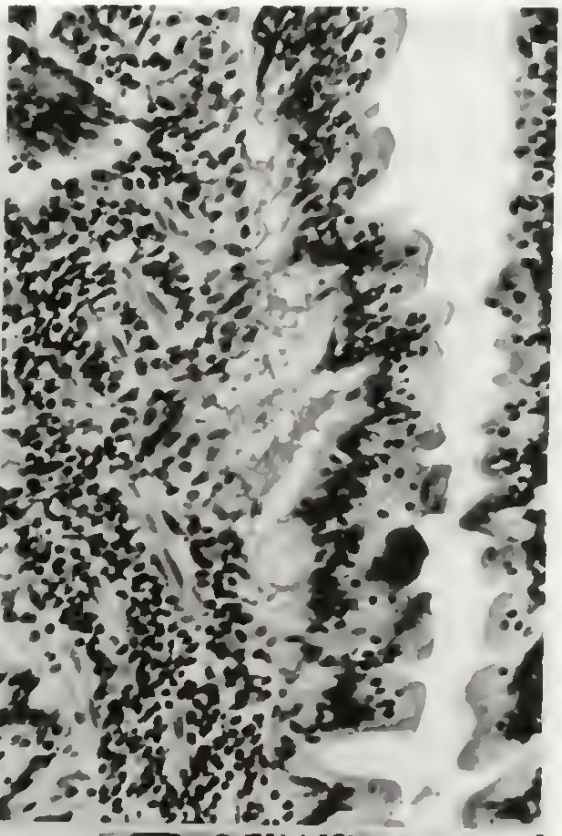


Figure 43

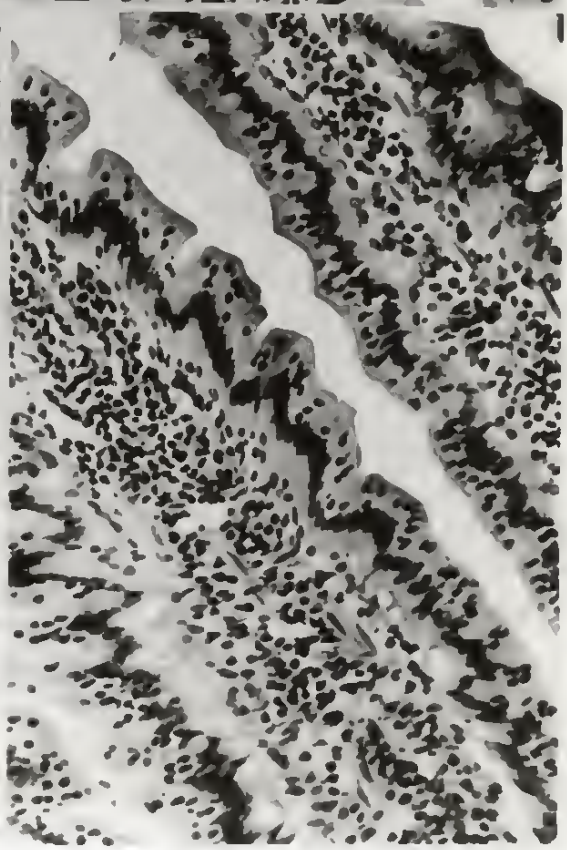


Figure 44

45 | 46
47 | 48

Fig 45. Ouodenum of dog 9, collection method 2. Separation of the epithelium from the lamina propria, graded moderate. Cellular debris and mucin present on the luminal surface (320X).

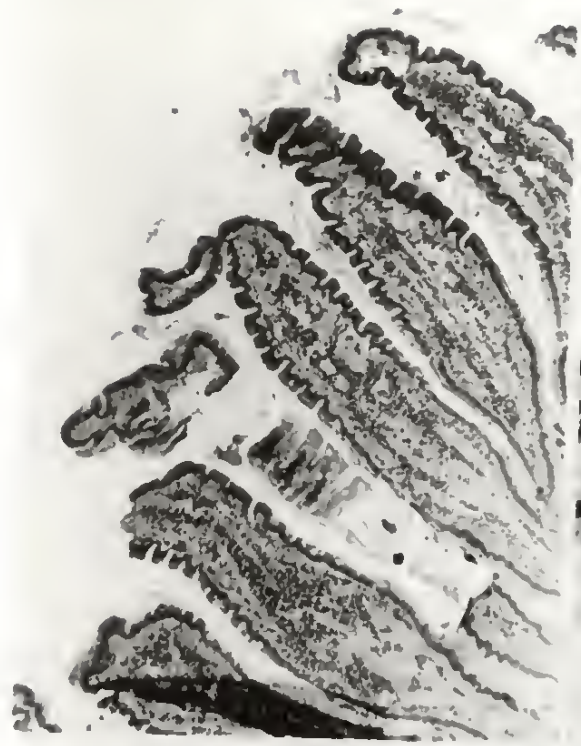
Fig 46. Jejunum of dog 5, collection method 1. Separation of the epithelium from the lamina propria, graded marked. Note the spaces produced by the separation (400X).

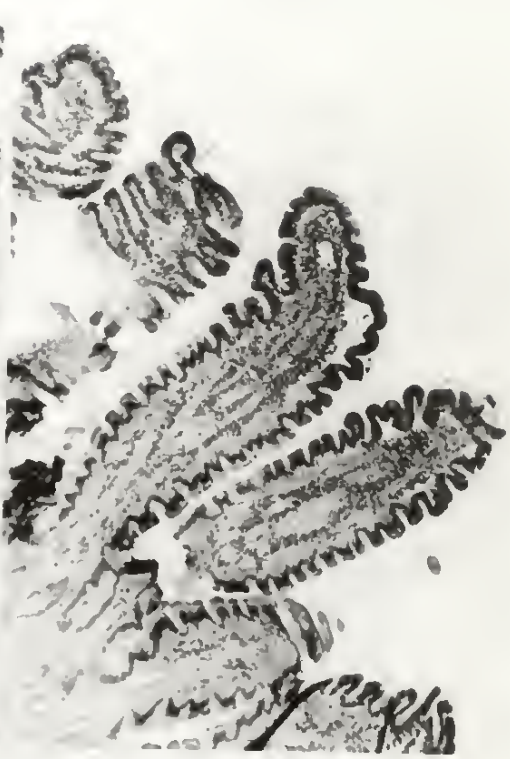
Fig 47. Ouodenum of dog 1, collection method 5. Separation and fracture artifacts between the epithelium and the lamina propria, graded marked (320X).

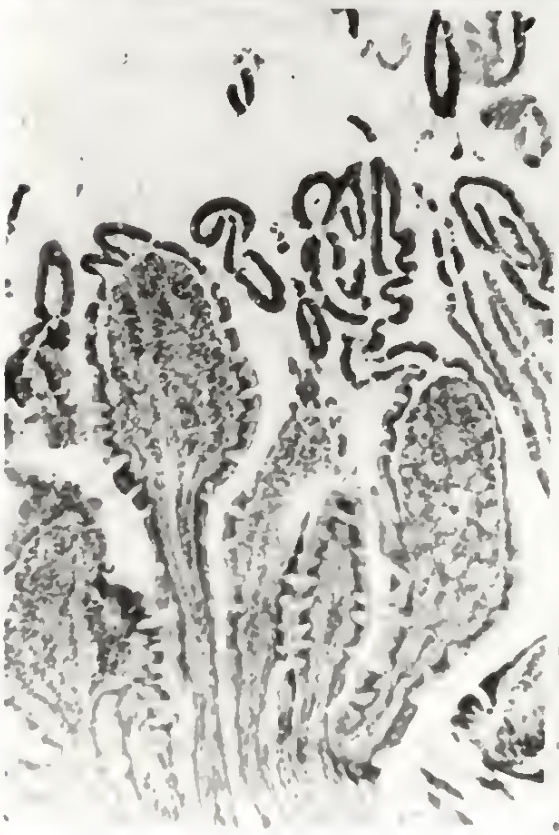
Fig 48. Ouodenum of dog 4, collection method 2. Separation between the epithelium and lamina propria, graded mild (400X)./

Figure 45

Figure 46







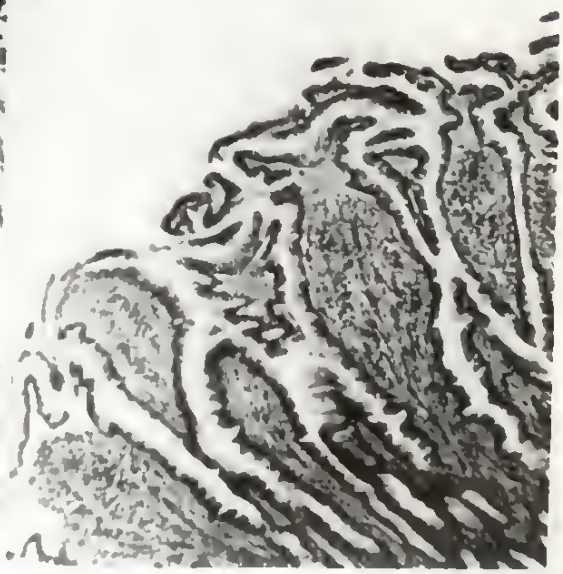


Figure 47

Figure 48

49	50
51	52

Fig 49. Ouodenum of dog 2, collection method 4. Separation artifact between the epithelium and lamina propria, graded marked. Note the central lacteal in the center of the villi (200X).

Fig 50. Ouodenum of dog 2, collection method 4. High magnification of Fig 4. (320X)

Fig 51. Duodenum of dog 9, collection method 5. Separation of the epithelium from the lamina propria, graded marked (320X).

Fig 52. Duodenum of dog 9, collection method 5. Lower magnification of Fig 51. Note the separation occurring both at the tip and along the sides of the villi (200X).

Figure 49

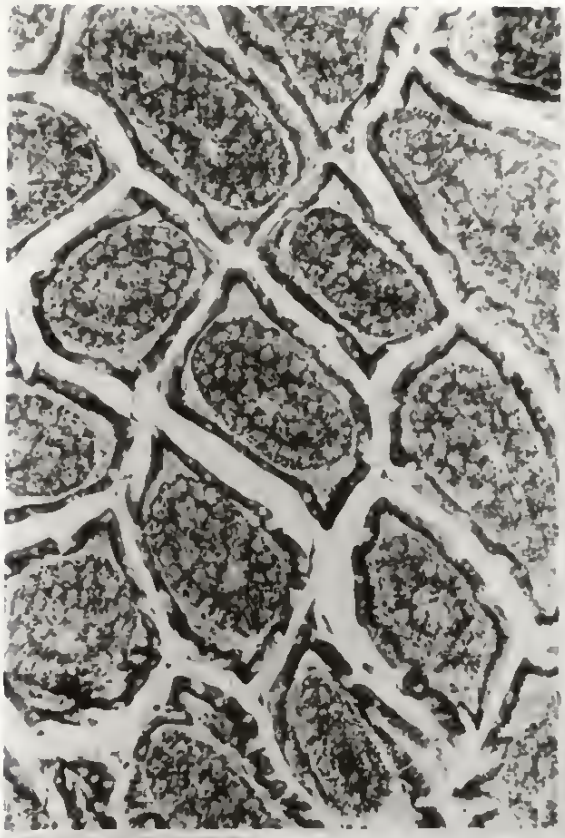
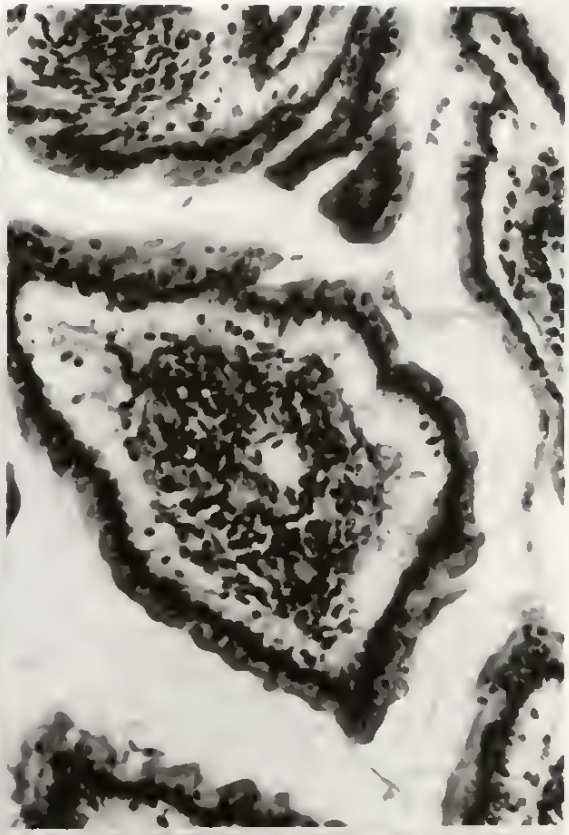


Figure 50



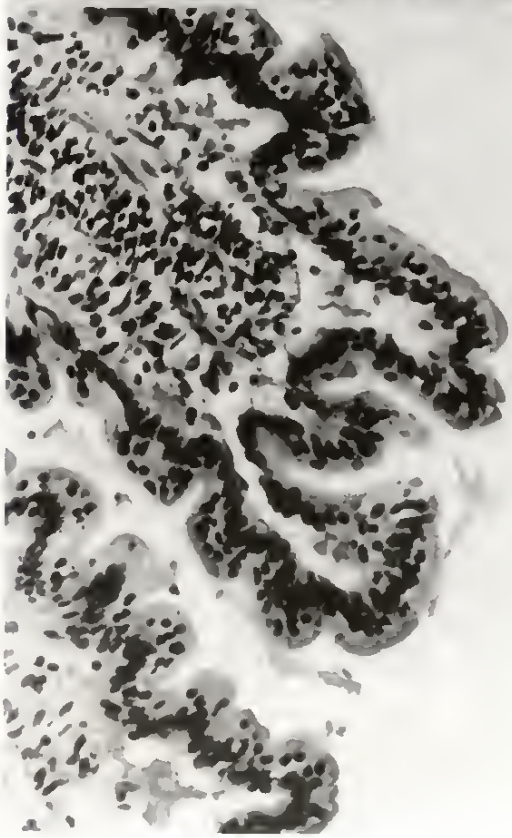


Figure 51

Figure 52

53	54
55	56

Fig 53. Duodenum of dog 4, collection method 4. Separations within the epithelium, Cellular individualization and sloughing, graded moderate. Separation between the epithelium and lamina propria, graded moderate (400X).

Fig 54. Ouodenum of dog 7, collection method 1. Separation between the epithelium and lamina propria, graded marked (400X).

Fig 55. Jejunum of dog 2, collection method 2. Separation between the epithelium and lamina propria, graded mild (400X).

Fig 56. Jejunum of dog 8, collection method 2. Separation between the epithelium and lamina propria, graded moderate (400X).

Figure 53

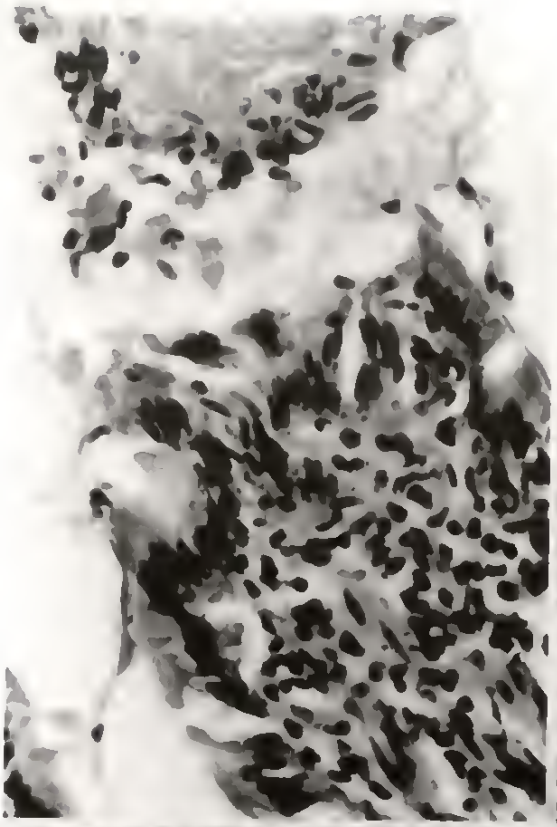
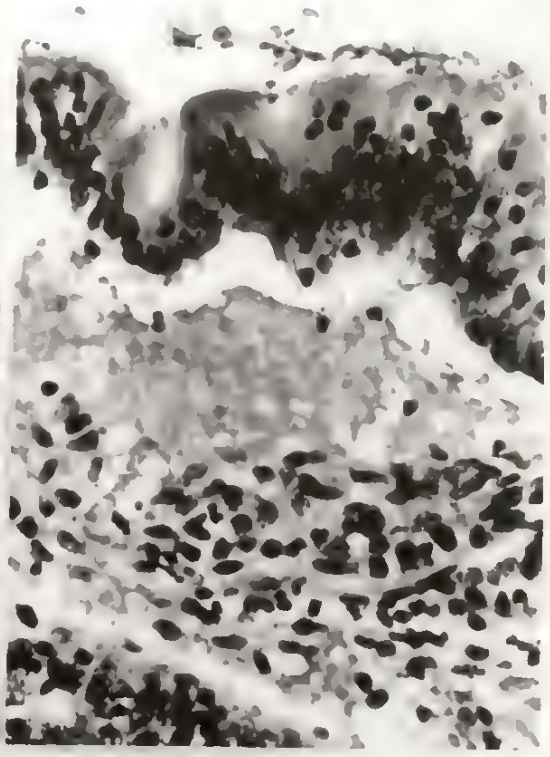


Figure 54



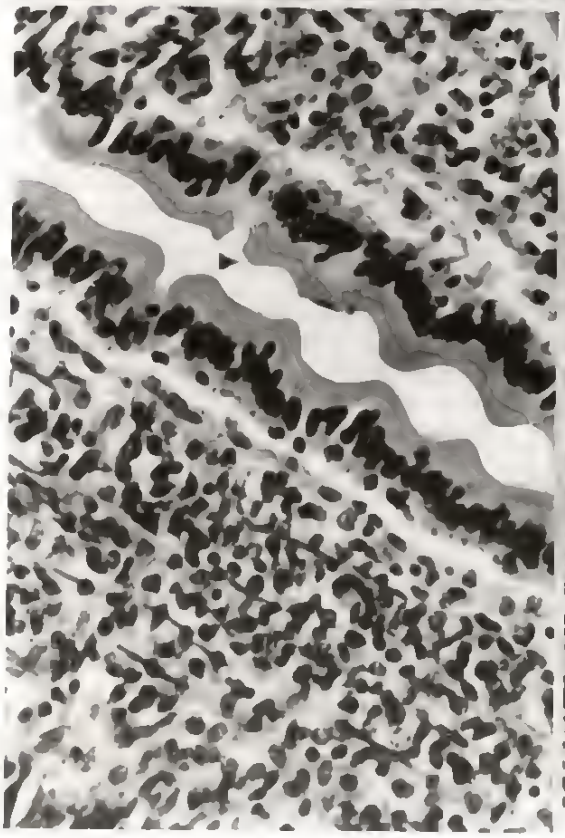


Figure 55

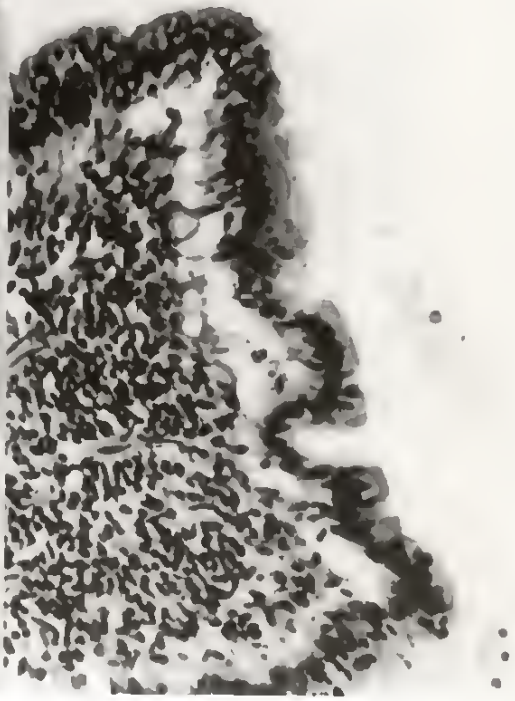


Figure 56

57	58
59	60

Fig 57. Ouodenum of dog 12, collection method 4. Autolysis at the tip of the villus, graded moderate. Note the presence of epithelium over autolysed lamina propria (120X).

Fig 58. Jejunum of dog 10, collection method 4. Autolysis of the villus tips, graded marked (100X).

Fig 59. Ouodenum of dog 2, collection method 5. Epithelial separation from the lamina propria, graded mild. Separation between epithelial cells, graded marked (320X).

Fig 60. Colon of dog 4, collection method 4. Autolysis of the villi, graded marked (120X).

Figure 57

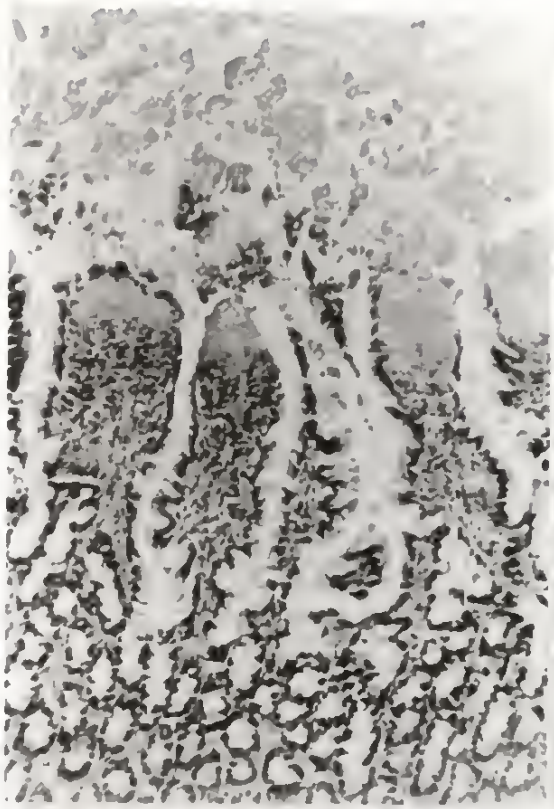
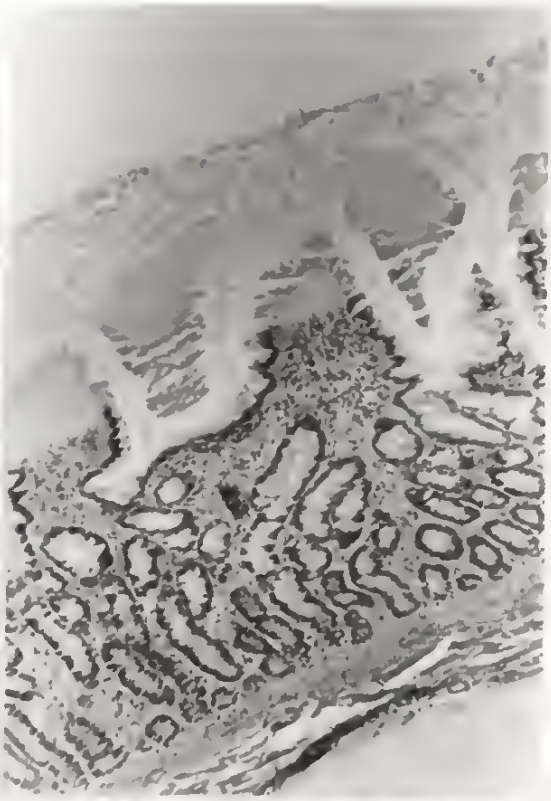


Figure 58



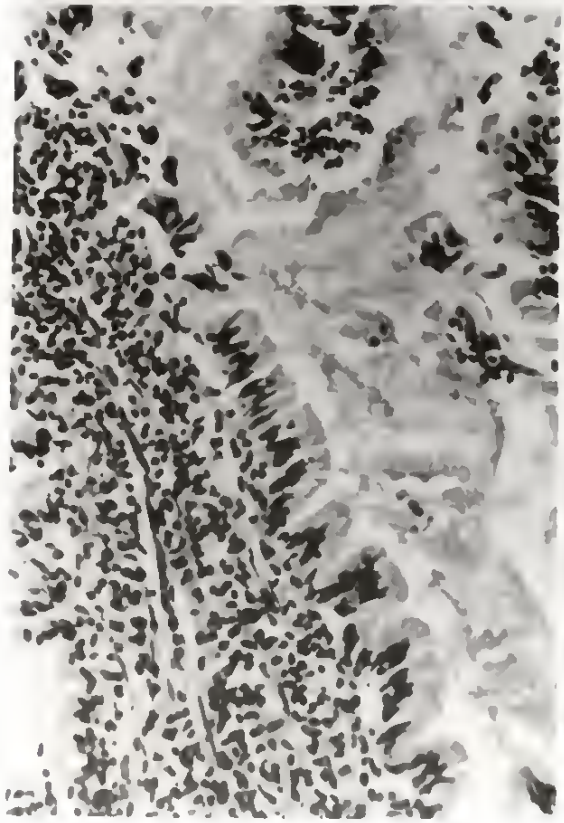


Figure 59

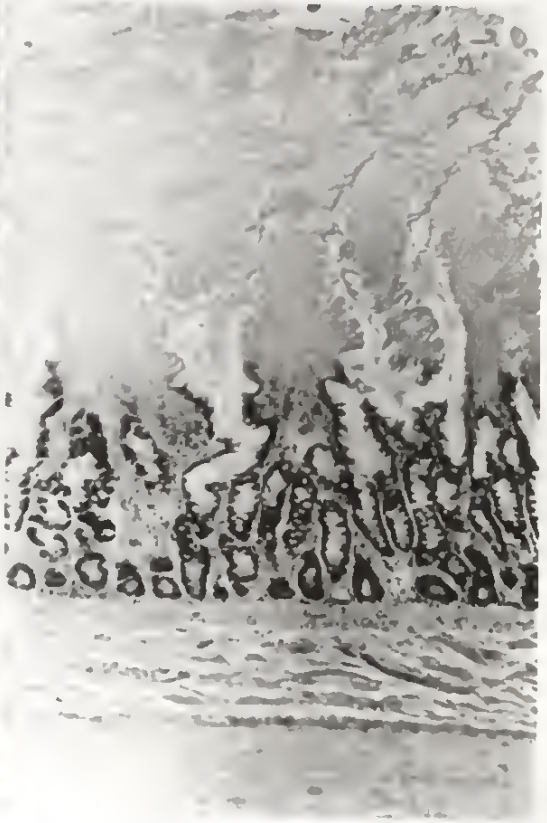


Figure 60

Fig 61. Duodenum of dog 12, collection method 1. Loss of epithelial cells from the tip of the villi. Note lamina propria remains intact. Numerous folds in the epithelium are present along the length of each villus.

Fig 62. Duodenum of dog 12, collection method 5. Loss of epithelium from the villus tip. Debris is noted trapped in epithelial folds.

Figure 61

Figure 62

Fig 63. Duodenum of dog 11, collection method 1. Loss of epithelium at the villus tip.

Fig 64. Duodenum of dog 11, collection method 1. Higher magnification of Fig 63. Note seperation line at the base of epithelial cells. An artifactual space has been formed between the epithelium and the lamina propria.

Figure 63

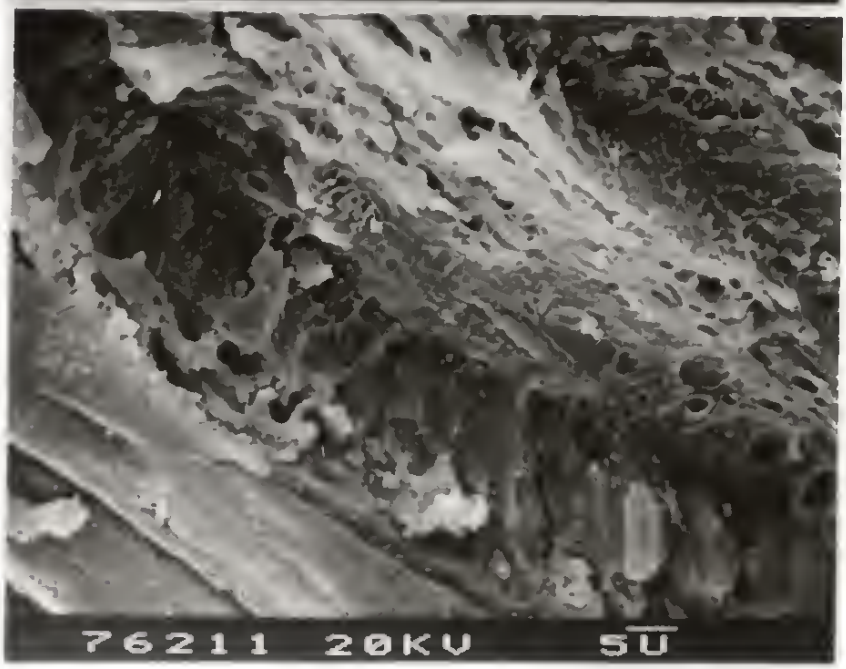


Figure 64

Fig 65. Duodenum of dog 12, collection method 3. Separation of epithelium from the lamina propria with sheets of cells being sloughed.

Fig 66. Duodenum of dog 12, collection method 3. Higher magnification of Fig 65. Note the detachment of sheets of epithelial cells from the lamina propria.

Figure 65

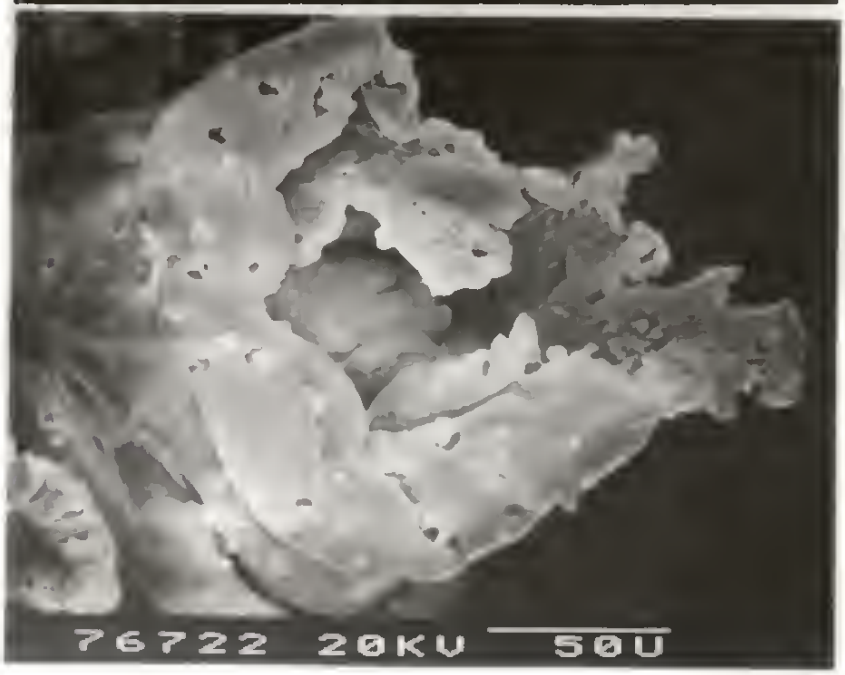


Figure 66

Fig 67. Jejunum of dog 11, collection method 4. Epithelial cells are noted piling up at the tip of the villus. Villus in the upper right corner is covered with mucous.

Fig 68. Duodenum of dog 12, collection method 2. Epithelial cells are separating from the lamina propria in large sheets.

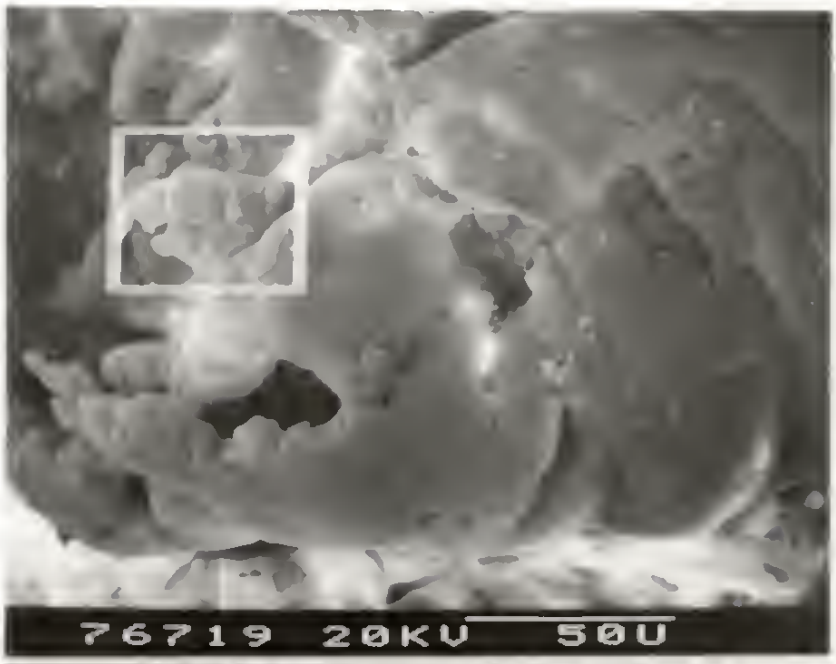
Figure 67

Figure 68

Fig 69. Duodenum of dog 12, collection method 3. Epithelium separating from the lamina propria.

Fig 70. Duodenum of dog 12, collection method 3. Higher magnification of Fig 69. Note microvilli present of the surface of the cells remain intact.

Figure 69



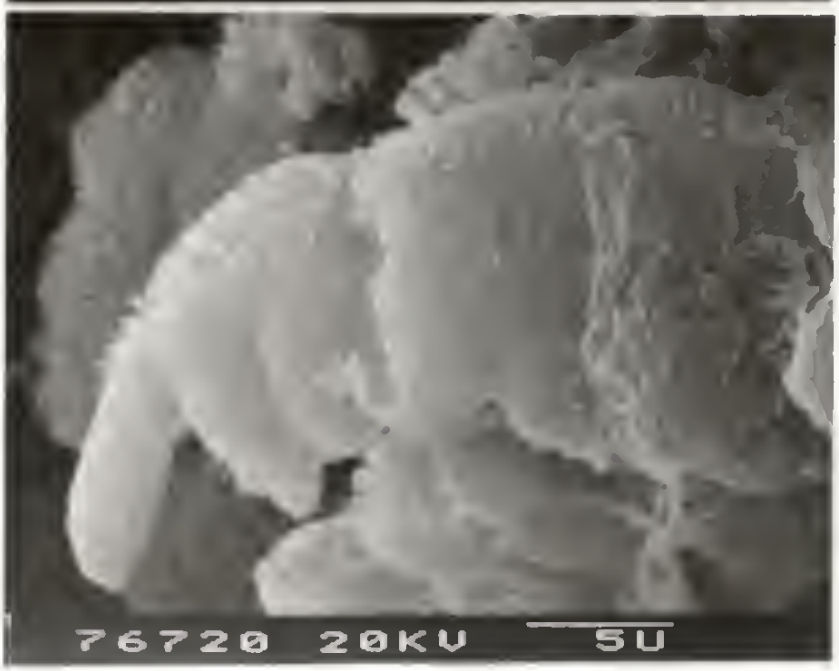


Figure 70

Fig 71. Jejunum of dog 11, collection method 1. Numerous separations of muscle bundles in the tunica muscularis. Extensive fracture artifacts are noted in the submucosa.

Fig 72. Duodenum of dog 12, collection method 4. Extensive separation artifcats are present in the submucosa where as none are noted in the muscularis. Mucous is present on the surface of the villi.

Figure 71

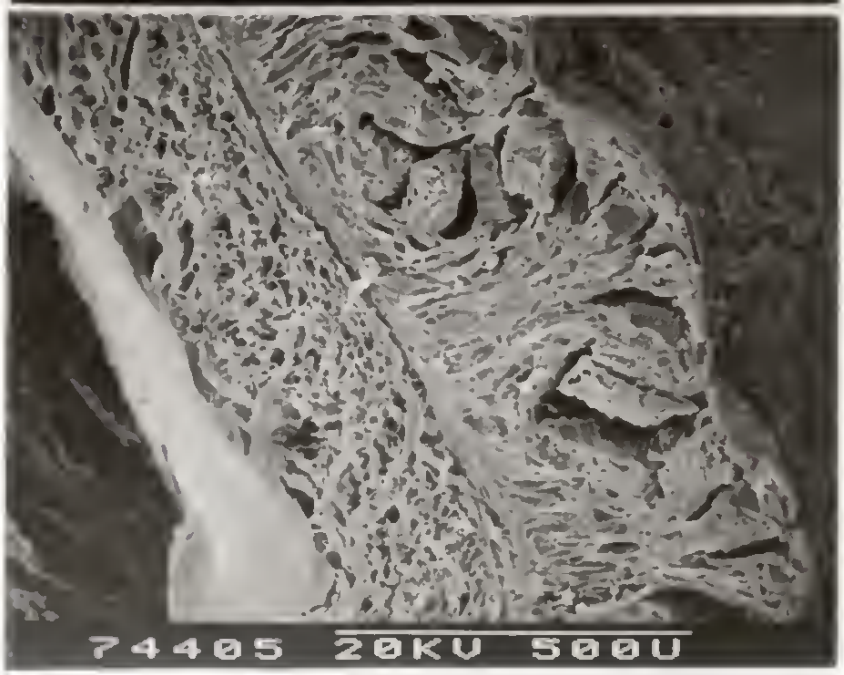


Figure 72

Fig 73. Duodenum of dog 11, collection method 5. Separation and loss of epithelium plus cellular individualization is noted. A prominate line is present at the site of separation between the epithelium and lamina propria.

Fig 74. Duodenum of dog 11, collection method 5. Higher magnification of Fig 73. Note the area where separation of the epithelium is occurring is characterized by large open spaces.

Figure 73

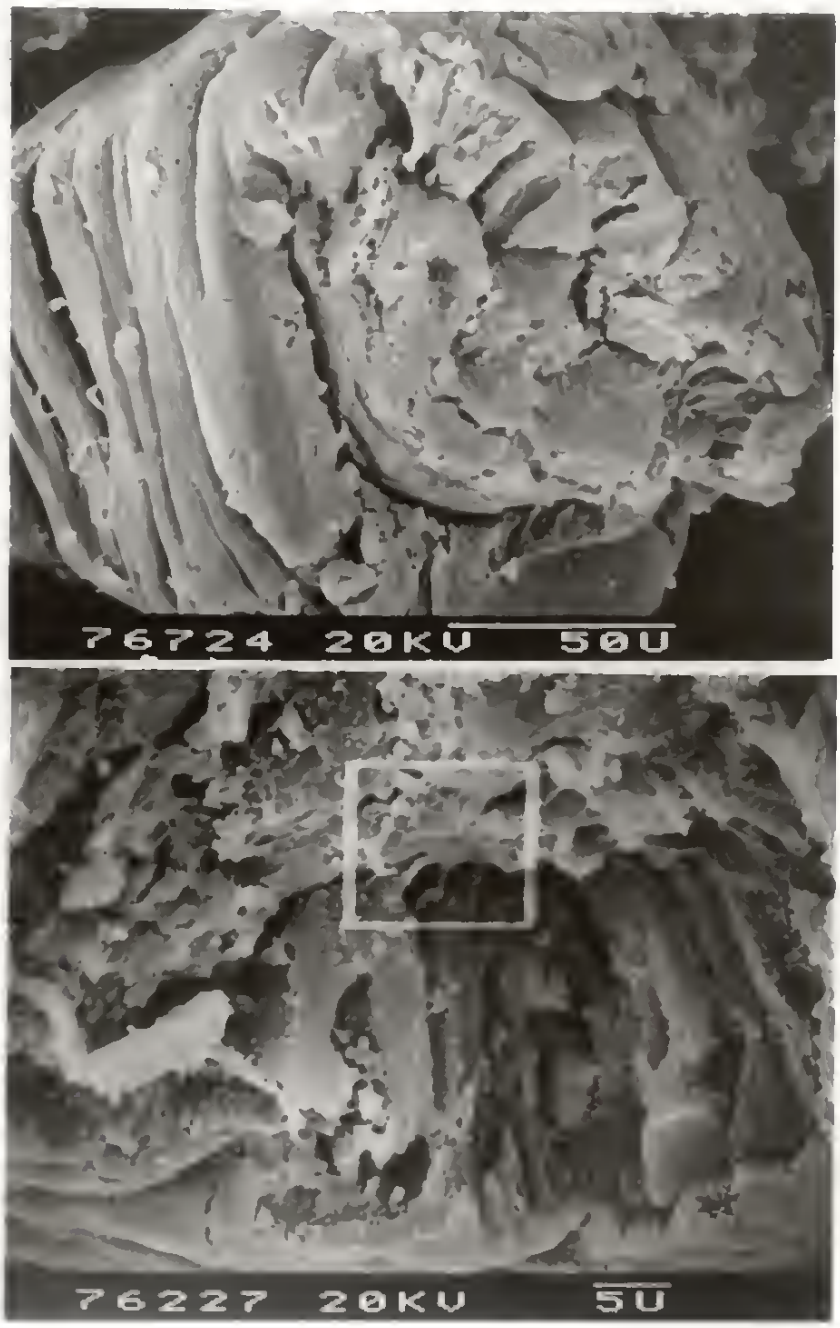
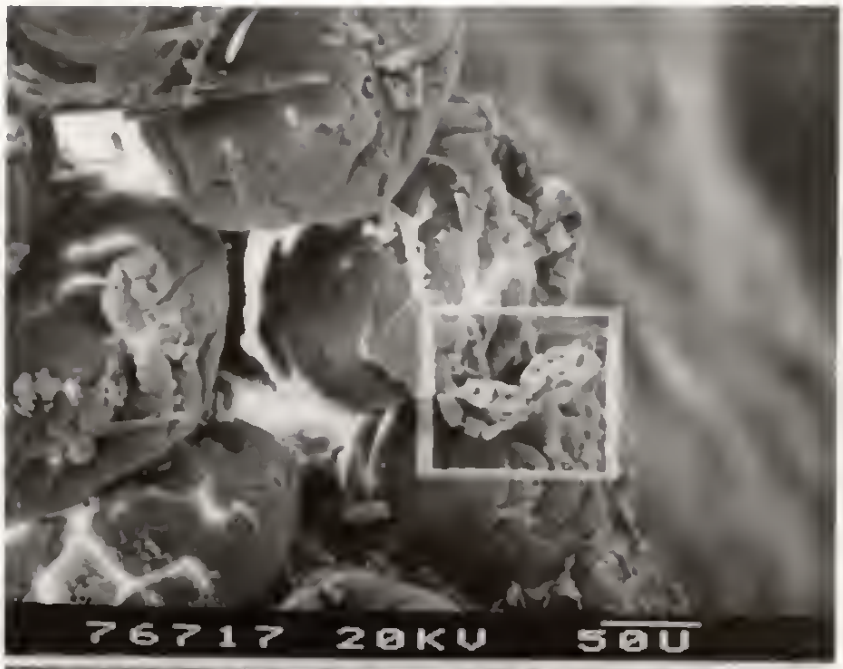


Figure 74

Fig 75. Duodenum of dog 12, collection method 2. Epithelial cells are being sloughed in large sheets.

Fig 76. Duodenum of dog 12, collection method 2. Higher magnification of Fig 75. Cellular individualization and separation can be seen within the sheet of sloughed cells.

Figure 75



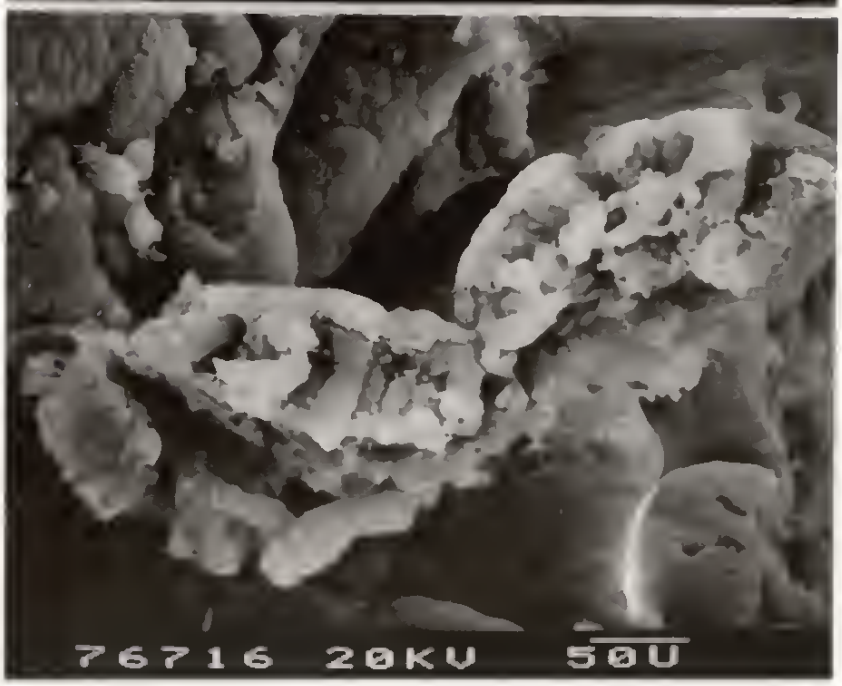


Figure 76

APPENDIX

Fig 1. Ligation of the esophagus just anterior to the stomach.

Fig 2. Transection of the esophagus anterior to the ligation.

Fig 3. Ligation of the distal colon.

Figure 1

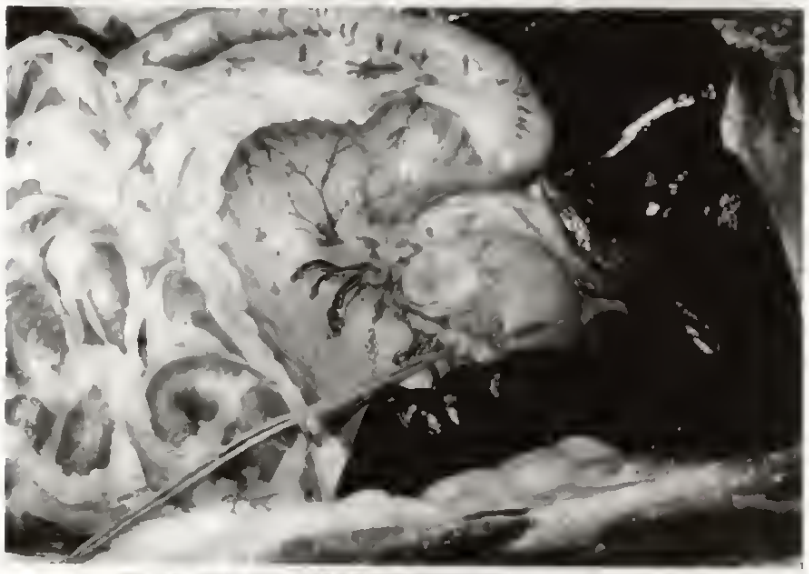


Figure 2

Figure 3

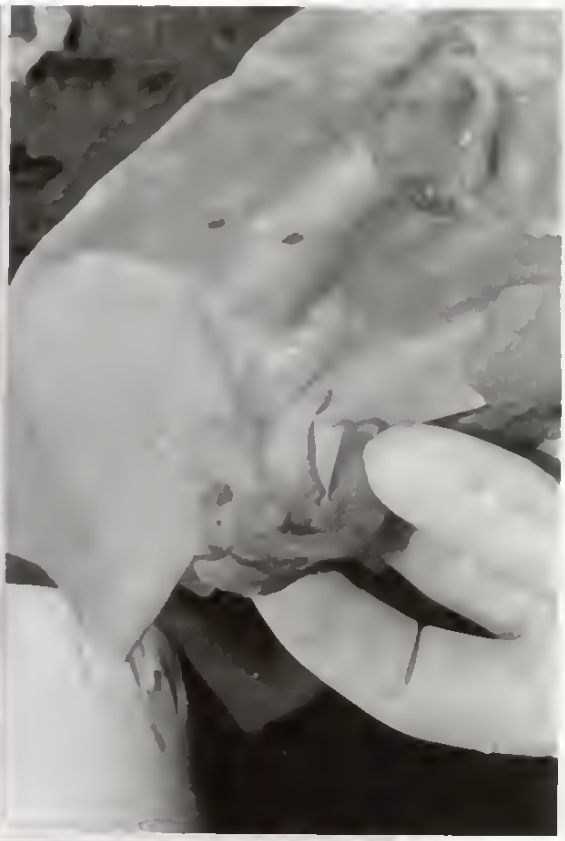
Fig 4. Fastening a tissue sample to a tongue depressor with staples, collection method 1.

Fig 5. Tissue adhering to a paper towel after fixation, collection method 3.

Fig 6. Injection of an intestinal segment with fixative after ligation at each end, collection method 2.

Figure 4

Figure 5



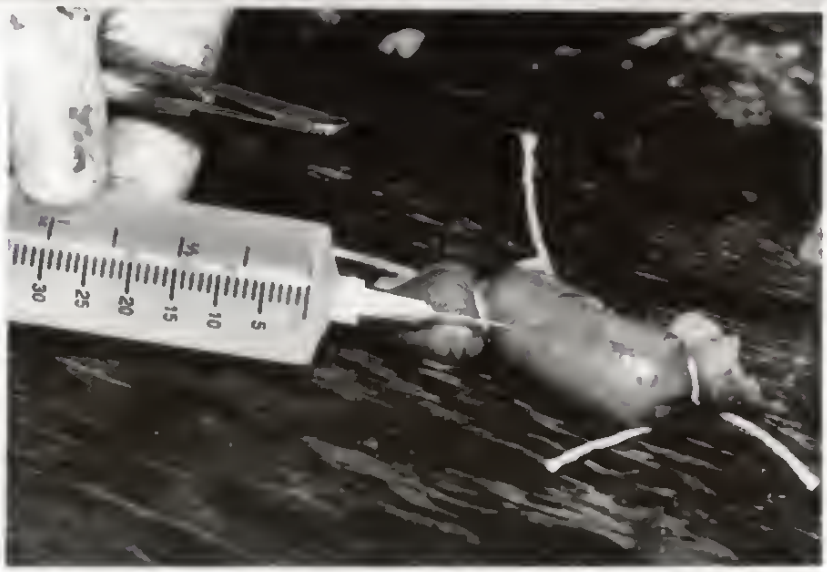


Figure 6

Fig 7. Trimming sections of the jejunum prior to histological processing, collection method 4.

Fig 8. Same as in Fig 7. Note loss of luminal dimension.

Fig 9. Section of colon with fecal material present in the lumen, collection method 4.

Figure 7

Figure 8

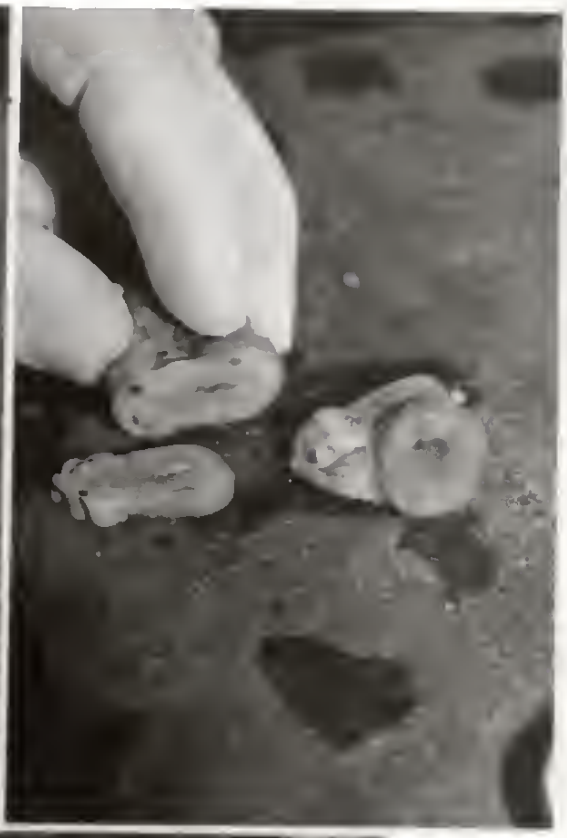


Figure 9

Fig 10. Trimming a stapled tissue sample, making a single longitudinal cut, collection method 1.

Fig 11. Transverse cuts are made in the tissue, collection method 1.

Fig 12. Removal of the tissue sample with forceps, collection method 1.

Figure 10

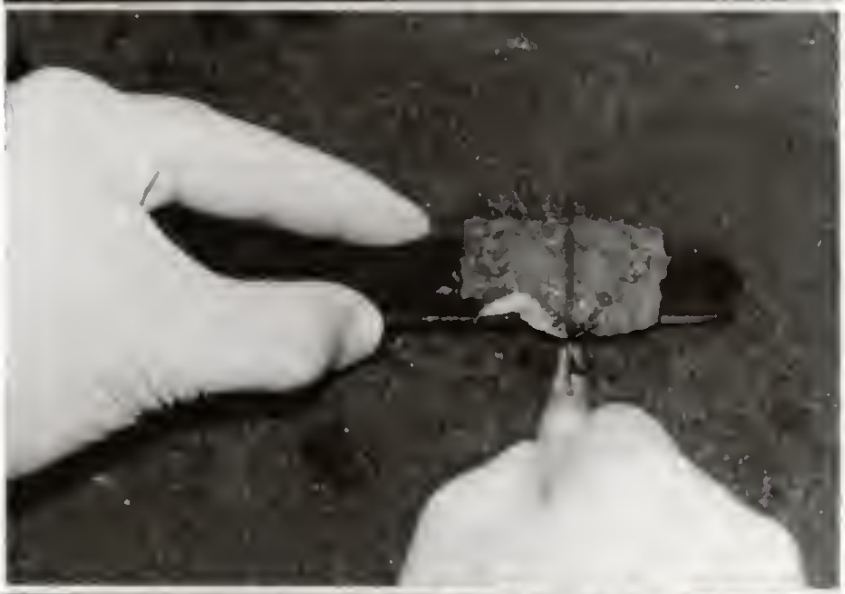


Figure 11

Figure 12

Fig 13. Trimming of tissue sample which were collected by method 2 A transverse cut is being made.

Fig 14. Same tissue as in Fig 13, note the open prominent lumin.

Fig 15. Tissue sample being removed with forceps, only the mesentery is handled.

Figure 13

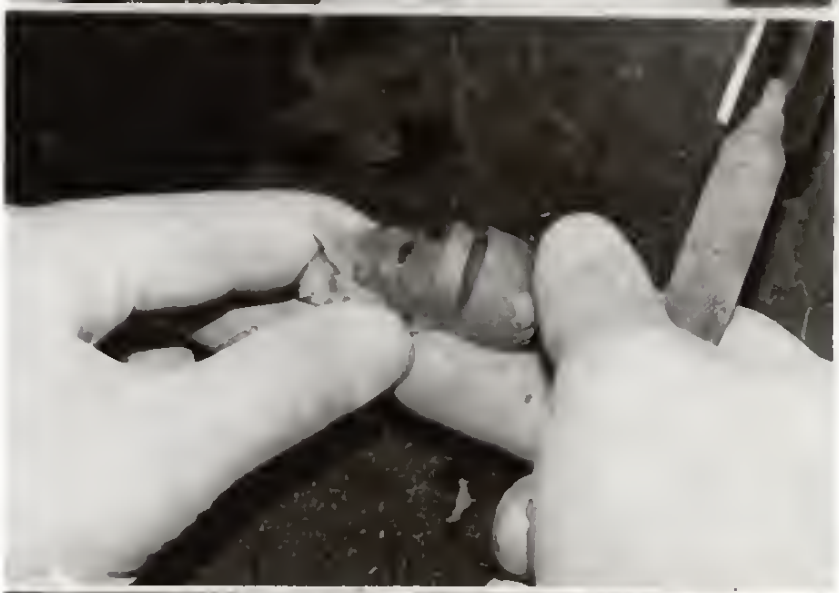


Figure 14

Figure 15

Fig 16. Trimming a tissue sample which was collected by method 3. Note how the tissue has become curled during fixation.

Fig 17. Tissue sample ready for histological processing, collection method 3.

Fig 18. Tissue sample collected by method 3. Curling of the tissue after fixation has cause the tissue to evert with the mucosal surface now being on the out side.

Figure 16

Figure 17

Figure 18

Fig 19. Tissue being placed directly in the fixative, collection method 4.

Fig 20. Tissue being placed in fixative after being cut longitudinally to expose the mucosal surface, collection method 5.

Fig 21. Tissues are ready to be histologically processed. Post fixative distortion of the tissue varies with the collection method. The first row is duodenum, second is jejunum and then ileum and colon respectfully. Beginning on the far left the columns are the collection method 3, 1, 2, 4 and 5 respectfully. Notice that the degree of tissue curling is dependent on the collection method.

Figure 19

Figure 20

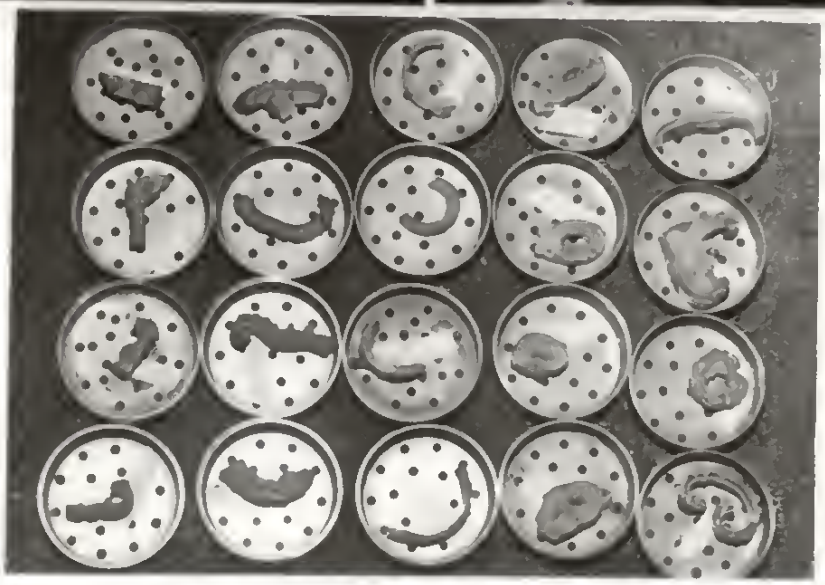


Figure 21

LIGHT AND SCANNING ELECTRON MICROSCOPIC EVALUATION OF COLLECTION METHODS USED IN THE PRESERVATION OF CANINE INTESTINE

by

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A.A. (Hutchinson Community Junior College) 1975
B.S. (Kansas State University) 1977
D.V.M. (Kansas State University) 1981

AN ABSTRACT OF A THESIS

submitted in partial fulfillment of the
requirements for the degree

MASTER OF SCIENCE

Department of Veterinary Pathology

KANSAS STATE UNIVERSITY

Manhattan, Kansas

1984

ABSTRACT

Twelve adult dogs weighing 19.5 to 25.0 Kg. were used in this study. The dogs were divided into two groups. Ten of the dogs were killed and intestinal tissue samples immediately collected. The remaining two dogs were anesthetized and perfused intravascularly with 10% buffered neutral formalin (10% BNF), and tissues collected immediately thereafter. The time between death and placement of all tissues in 10% BNF at room temperature was 15 to 22 minutes with a mean of 19 minutes.

Tissues were collected from the duodenum, jejunum, ileum and colon. Five different tissue samples were taken from each area. Each sample was randomly collected by one of the following methods:

Method 1

Longitudinal incision along the antimesenteric border and the ends of the intestine stapled to a wooden tongue depressor and placed in 10% BNF.

Method 2

Ends of the intestine ligated and the lumen injected with 10% BNF until slightly distended and place in 10% BNF.

Method 3

Longitudinal incision along the antimesenteric border and then placed on a dry paper towel with the serosal surface down, then place in 10% BNF.

Method 4

Intestine not longitudinally incised or ends ligated before being place in 10% BNF.

Method 5

Longitudinal incision along the antimesenteric border and then placed directly into 10% BNF.

All tissues were allowed to fix in 10% BNF for at least 10 days before routine histological processing and staining with hematoxylin and eosin. Five sections from each of the four regions of the intestine, one from each of the five collection methods, were examined. A total of 20 sections were examined from each dog.

Tissue sections were examined randomly without knowledge of location or collection method. Artifacts were scored as to severity using a system developed for this purpose. The parameters measured were: autolysis of the serosa, muscularis, submucosa and mucosa; folding of the serosa, outer muscularis, inner muscularis, submucosa, lyphoid nodules and mucosa; separations between the serosa and muscularis, outer and inner muscularis, inner muscularis and submucosa, and submucosa and mucosa; fractures in the serosa, outer muscularis, inner muscularis, submucosa, lymphoid nodules and mucosa; and miscellaneous artifacts including stain precipitate and variable tissue thickness. Surface changes were evaluated by scanning electron microscopy and compared with the light microscopy findings.

Comparisons were made between the various collection methods and regions of the intestine by evlauating trhe means of the total artifact scores using the Duncan multiple range analysis. Significantly more artifacts of greater severity occurred in the duodenum and jejunum than occurred in the ileum and colon irrespective of the collection method. Collection method 2 was shown to result in signficantly fewer artifacts than any of the other collection methods. Methods 1, 3, and 5 were not significantly different and collection method 4 caused significantly more artifacts. The artifact scores of the collection methods were not significantly different between regions of the intestine.

From these results it was concluded that canine intestine can best be preserved and collection artifacts avoided by utilizing method 2. It was also concluded that a significant inherent difference in total artifact frequency and severity exists between the duodenum and jejunum, and the ileum and colon, with the anterior regions of the intestine being more difficult to preserve artifact-free than the posterior region irrespective of the collection method used.