EVALUATION OF THE MICROCIRCULATION OF THE EQUINE SMALL INTESTINE FOLLOWING INTRAMURAL DISTENTION AND REPERFUSION

by

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(ABSTRACT)

The effects of intraluminal distention (25 cm H_2O, 120 minutes) and subsequent decompression (60 minutes) on the intramural vascular patterns of the equine small intestine was evaluated in 7 anesthetized horses. The vascular system of experimental and control segments were injected with a blue-colored radiopaque medium for microangiography and histology or a diluted methyl methacrylate (MERCOX CL-2B) for scanning electron microscopy.

The distended segments had shortened villi that were separated by expanded crypts and mesothelial cell loss, neutrophil infiltration and edema in the seromuscular layer. The number of filled vessels was decreased in the seromuscular layer and to a lesser extent in the mucosal layer in the distended segments compared to controls. Following reperfusion, the morphologic lesions progressed and the number of observed vessels increased in all layers; however the vascular density did not return to the pre-distention state.

This study identifies altered intramural vascular patterns in the equine jejunum during luminal distention and reperfusion.

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INTRODUCTION

Simple or strangulating obstructions of the small intestine are common in horses and often require surgical correction.[1] Despite improvements in surgical technique and post-operative care, horses continue to have numerous complications and poor survival rates following the relief of small intestinal obstructions.[2] Commonly encountered complications include continued tissue necrosis, ileus and adhesion formation.[2]

During exploratory abdominal surgery, luminal distention is frequently discovered proximal to the obstructive lesion and results from the accumulation of ingesta, intestinal secretions and gas from bacterial fermentation. The magnitude of the intraluminal pressure may affect horses' survival. Horses surviving naturally occurring small intestinal obstruction had a mean intraluminal pressure of 6.3 cm H_2O; whereas those that died had a mean of 15 cm H_2O.[3]

In clinical cases, distention was associated with mucosal lesions, however, experimental intestinal studies in the horse show that luminal distention induces minimal mucosal lesions.[4] In foals, the serosal damage was severe enough to stimulate post operative adhesion formation.[5]

Intestinal damage is often dependent on the amount of

vascular compromise to the involved bowel segment. A complete understanding of the intestinal microvasculature during bowel obstruction is essential to define the pathophysiology of these conditions. Numerous studies[6] have documented a reduction in blood flow during increased intraluminal pressure, but the effects of distention on the intramural vascular architecture has not been studied. The purpose of this study was to identify the altered perfusion patterns during intraluminal distention and subsequent decompression of the equine small intestine using microangiography and light microscopy of injected specimens and scanning electron microscopy of vascular corrosion casts.

LITERATURE REVIEW

Gross Anatomy of the Equine Small Intestine

The small intestine extends from the pylorus to the ileocecal valve and is divided into the duodenum, jejunum, and ileum. Its average length is 22 meters and is distinguished from the rest of the gastrointestinal tract by its suspension from a sheet of mesentery suspended from the dorsal midline of the abdomen and the presence of villi on its mucosal surface.[7] The majority of the small intestine is jejunum which lies in numerous coils, with the small colon, in the left caudal and ventral regions of the abdomen. The jejunum is suspended by mesentery which increases in length distally and allows the distal jejunum and ileum a large range of movement increasing susceptibility to volvulus and incarcerations.[8] The mesentery is a wide, fan-shaped fold, consisting of two layers of peritoneum between which the blood vessels and nerves pass to reach the bowel. The root of the mesentery is attached to a small area around the cranial mesenteric artery under the first and second lumbar vertebrae.[7] The ileum has a thicker muscular wall than the duodenum or

jejunum and terminates at the ileocecal valve. The ileum is fixed by its attachment to the cecum and has been suspected as being a pivot for the development of volvulus of the jejunum.[8]

Small Intestinal Histology

The wall of the small intestine has four distinct layers including the tunica mucosa, tela submucosa, tunica muscularis and tunica serosa.[8,9] Absorptive function is enhanced by a large mucosal surface area.[8,9] Unique features which enhance surface area include mucosal-submucosal folds (plica circularis or Kerckring's plicae) located half way around the circumference of the proximal small intestine, finger-shaped villi that cover the mucosal surface, and the numerous microvilli on each mucosal epithelial cell.

The tunica mucosa is the luminal surface layer of the small intestine composed of an epithelium (lamina epithelialis) supported by a loose bed of connective tissue (lamina propria mucosa) and one or two layers of smooth muscle fibers (lamina muscularis mucosa). The mucosal epithelium is composed of simple columnar absorptive cells

with interspersed mucus secreting goblet cells. Occasional endocrine cells are also scattered among the absorptive cells. Tight junctional complexes prevent intestinal contents from diffusing between the cells and are important in electrolyte transport function.[8] At the base of the intestinal villi are simple tubular intestinal glands (crypts of Lieberkuhn) which penetrate as far as the muscularis mucosa. Undifferentiated cuboidal cells in the crypts multiply, differentiate and are pushed to the villus tip by succeeding cells while the oldest cells are sloughed into the intestinal lumen.[9] The cell replacement creates a new epithelium every two to four days with crypt cells differentiating into absorptive or goblet cells.[8-10] The enterochromaffin cells are located in the crypts and secrete endocrine hormones such as serotonin, motilin and substance P. The paneth cells produce enzymes in the crypts which may aid in protein digestion.[11]

Each villus consists of a core of loose connective tissue (lamina propria) with a single blind-ended lacteal located in its center which drains into a lymphatic plexus at the villous base. Smooth muscle fibers originating in the muscularis mucosa ascend the villus and are responsible for villus motility which aids in emptying the lacteal of chyme[9].

The tela submucosa contains areolar connective tissue composed of collagen and elastin which support the major intramural blood vessels, lymphatics and nerves (Meissner's plexus). Tubuloalveolar submucosal glands (Brunner glands) are located in the proximal third of the equine small intestine and produce both mucous and serous secretions. Dense aggregates of lymphatic tissue are present in the submucosa (Peyer's patches) and are more numerous in the horse compared to other species.[9]

The tunica muscularis consists of an inner circular and outer longitudinal smooth muscle layer and is thickest in the horse with a total thickness of 1,150 um.[8,9] The muscle fibers in the inner layer are oriented circularly, whereas the outer layer fibers run parallel to the long axis of the intestine.[8] Numerous parasympathetic ganglionic cells and fibers are located between these muscle layers within the myenteric nerve plexus (Auerbach's plexus).[9] The myenteric plexus is a network of neurons and nerve fibers which relay and modify signals from the central nervous system and influence smooth muscle activity via local reflexes. The outermost layer of the intestine is the tunica serosa which is thin connective tissue covered by squamous mesothelium.

Gross Vascular Anatomy of the Equine Small Intestine

The visceral branches of the abdominal aorta are the celiac, cranial mesenteric, renal, caudal mesenteric, and testicular or ovarian arteries. The celiac artery is an unpaired vessel arising from the aorta near the aortic hiatus and branches into the left gastric, hepatic and splenic arteries. The cranial mesenteric artery arises from the ventral aspect of the aorta at the level of the first lumbar vertebrae. It is a large unpaired vessel which passes ventrally between the caudal vena cava and the left adrenal gland into the root of the mesentery. The first branch of the cranial mesenteric is the caudal pancreaticoduodenal artery which anastomoses with the cranial pancreaticoduodenal branch of the hepatic artery.[7] Fifteen to twenty jejunal arteries branch from the cranial mesenteric artery. Each jejunal artery divides into two branches which anastomose with adjacent jejunal arteries in the mesentery to form a series of vascular arches which are accompanied by corresponding veins, nerves and lymph vessels. The ileocecocolic artery is a continuation of the cranial mesenteric artery and becomes the ileal, two cecal and colic arteries. The ileal arteries parallel the terminal ileum in an oral direction and form a continuous

arch with the last jejunal artery.

All venous drainage from the small intestine enters the portal vein. The lymphatic drainage from the duodenum is to the hepatic, pancreaticoduodenal, cranial mesenteric, and cecal lymph nodes. The jejunal lymph drains into the jejunal lymph nodes, and ileal lymph drains into the jejunal, ileal, cecal, and colic lymph nodes.

Microcirculation of the Small Intestine

The microcirculation of the small intestine has been studied in many species,[12-15] and that of the equine small intestine has recently been described.[16] Small arteries branch from the jejunal vascular arcade close to the bowel wall and penetrate the serosa either directly at the mesenteric attachment or after traveling in both directions around the outer circumference of the intestine toward the antimesenteric border. The artery and corresponding vein then penetrate the muscularis externa forming the submucosal vascular plexus which anastomoses with vessels around the circumference of the intestine. The directly penetrating vessels from the marginal vascular arcade supply the

submucosal plexus at the mesenteric angle. The vessels to the mucosa and seromuscular layers originate from the submucosal plexus.

The arterial supply to the muscularis and the serosa branches at right angles from the submucosal artery and forms a smaller parallel vascular plexus traveling between the two muscle planes. Numerous branches supply the circular and longitudinal muscles and the serosa. These vessels run parallel to direction of the muscle fibers similar to reports in other species.[17,18] The submucosal plexus supplies the mucosa via one of two capillary networks. The glands in the intestinal crypts are supplied by arterioles branching from the arterial plexus as it ascends towards the mucosa giving rise to a polygonal "basket" of pericryptal capillaries. A second arteriole originating from the submucosal plexus ascends the villus eccentrically and arborizes at the villous tip into a network of capillaries which descend to the villus base and drain through 1-3 peripheral venules. The crypt capillary network joins the villus capillary network at the base of the villus to drain through these venules. Numerous venules from the villi unite to form a larger venule which empties into the submucosal vein (Figure A).

The largest species difference in intestinal

microvascular anatomy is the architecture in the villus.[18,19] Spanner classified the villous vascular structure according to the architecture of arterial breakup and described a "tuft", "stepladder" and "fountain" pattern.[20] The tuft pattern consists of arterial division into capillaries at the villous base with drainage from a venule originating at the villus tip. The stepladder pattern contains an arteriole and venule ascending to the villus tip with multiple capillaries joining between the two vessels. The fountain pattern (Figure B) has a single unbranched arteriole ascend the villus and arborize into a network of subepithelial capillaries that drain into 1-3 venules at the base of the villus. This traditional classification of the villus architecture appears inadequate since most of the species studied do not exhibit a single specific villus vascular pattern.[18] The rat, rabbit, and human have a capillary network in a fountain pattern at the villus tip and a tuft pattern at the base.[12,14,15] The dog demonstrates a complex capillary pattern with a fountain network at the very tip, a stepladder pattern in the proximal villus and a tuft pattern for the distal half of the villus.[18] Both the cat and horse villous capillaries have structures which fit the classic description of the "fountain" pattern.[18,20]

A unique feature of the villus vascular architecture is

the close approximation of the central arteriole to the peripherally located capillaries in which the blood flows in an opposite direction.[21,22] This arrangement is similar to the countercurrent multiplier in the renal medulla. Results of experiments in the dog,[48] man[49] and cat[50] suggest that a gradient of osmolality exists between the villus tip and base, which facilitates absorption and fluid uptake.[48] It has been suggested that the countercurrent exchange (CCE) mechanism plays a role in mucosal necrosis following intestinal ischemia. Normally dissolved arterial oxygen is diffused to the relatively oxygen deficient venule at the base of the villus, changing the oxygen gradient from villus base to tip. This gradient may be a physiologic factor in the normal death and high turnover rate of the villus tip epithelial cells.[22] During ischemia or low blood flow states, arterial blood transit time is increased allowing more time for oxygen diffusion, thereby exacerbating the relative hypoxia of the villus tip.[22,50] Evidence for a functional CCE has been demonstrated in the cat using microelectrodes to measure an increasing oxygen gradient from villus base to villus tip.[47] Although the existence of a functional CCE has not been studied in the horse; the similarity between the equine and feline villous vascular structure suggests that it could exist.

The Microcirculation and Transcapillary Fluid Exchange

The microcirculation of the intestine is composed of an anatomically and functionally defined series of parallel-coupled vascular circuits each supplying a different tissue layer of the intestinal wall.[21] Each vascular bed is composed of several series-coupled (consecutive) vascular sections. The precapillary resistance vessels (arterioles and metarterioles) have a smooth muscle wall which is highly responsive to local and remote stimulation. These vessels regulate the rate of blood flow from moment to moment by changes in the vascular smooth muscle tone. The arterioles to the villi have smooth muscle in the segment in the crypt area, but lack smooth muscle as they ascend the villus.[19] At the junction of the capillary and arteriole there is a smooth muscle structure called the "precapillary sphincter" which can open or close the entry to the capillary. These sphincters contribute very little to the total vascular resistance, but are major determinants in controlling the number of capillaries perfused and therefore are important in determining the oxygen diffusion distance.[23]

The endothelial cells lining the intestinal capillaries have characteristics that allow fluid and solute transport of which the fenestrae or circular openings (2-3

um radius) appear most important.[23] The fenestrae face the enterocytes and increase in frequency from arterial to venous ends of the capillary.[24] Distal to the capillaries are the postcapillary resistance vessels (veins and venules). Contraction of the smooth muscle of these vessels results in an increased resistance to the flow of blood creating a back-pressure on the capillary bed. This back-pressure increases the mean hydrostatic capillary pressure and rate of fluid movement across the capillary wall between vascular and extravascular compartments.[22] The veins and venules also act as "capacitance vessels" or blood reservoirs for the rest of the body. The presence of arteriovenous shunts in the gastrointestinal tract is controversial and their functional significance seems to be small.[21,22]

The magnitude and direction of the movement of water and solutes between the capillaries and interstitium are described by Starling's hypothesis[25]:

$$Jvc = Kfc\ [(Pc - Pi) - od\ (Oc - Oi)]$$

where,

Jvc = net rate of capillary filtration (or absorption)

Kfc = capillary filtration coefficient

Pc = capillary hydrostatic pressure

Pi = interstitial hydrostatic pressure

od = osmotic reflection coefficient

Oc = capillary oncotic pressure

Oi = interstitial oncotic pressure

The capillary filtration coefficient measures the hydraulic conductance of a capillary bed and is influenced by both the number of perfused capillaries (determined by the precapillary sphincter) and the capillary pore size.[24] Many humoral and pharmacologic agents increase the capillary filtration coefficient by increasing capillary pore size (glucose absorption, bradykinin and histamine) or increasing the number of filtering capillaries (prostaglandins and endotoxin).[23] In general, conditions or agents that produce vasodilation increase Kfc, while vasoconstriction i.e. hypothermia, sympathetic stimulation and angiotensin II reduces it.[23] A change in Kfc reflects an alteration in either the number of perfused capillaries or microvessel permeability. The alterations in Kfc produced by capillary pressure changes are generally assumed to represent capillary recruitment or closure.[19]

Capillary hydrostatic pressure pushes fluid out of the capillaries into the interstitium (Pc) where as, interstitial hydrostatic pressure pushes fluid in the

reverse direction (Pi). In the small intestine, the capillary hydrostatic pressure is 16 mmHg and the interstitial fluid pressure is from -3 to 0 mmHg [26]. Changes in both arterial and venous pressures will influence the capillary hydrostatic pressure; however venous pressure has a much greater effect than arterial pressure. Only 5-10% of the arterial pressure increase is transmitted to the capillaries; where as 60-70% of the venous pressure increase affects the capillary bed.[23]

The osmotic reflection coefficient measures the osmotic pressure exerted across the capillary membrane and is determined by each compartment's protein concentration. The osmotic coefficient of the capillaries in the feline small intestine is .92 which means that a macromolecule striking a capillary pore has an 8% chance of passing through.[27] A capillary that is totally impermeable to proteins would have a od = 1.0 and a freely permeable capillary would have an od = 0. The osmotic coefficient decreases (capillary is more permeable) when a capillary is subjected to systemic endotoxin, histamine, prostaglandins, oxygen radicals and ischemia.[28-30] Ischemia for 1 hour decreased the osmotic coefficient from .92 to .59 in the feline small intestine.[23] The osmotic coefficient has not been determined for the equine small intestine.

The oncotic and hydrostatic pressures of the capillaries and interstitium normally are balanced to prevent overhydration of the interstitium referred as the "edema safety factor".[31] Adjustments in these forces allows the microcirculation to provide fluid for the epithelial transport during secretion and to remove fluid from the interstitial tissue during absorption. Unbalanced hydrostatic and oncotic pressures, alterations in vascular permeability, and lymphatic obstruction can lead to overexpansion of interstitial spaces with capillary filtrate.[23,32] This edema is a common feature of many gastrointestinal diseases such as enteritis, simple and strangulating obstructions.[5,25,31]

Intestinal Blood Flow

Although the "resting" values of splanchnic blood flow vary considerably within a species and between species, some generalizations can be made. Blood flow to the small intestine is greatest compared to the stomach or colon, and the proximal jejunum receives a greater share of blood flow than the ileum.[33]

The degree of vascularity differs in the various intramural segments depending on the metabolic requirements

of each layer. In dogs, 75% of the total resting intestinal blood flow is distributed to the mucosa and submucosa, presumably reflecting the high metabolic demands of the epithelial transport processes involved in absorption.[22] The resting blood flow distribution to the mucosa-submucosa is 75% in humans and 80% in cats with the remainder going to the seromuscular layer.[34] Physiologic and pharmacologic interventions which alter total intestinal blood flow may affect the intramural circulation in the same or varying degrees; however only a small fraction of the published literature dealing with intestinal hemodynamics includes estimates of intramural blood flow distribution.[35] Intraluminal glucose, digested food and cholera toxin increased the fractional mucosal blood flow and subsequently decreased blood flow to the muscularis.[36] Adenosine, contractile motility and surgical manipulation increased seromuscular blood flow but did not alter the submucosa-mucosa flow in the canine small intestine.[37]

Intestinal motility can influence mesenteric blood flow by altering intramural blood distribution and oxygen consumption.[35] Mild rhythmic contractions increase total mesenteric blood flow causing an increase in fractional flow to the muscularis (exercise hyperemia of the contracting muscles); whereas flow to the mucosa is either reduced by

mechanical compression or not altered.[37] Tonic contractions decrease total intestinal blood flow, presumably because of substantial increases in extravascular compression.[37]

Intestinal blood flow is regulated by intrinsic, extrinsic and circulating humoral factors. The intrinsic ability of the intestine to control perfusion and oxygenation during changing tissue demands is attributed to metabolic and myogenic influences on the local resistance and exchange vessels.[33] The metabolic theory of autoregulation is based on the release of metabolites during tissue hypoxia (low blood flow) which enhance blood flow by vasodilation.[42] The myogenic theory is based on the assumption that a passive increase in vascular wall tension will elicit an increased arteriole resistance; thereby decreasing capillary hydrostatic pressure and reversing abnormal transcapillary fluid exchange.[95] Evidence for the existence of local vascular control mechanisms include pressure-flow autoregulation, reactive hyperemia, hypoxic vasodilation, response to acute venous pressure elevation and functional hyperemia.[33,35]

Autoregulation describes the intrinsic ability of an organ to maintain a constant blood flow despite a fluctuating arterial pressure and the site is localized to the precapillary resistance vessel (arteriole).[38] Relaxation

of the precapillary sphincter results in capillary recruitment facilitating the extraction of additional oxygen during low flow states by decreasing the vessel to cell diffusion distance.[33,35] Autoregulation is unaffected by denervation or sympatholytic agents, but is reduced or abolished with increased intraluminal pressure.[35,39]

The small intestine exhibits a characteristic hyperemia after brief periods of arterial occlusion in which the magnitude and duration of the response is dependent on the duration of occlusion.[33,35,40] Arterial hypoxia and hypercapnia in the small intestine elicit vasodilation and capillary recruitment.[41] The functional hyperemia associated with food digestion has also been well documented.[35] Blood flow increases by 30 to 130% after a meal depending on the type of food ingested. The metabolic theory of intrinsic blood flow regulation has been proposed to explain the above mechanisms.[33,35,42] According to the metabolic theory, any condition which reduces O_2 delivery or increases O_2 demand will cause a reduction in tissue PO2 and increase vasodilator metabolites, i.e. CO_2, H+, adenosine, in the interstitial tissue. These metabolites, (adenosine seems most important) cause relaxation of the precapillary sphincter resulting in increases in blood flow and capillary recruitment.[35,42] This increases the surface area for O_2

exchange and decreases the blood-to-cell diffusion distance. The ultimate effect is to maintain cell PO_2 above the critical level where O_2 availability limits energy metabolism.[42] The metabolic mechanism is important in maintaining adequate blood flow and oxygen delivery to the tissues.

The small intestine has numerous capillary networks characterized by high capillary hydraulic conductance, where a sudden increase in capillary hydraulic pressure (from venous hypertension) will greatly increase capillary filtration leading to interstitial edema.[33,35,43] Venous hypertension causes an increased vascular resistance which is explained by the myogenic theory of intrinsic regulation.[35,43] The myogenic-induced response is to increase precapillary resistance (arteriole); thereby decreasing capillary hydrostatic pressure and protecting the tissue from edema. According to this theory, arterial smooth muscle tension receptors modulate vascular tone in response to capillary transmural pressure.[35] An increase in venous pressure will cause "back pressure" [23] through the capillary bed to the arteriole which increases vascular transmural pressure resulting in an increased arteriolar smooth muscle tone. This increase in arteriolar resistance causes vasoconstriction and decreased capillary filtration due to

capillary closing.[95] This myogenic mechanism functions to maintain a constant capillary pressure and transcapillary fluid exchange protecting the intestinal tissue from edema. A recent study showed that the myogenic response to venous pressure elevation was diminished during arterial hypoxia suggesting that the metabolic mechanism has a dominant role in the autoregulation of capillary filtration in the small intestine.[44]

Extrinsic nervous control of the intestinal vasculature is primarily sympathetic. Stimulation of the regional sympathetic nerves reduces intestinal blood flow via stimulation of adrenergic receptors following catecholamine release which constrict the vascular smooth muscle.[45] The intense vasoconstriction elicited by sympathetic stimulation is rapidly diminished (even with continued catecholamine release) to a moderate vascular resistance. The physiologic basis of this phenomenon termed "autoregulatory escape" has not been clearly defined.[21,33,45] Although the small intestine is innervated by parasympathetic fibers originating from the vagus, no direct innervation of the mesenteric vasculature has been identified.[33] Stimulation of the vagus nerve will increase jejunal motility, but has little effect on intestinal blood flow.[46]

Several substances also effect intestinal blood flow.

These include intestinal vasodilators gastrin, secretin, cholecystokinin, glucagon, vasoactive polypeptide and histamine and vasoconstrictors catecholamines, angiotensin II and prostaglandins of the F and D series.[21,35]

Measurement of Intestinal Blood Flow

Many methods of measuring intestinal blood flow exist such as direct microscopic observation, flowmetry, capillary fluid exchange methods, microsphere distribution, venous outflow methods, indicator dilution techniques, inert gas washout and laser Doppler velocimetry.[21,51-55] These methods have been reviewed extensively,[51] however this discussion will be limited to the techniques used by previous investigators to the study the effects of intraluminal distention on blood flow. Although blood flow was not measured in this study, reference is made to the previous distention studies and therefore the measurement techniques are discussed including the advantages and limitations of the venous outflow, electromagnetic flowmeter and microsphere injection techniques.

A direct method of measuring mesenteric circulation is the venous outflow technique which is performed assuming that venous drainage reflects arterial flow.[52] With this

method, intestinal blood flow and oxygen consumption can readily be determined and its advantages include accuracy, directness and simplicity. A disadvantage is that the invasive surgical preparation can itself reduce blood flow. Additionally, no information is provided on the intramural distribution of blood flow.[51]

Electromagnetic flowmeters are based on the principle that, when blood moves through a magnetic field, an electromagnetic force proportional to the flow rate is created by the moving ions.[51] Cuff transducers are placed on any vessel of greater than 0.5 mm diameter and the electric signal is recorded and calibrated. This method is highly accurate for determining blood flow in large vessels and allows instantaneous and continuous measurements of blood flow; however, measurements can be altered if blood pH, hematocrit or ion concentrations are changed between measurements.[52] Like the venous outflow method, no information on the intramural distribution of blood is provided.

Radioactive microspheres injected into the central arterial system are presumed to distribute according to the precapillary distribution of blood flow where they lodge in the capillary beds and are not recoverable in the venous effluent.[51,52] The radioactivity of the sample is measured and reflects the blood flow distribution to each intestinal

layer. Another advantage of the microsphere method is that it can quantify the mucosa-submucosa or seromuscular layer blood flow; however, it cannot distinguish submucosa or serosa blood flow alone.[55] Additional limitations include the migration of previously lodged microspheres from the submucosa to mucosa or from the tissue to venous blood in response to changing arterial blood pressure.[21]

Ischemia and Reperfusion Injury

Intestinal ischemia is produced by strangulation obstructions (volvulus or incarcerations), simple obstructions, nonstrangulating infarctions or low blood flow states (shock) and is common in both man[56] and horses.[1,2,59,60] Common experimental strangulation obstructions include total vascular occlusion (Ischemic Strangulation Obstruction, ISO) or venous obstruction (Hemorrhagic Strangulation Obstruction, HSO).[60] Most clinical cases of strangulation obstruction in the horse, resemble initial venous occlusion similar to HSO.[60]

Ischemia induces characteristic mucosal lesions within a short period of time. Morphologic mucosal alterations were observed by light microscopy after 30 minutes of small

intestinal ischemia in the dog,[58] rat[63] and pony.[59,61] Using electron microscopy, mucosal cellular damage was observed following 5 to 10 minutes of intestinal arteriovenous occlusion.[64] The severity of the tissue damage is dependent upon the degree and duration of the ischemia. The progressive development of the mucosal lesions during experimentally induced strangulation obstructions has been described and graded by Chiu and coworkers:[58]

Grade 0: Normal

Grade I: Development of a subepithelial space (Gruhagen's) at the very tip of the villus

Grade II: Loss of epithelial cells from the villus tip and minimal hemorrhage into the lamina propria

Grade III: Continued lifting of the surface epithelial cells down the sides of the villus and hemorrhage in the lamina propria and submucosa

Grade IV: Complete separation of the villus epithelium with marked lamina propria and submucosal hemorrhage and edema

Grade V: Loss of villus architecture and early necrosis of crypt cells

Reports on both experimentally induced[59,60,61] and naturally

occurring[62] intestinal strangulations in the horse describe similar lesions. The pathogenesis of the mechanical epithelial separation is thought to be a ischemia-induced de-energized sodium-potassium pump which allows cellular influxes of sodium and subsequently water.[63] Other mechanisms contributing to the mucosal lesion are controversial, but three major factors have been implicated: pancreatic proteases, tissue hypoxia and reactive oxygen metabolites.[56] Bounous and coworkers proposed that the ischemic, and therefore energy deficient, jejunum ceases to produce mucus making the mucosal epithelium vulnerable to intraluminal enzymes such as trypsin and chymotrypsin.[65] Protease inhibitors and pancreatic duct ligation minimized the mucosal lesions supporting this theory; however, pancreatectomy had no effect on the lesions suggesting the enzymes probably play a secondary role.[66]

Intraluminal oxygen minimized mucosal damage in the pony [67] and cat and therefore supports the importance of hypoxia in intestinal ischemic injury. Although the countercurrent exchange mechanism has not been studied in the horse, extravascular diffusion of oxygen through the villus causing hypoxia at the tip could contribute to the pattern of progressive mucosal damage.

Numerous studies have shown an increase in intestinal

morphologic damage following the return of blood flow; this progression of the mucosal lesion has been termed reperfusion injury.[57,69,70,71] Parks and Granger demonstrated that the mucosal injury produced by 2 hours of ischemia and 1 hour of reperfusion was significantly greater than that produced by 4 hours of ischemia alone.[70] Tissue damage is attributed to the release of oxygen metabolites (oxygen radicals) which cause lipid peroxidation and protein denaturation resulting in increased membrane permeability and enzyme activation.[57] Neutrophil infiltration stimulated by tissue damage substantially increases during reperfusion.[71] Activated neutrophils have been reported to cause capillary plugging and extensive damage during myocardial reperfusion and may play a role in intestinal ischemic injury.[72]

Previous small intestine ischemia/reperfusion studies in the horse demonstrated continued mucosal necrosis and edema following occlusion release.[59,61] A recent study in foals reported meso-thelial cell loss, serosal edema and erythrocyte leakage after 70 minutes of arteriovenous ischemia with neutrophil infiltration and increased edema following 60 minutes of reperfusion.[5] All foals developed significant peritoneal adhesions 10 days after the experiment.

Ischemia has been reported as a potent stimulus for

adhesion formation in many species, including the horse.[7,73,74,75] Visceral or parietal peritoneal injury results in the release of vasoactive substances such as histamine, bradykinin and prostaglandins which increase capillary permeability and allow a serofibrinous exudate onto the damaged serosal surface. Platelets aggregate and the coagulation cascade is activated by tissue thromboplastin, converting fibrinogen to fibrinous adhesions where surfaces are intact. Although the majority are transient, some fibrinous adhesions persist and organize to form permanent fibrous adhesions.[76] Buckman suggested that the local fibrinolytic system determines which adhesions are absorbed and which will remain.[76] The local fibrinolytic system is dependent on local plasminogen activator activity which converts plasminogen to plasmin. [77,78] Plasminogen activator activity has been localized to peritoneal macrophages, mesothelium and submesothelial blood vessels.[79] It has been suggested that during intestinal ischemia, the mesenchymal cells preferably differentiate to fibroblasts versus mesothelial cells resulting in a decrease in total plasminogen activator activity and therefore, more permanent fibrous adhesions.[77,80]

Intestinal ischemia produces a biphasic change in both electrical and contractile activities of the gut.[82] Occlusion of the mesenteric artery causes an initial hypermotile

response followed by a quiescent period.[37] The duration of the ischemic insult appears to be a critical factor in restoring normal intestinal motility after reperfusion.[83] Mesenteric vascular occlusion for 2 hours allowed complete recovery of motility; however, 3 hours of occlusion led to irreversible tissue damage and muscle paralysis.[102]

Intraluminal Distention

Intestinal obstructions are common in horses and man which are caused by simple obstruction, strangulation or ileus.[84] A feature common to all intestinal obstructions is fluid and gas accumulations and intraluminal distention proximal to the lesion.[81,84-86] In the small intestine of the cat, dog and man intraluminal pressures range between 4-10 mmHg in relaxed bowel and can increase to 40 mmHg in obstructed bowel.[93] In clinical cases of small intestinal obstruction, surviving horses had extra-abdominal intraluminal pressures of 4.5-10 cm H2O; whereas nonsurvivors had 8-21 cm H2O.[3] Because the intraluminal pressure measurements were made with the bowel outside the abdominal cavity, the pressures are artificially lower than actual intra-abdominal pressures.[3]

The increase in intraluminal pressure compromises the

intestinal transport of water and electrolytes, further increasing the intraluminal fluid volume and exacerbating the distention.[87] In 1933, Herrin and Meek observed that intestinal distention produced by a water-filled balloon caused secretion of fluid into the lumen.[88] Others have reported that intraluminal distention either decreased absorption[90] or increased secretion of intestinal fluid depending on the magnitude and duration of the increased intraluminal pressure.[90,91] Swabb *et al* showed that an intraluminal pressure of 15-30 cm H_2O induced secretion of water and electrolytes in the ileum, decreased absorption in the jejunum, and produced no changes in transport in the rabbit colon.[91] Adjacent control segments had no transport changes suggesting a locally induced phenomenon. Intraluminal pressures up to 70 cm H_2O had no effect on the colonic transport functions. Intraluminal pressures of 15 mm Hg caused decreased absorption in ileum of ponies[93]; whereas higher pressures (25-50 mm Hg) produced secretion of water, sodium and potassium.[93] The distention-induced secretory process in the ileum was reversible at 20 cm H_2O pressure after 100 minutes of decompression; but transport changes were irreversible after 200 minutes at 30 cm H_2O.[91] Reversibility of transport rates suggests that the mucosal epithelium was not significantly damaged during 20 cm H_2O disten-

tion pressure; however, morphologic studies were not done.

The mechanism involved in the distention-induced secretion has been investigated.[90,93-95] Caren *et al* showed that hexamethonium, a ganglionic blocking agent, modified the secretory response to distention suggesting that intrinsic neural reflexes may be involved.[90] In rats, a neural reflex arc involving Vasoactive Intestinal Polypeptide (VIP) is activated with mechanical stimulation of the mucosa and increases intestinal secretion by activating adenylate cyclase production of cyclic AMP suggesting its role in the secretory response.[90] Processes that increase intracellular levels of cyclic AMP reduce intestinal absorption by reducing the coupled sodium and chloride transport.[89,94] In another study, a prostaglandin synthetase inhibitor, indomethacin, prevented the secretory response to distention suggesting that prostaglandins play a role in mediating the secretion.[95]

The increased secretion may involve either active or passive mechanisms. Enzymes, adenylate cyclase and Na-K-ATPase, are involved in regulating active intestinal electrolyte transport, but were unchanged in distended rabbit and rat jejunum.[92] The passive driving force for intestinal secretion is produced by an increase in tissue interstitial pressure above intraluminal hydrostatic

pressure (IHP) which is influenced by the intestinal microcirculation and explained by the Starling equation.[25,93,95,96] Under physiologic conditions, the driving pressure for blood flow through the intestinal vasculature is the difference between arterial (Pa) and venous (Pv) pressure. Intestinal distention causes an increased tissue pressure (Pt) which collapses the thin-walled venules.[93,95,96] Under these pathologic conditions venous outflow no longer influences capillary pressure (blood flow).[96] Instead the capillary hydrostatic pressure is determined by the arterial pressure minus the tissue pressure.[96] A progressive elevation of intraluminal pressure induces a concomitant increase in capillary hydrostatic pressure and capillary filtration rate.[96]

Granger *et al* demonstrated an increased capillary filtration rate in the cat jejunum during increased luminal distention.[96] Using a non-absorbable solution, Granger found a fifteen to twentyfold increase in intestinal lymph flow and pressure when intraluminal pressure was elevated from 0 to 20 mm Hg. In the absence of net water absorption, the sustained increase in lymph flow (and decreased lymph oncotic pressure) must be attributed to enhanced capillary filtration. Furthermore, increased capillary pressure and filtration drives fluid from the vascular to extravascular space causing increased tissue interstitial pressure. This

increased interstitial pressure augments distention-induced extrinsic pressure, further compressing the intestinal veins much like the "compartment syndrome" described in other species.[97] The result is that fluid is secreted into the intestinal lumen when tissue interstitial pressure exceeds the intraluminal pressure by 4-6 cm H2O.[93] Secretion can also be caused by increasing venous pressure to 10-30 cm H2O in the rabbit ileum distended to 20 cm H2O.[92] Support for this mechanism was demonstrated by a recent study.[96] When luminal pressure was 0 mmHg, an increase in venous pressure causes a progressive increase in lymph flow and decrease in blood flow; however when luminal pressure was held at 20 mmHg, venous pressure elevation had no effect on blood or lymph flow until it exceeded the luminal pressure.[96]

Other mechanisms have been proposed to explain the increased capillary filtration rate during luminal distention. These include a myogenically mediated increase in capillary hydrostatic pressure and surface area or increased capillary permeability.[96]

According to the myogenic theory, a reduction in vascular transmural pressure (caused by luminal distention) should relax the precapillary sphincter causing vasodilation (increased blood flow) which would increase capillary hydrostatic pressure and capillary filtration rate.[98] This mecha-

nism would amplify the effect of venous collapse on capillary hydrostatic pressure, because arteriolar relaxation will increase the capillary pressure during venous collapse.[96] A recent study in ponies, showed an increase in vascular resistance at an intraluminal pressure of 10 mmHg which conflicts with the myogenic theory.[99] Presently the explanation of the effects of luminal distention on blood flow using the myogenic theory are not supported.

Several humoral agents, ischemia and oxygen radicals are known to increase intestinal capillary permeability which would enhance protein leakage and increase capillary filtration.[23,27,28] If luminal distention results in ischemia or reperfusion, it may increase the intestinal capillary permeability and ultimately increase capillary filtration. Increased tissue permeability in response to increased intraluminal pressures has been reported in both the rabbit and rat jejunum as measured by osmotically induced fluid flow.[93,107]

Intraluminal distention decreases intestinal motility promoting the accumulation of gas and fluid with small intestinal obstructions.[94] Numerous studies report normal peristalsis at intraluminal pressures of 0-5 mm Hg, a moderate decrease at 15 mmHg and no motility at 20 mm Hg.[100,101] Hanson observed an initial increase or spasm of intestinal

motility lasting for 1-3 minutes followed by longer periods of quiescence, which was dependent on the magnitude and duration of luminal distention.[102] Luminal distention also has an indirect affect on intestinal motility. Distention increases intestinal wall tension resulting in mechanoreceptor stimulation and pain. Intestinal motility is inhibited and blood flow is reduced from the sympathoadrenergic induced mesenteric vasoconstriction which exacerbates intestinal ileus and increases luminal distention.[102] Intestinal distention results in the pathophysiologic loss of fluids and electrolytes from the extravascular fluid into the intestinal lumen by secretion. The combination of secreted fluid with ingesta and gas exacerbates the intraluminal distention resulting in pain and ileus, further compromising intestinal function. Extracellular fluid losses can be considerable, resulting in plasma volume depletion, hypovolemia and shock.[8,116]

Morphologic Effects of Intraluminal Distention

Many intestinal distention studies report gross lesions of a thinned intestinal wall and venous engorgement with increased intraluminal pressure;[93,101,104] but only a few describe histologic lesions.[4,105] In clinical cases of intesti-

nal obstruction in the horse, small intestinal distention was associated with mucosal lesions proximal to the primary lesion.[62] Intestinal lesions proximal to the obstruction are more severe in horses with higher intraluminal pressures and are similar to the lesions reported with induced intestinal ischemia.[59] Experimentally, very little morphologic mucosal damage occurred in equine small intestine with intraluminal pressures of 9 and 18 cm H_2O for 4 hours.[4] Lamina propria edema and dilated villous lacteals formed at 9 cm H2O and were more pronounced at 18 cm H2O. Mucosal necrosis was not observed histologically and there was no evidence of epithelial necrosis using transmission electron microscopy. Lamina propria edema was attributed to the increased capillary hydrostatic pressure and capillary filtration seen in response to luminal distention. In rabbits, 72 hours of simple obstruction produced dilation of villous lacteals and lamina propria edema with no evidence of mucosal degeneration; however the intraluminal pressure was not measured.[105] In the rabbit ileum, higher intraluminal pressures (30-60 cm H_2O) collapsed the villus central lacteal[92] which may explain the progressive decrease in lymph pressure and flow at high (> 25 mm Hg) lumen pressures in the feline small intestine.[96] This lymphatic obstruction should enhance tissue hydraulic conductance at high intraluminal pressures.

Using scanning electron micrographs, Harris demonstrated a sixfold increase in intervillous distance and a 30% reduction in villus height at intraluminal pressures of 12.5 cm H_2O in rat small intestine.[106] He also described widening and shortening of the crypt glands resulting in an increased non-villus and decreased villus surface area with no net change in total mucosal absorptive area. He proposed that the widening of the villus base could increase the diffusion distance between villus core arterial and venous structures thereby inhibiting countercurrent exchange mechanisms.[106] However, the countercurrent exchanger has not been demonstrated in the rat intestine.[15] Harris *et al* also suggested that the increased intervillous space promotes access of solutes to intervillous transport sites which would augment absorption during luminal distention. Other studies report decreased absorption and increased secretion during intraluminal distention.[90,92,93,95]

The clinically adverse effects of intraluminal distention may not depend solely on the morphologic mucosal injury. In 1943, Oppenheimer *et al* observed no histologic changes at 15-30 mm Hg, but hemorrhage into the seromuscular layer was seen at higher distending pressures (50-60 mm Hg).[104] In a more recent study, two hours of small intestinal distention at 25 cm H_2O resulted in mesothelial cell

loss, serosal edema, neutrophil infiltration and hemorrhage which increased after sixty minutes of decompression and caused subsequent post-operative peritoneal adhesion formation in foals.[5] Adjacent control segments showed mesothelial cell loss and mild serosal edema which was attributed to a peritoneal inflammatory response from bowel manipulations but no subsequent adhesions.[5] The serosal edema may result from venous collapse and subsequent increased capillary filtration, much like the mucosal response to luminal distention.

Effects of Luminal Distention on the Intestinal Blood Flow

Although simple mechanical obstruction is defined as occlusion of the intestinal lumen without compromise of the vascular supply, numerous studies have reported a reduction in blood flow subsequent to increased luminal distention.[6,39,93,100,101,104,107]

In 1907, Van Zwalenburg inserted a small lamp into the small intestine of the dog and studied the effect of increased pressures on the blood flow through the intestinal wall using low power microscopy.[108] He observed capillary arrest at 30 mm Hg, collapse of small veins at 60 mm Hg and minimal blood flow at 90 mm Hg pressure. Gatch and

Culbertson (1925)[109] and Gatch, Trusler and Ayers (1927)[110] collected all venous return from obstructed bowel loops in the dog and found that the rate of return flow was inversely proportional to the degree of luminal distention. They also reported a constant residual blood flow at the mesenteric region of the bowel wall which remained despite extreme pressures. Dragstedt *et al* (1927) used similar venous outflow methods and confirmed that increasing distention produced progressive impairment of the venous return which occurred at pressures of 35-40 mm Hg in the duodenum and jejunum compared to 55-60 mm Hg in the ileum and colon of the dog.[100] He attributed the variation in susceptibility to the distribution of the intramural blood vessels in the various intestinal segments.

In 1935, Gatch and Culbertson reported that pressures as low as 20 mm Hg produced mucosal ischemia in the canine small intestine.[109] Lawson and Chumley (1940) observed no reduction in blood flow until 30 mm Hg pressure was reached and were the first to describe intense motility and hyperemia after deflation.[111] Since the post-inflation hyperemia was not observed in intestinal loops that were encased in plaster-of-Paris, these investigators suggested that the stretch of the intestinal wall is an intrinsic mechanism which compensates for the increased luminal distention.[111]

Oppenheimer and Mann (1943) transilluminated the rat intestinal wall and found no change in the intraparietal capillary circulation at pressures of 10-20 mm Hg, and progressive interference in capillary flow from 30-60 mm Hg.[104] Peristalsis was uniformally absent during the period of distention, but returned after deflation. Deflation was also followed by a reactive hyperemia lasting less than one minute and scattered seromuscular ecchymoses and erythrocyte plugged capillaries. Noer *et al* used quartz rod transillumination studies of rabbit intestine and described a rectangular pattern of the superficial seromuscular capillaries and a progressive decrease in peristaltic activity and blood flow with increasing luminal pressures.[101] The vascular compromise initiated in the small venules at 18-20 mm Hg pressure before being observed in the capillaries at 30 mm Hg and then in arterioles (40 mm Hg) at higher intraluminal pressures. Thirty minutes after deflation, capillary flow returned to normal in segments having the lower but not higher intraluminal pressures. Derblom *et al* observed progressive obstruction of the villous vascular capillaries after 4 days of simple obstruction of the rat jejunum; however, luminal pressures were not measured.[113]

Boley *et al* used electromagnetic flow meters to determine the effects of intraluminal distention of 0-210 mm Hg

on intestinal blood flow in the dog.[114] Blood flow to the isolated intestinal segments was unchanged until 30 mm Hg intraluminal pressures was reached and then a stepwise decrease in flow occurred as the pressure was increased to 60 mm Hg. The maximum decrease in flow occurred 90-120 mm Hg; however, even at 210 mm Hg, 30% of control blood flow remained. Even though only 30% of blood remained at these high pressures, the external appearance of the bowel was a normal pink color which he proposed could mask underlying tissue damage. Systemic and mesenteric arterial pressures were unchanged despite the reduction in blood flow to the segments being studied. Deflation resulted in an immediate increase in blood flow above control levels; however measurements were only made within the initial 5 minutes of decompression. Additional biochemical studies, by the same investigators, determined that as blood flow decreased during distention, there was an associated increase in intestinal venous PO_2 (decrease in the arteriovenous difference) suggesting diminished oxygen extraction by the intestine which returned to normal upon decompression.[114]

Oxygen uptake by the intestine is independent of blood flow despite a wide range of flows in the normal "resting" intestine.[18,22,33] Oxygen uptake was unaffected by intraluminal pressures of 20 mmHg in the feline[107], canine[116] or rabbit[93]

small bowel. At moderate distention pressure (30-60 mm Hg), oxygen uptake becomes a function of blood flow and it is reduced significantly with higher pressures (60-120 mm Hg).[6,107,114,116] The autoregulation of oxygen uptake is an intrinsic mechanism which satisfy the oxygen demands of the intestine regardless of blood flow.[18,21,33] Other studies have shown that autoregulation of blood flow to the intestine is abolished with luminal distention[21,6,114] and it has been suggested that the autoregulation of oxygen uptake is also impaired with distention.[107]

In 1970, Tunik and coworkers performed similar experiments, using electromagnetic flowmeters, and observed a 64% decrease in blood flow in intestine that was distended intermittently (5 minutes distention and 5 minutes decompression) at 45 mm Hg for 60 minutes.[115] A decrease in blood flow (35% below controls) persisted for 2 hours following decompression. These investigators confirmed earlier reports[93,114] of decreased arteriovenous oxygen difference during luminal distention which was not inhibited with cocaine in this study. These results supported the theory that the oxygen shunting is caused by mechanical factors rather than by an intrinsic neural reflex.

Ohman studied the circulatory effects of intraluminal distention in the feline small intestine and reported an in-

creased vascular resistance and capillary filtration rate with decreased blood flow and oxygen consumption as luminal pressures increased.[6] After 60 minutes of decompression, capillary filtration and oxygen extraction returned to control levels; however blood flow remained low and vascular resistance was elevated compared to control segments. He concluded that intestinal distention augments vascular resistance through extravascular compression; thereby causing a reduction in blood flow.[106] In a similar study, Ohman showed that capillary perfusion, unlike blood flow, was not reduced by 20 mm Hg intraluminal pressure, however at higher pressures both decreased.[107] At 100 mm Hg, 30% of basal blood flow remained and, but only 15% of the intestinal capillaries were functional. Oxygen uptake paralleled blood flow rate. Ohman explained these results by the myogenic resistance response to venous-pressure elevation, where distention causes an increased transmural vascular pressure resulting in increased precapillary sphincter tone which decreases the number of perfused capillaries.[39] Decompression of the bowel from 100 mm Hg to 0 mm Hg returned the transcapillary exchange and oxygen uptake to normal; however, blood flow did not return to predistention levels.[107]

Hanson inflated segments of ileum to 20 mm Hg in the dog and observed an initial decrease in blood flow and

increase in vascular resistance which recovered as the intestinal wall was stretched and subsequently reduced the intraluminal pressure.[39] Stress relaxation or "delayed compliance" has been reported in many distention studies.[5,6,39,107,111] Each increment of added fluid or gas causes a steep rise in luminal pressure and decrease in blood flow, but the bowel wall responds by relaxing to accommodate the increased volume; thereby reducing the intraluminal pressure. This relaxation is followed by an immediate recovery of blood flow which is less complete as the distention progresses.[39] Maintenance of a fixed level of intraluminal pressure requires repeated increments of distending volume until a steady level is reached. At this plateau (40 mm Hg in this study), there is no hemodynamic recovery and the effects of distention on blood flow and resistance are maintained.[6,39,] Earlier studies[111] showed that treating the intestine with cocaine or encasing it in Plaster-of-Paris prevented the recovery of blood flow. Hanson suggests that delayed compliance of the intestinal wall decreases extravascular pressure; and therefore, is responsible for the recovery of blood flow and vascular resistance during the initial period of distention.[39]

Ruf and co-workers used microspheres to study luminal distention in the closed abdomen of piglets, and observed a

pressure related decrease in blood flow.[117] The large intestine maintained a significantly higher blood flow rate at 45 mm Hg luminal pressure; however at 60 mm Hg, blood flow was reduced to 25% of the basal level in both segments. At 15 mm Hg pressure in the small intestine, mucosal blood flow was reduced to 45% basal level but muscularis flow greatly increased (218%); however, increasing pressures up to 60 mm Hg caused a stepwise decrease in blood flow in all intestinal layers. Decompression of the distended bowel (30-60 mm Hg) resulted in an initial hyperemic response (118% of basal levels) followed by a sustained 33% reduction in blood flow. The mucosa and muscularis had equal reductions in blood flow. It appears that lower intraluminal pressures are required to impair the intestinal blood flow in the closed abdomen. This may be similar to physiologic conditions where intra-abdominal pressures can compress the intestine from the outside and compound the effects of luminal distention.

Decompression of obstructed bowel enhances intramural edema and vascular resistance which decreases blood flow; however, by opening a significant number of capillaries, deflation restores the capacities for autoregulation and oxygen uptake emphasizing the importance of intra-operative decompression.

Effects of Luminal Distention on Intramural Blood Distribution

Although numerous studies[39,91,93,100,101,104,107,109,111,112,114-117] report a reduction in blood flow during intraluminal distention, only a few have investigated the intramural blood distribution of flow with increased intraluminal hydrostatic pressure.[104,114,117]

Using india ink injection studies, Noer and Derr reported capillary compromise throughout the intestinal wall at 20 mm Hg pressure in both living animal intestine and excised human intestine suggesting the importance of the mechanical versus physiologic effects of luminal pressure on the blood vessels.[112]

Using silicone rubber, Boley *et al* and then Tunik showed a selective ischemia of the mucosa and muscularis at 60-90 mm Hg intraluminal pressures in the canine small intestine.[114] Ruf *et al* tried to quantify the changes in intramural blood flow distribution using microspheres.[117] At 15 mm Hg intraluminal pressure, mucosal blood flow was reduced to less than half basal values; whereas muscularis flow increased 110%.

Methods of Studying the Microvascular Architecture

Although studies of the mesenteric circulation involving perfusion, oxygen consumption and fluid exchange are of primary importance, knowledge of the vascular architecture is essential to understanding both normal and pathologic intestinal function. In 1932, Spanner used a fractionated india ink injection technique to visualize and describe the vascular system of the intestinal villi.[20] Contrast injection media was used in later studies which allowed for a more detailed anatomic description of the small intestine of the rat and dog.[118,119] Bismuth oxychloride or Diodrast contrast medium was used early on; however, the particle size prevented capillary filling. Nylander studied the vascular pattern of the rat small intestine by injecting a barium sulphate suspension 30 days after performing an end-to-end anastomosis.[118] This contrast solution allowed capillary identification. Others have utilized a combination of agar base and radiopaque lead phosphate to study mesenteric, kidney and coronary arteries; however, solidification and lead toxicity problems discouraged its use.[121] Schlesinger improved the injection solution by creating a gelatin-base barium sulfate mixture which remained liquid at room temperatures by the addition of potassium iodide (KI) and would

solidify only on the fixation of formalin.[122] In 1969, Kormano combined microangiography with routine histologic methods to study the relationship between filled capillaries and the surrounding tissue structures.[120] In addition, he determined that frozen tissue sections preserves the microvascular architecture better than formalin fixation. Wojtowicz combined angiography, microangiography and histology methods to describe the effects of simple or strangulation obstruction on exteriorized loops of rabbit small intestine after 6, 12, 24 and 48 hours using a colloid silver injection solution.[123]

Microangiography allows the visualization of large vessels and is useful when comparing the degree of vascularization between specimens.[113] Recently Snyder *et al* combined microangiography with histologic methods to describe the vascular injury associated with clinical cases of large colon strangulation obstruction in the horse.[124] The resolution of smaller vessels is limited by microangiography which prompted the use of other injection materials.

In 1965, Sobin and co-workers introduced a silicone-rubber method of injecting the microcirculation for a direct three-dimensional study of the vascular architecture.[125] A catylst is added to the liquid silicone rubber initiating a non-exothermic reaction which polymerizes in about 30 min-

utes and has an average viscosity of 15-25 centipoise.[126] Silicone rubber injection techniques have been used to determine the normal jejunal vascular architecture in many species[126] and also to describe the intestinal lesion in canine endotoxin shock.[127] This injection method requires minimal technology and can be performed in any laboratory; however, the technique is limited by the depth of field and resolution of the light microscope.

Within the last 10 years, scanning electron microscopy of vascular corrosion casts has overcome the limitations of silicone rubber injections. In this technique, a low viscosity plastic completely fills and replicates the microvasculature after injection. Following polymerization, the surrounding tissue is digested away in a strong alkaline solution, leaving an intact cast of the microcirculation. Murakami initially used methylmethacrylate at 60 centipoise viscosity as the casting medium to study the microcirculation of the renal glomeruli in rats; however, difficult mixing procedures discouraged its use.[128] Another polymerized methacrylate material was developed and modified by Batson as a high viscosity (260 centipoise) compound for large arteries, but did not allow casting of small arteries, capillaries or venules.[126] Many studies followed with numerous recipes for improved casting medium.[129,130] The

compound in use today is an 80% dilute methylmethacrylate solution which has a viscosity similar to blood, (5 centipoise) and polyermerizes when a catylst is added.[126] The low viscosity permits the complete filling of all vessels and allows distinction between arterial and venous sides of the circulation. Arterial endothelial cell nuclear imprints are elongated and oriented parallel to the long axis of the vessel; whereas veins have random circular impressions.[124,126,129,130]

Recently, a similar casting material was used to describe the microvascular circulation of the equine large colon, small intestine and cecum.[130,16,131] The advantages of this technique are the high resolution, depth of field, and wide magnification range of the scanning electron microscope to visualize the three-dimensional microvascular organization. Important limitations are the inability to determine exact vessel diameters due to cast shrinkage or vessel stretch during perfusion.[126] The importance of perfusion pressures at the time of infusion is debated. Most intestinal studies measure pressures at the injection syringe and maintain physiologic mesenteric pressures (120 mm Hg) during perfusion; however, syringe pressures probably do not estimate intravascular pressure. In one study, it was difficult to reach a perfusion pressure above 100 mm Hg when injec-

tions were performed while monitoring intravascular pressure.[126] The end point of injection is determined when the tissue is a uniform color of the injected compound and when the solution is flowing freely from the venous circulation. Specimen comparisons can be made if perfusion techniques are kept constant.[126]

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FIGURES

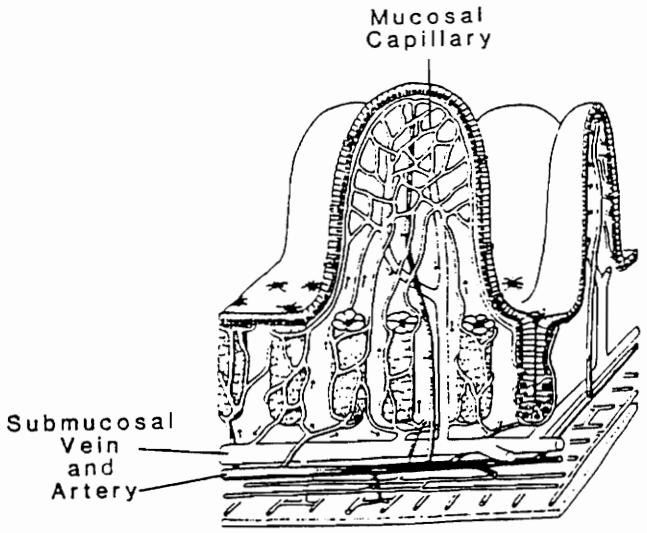

Figure A. Microvascular organization of the small intestine. (From Ohtani O, Kikuta A, Taguchi T, and Murakami T: Arch Histol Jpn 1, 46, 1983)

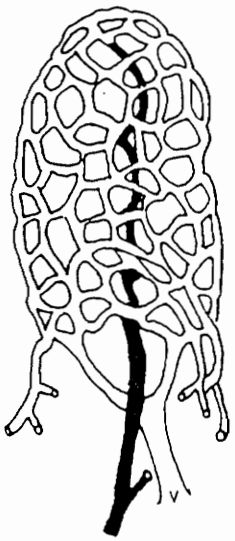

Figure B. Microvascular pattern of the equine small intestinal villus.(From Snyder JR: Vet Clin NA Eq Prac, 5, 247-270, 1989)

EVALUATION OF THE MICROCIRCULATION OF THE EQUINE SMALL INTESTINE FOLLOWING INTRALUMINAL DISTENTION AND REPERFUSION

INTRODUCTION

Despite improvements in surgical techniques and post operative care, horses continue to have poor long term survival rates following surgical relief of small intestinal obstruction. Small intestinal obstruction accounts for 19.1% of referral colic cases in the US with a fatality rate is 67.3%.[1] In a recent study, 51% of 140 horses with small intestinal strangulation obstruction died during the initial post operative period and only 24% were alive 36 months after surgery.[2] These poor survival rates are attributed to the rapid onset of ischemia and necrosis and the frequently encountered post-operative complications such as continuing necrosis, ileus, and adhesion formation.

Affected horses commonly have luminal distention of the small intestine proximal to the primary obstruction. A relationship between the degree of intraluminal distention and survival was observed in a series of horses having surgery for naturally occurring small intestinal obstructions. Horses which survived had a mean small intestinal intraluminal pressure of 6.3 cm H_2O whereas those that died had a mean of 15 cm H_2O.[3] In clinical cases, small intestinal distention was associated with mucosal lesions proximal to the primary lesion which were similar to the mucosal

lesions seen in experimentally induced stangulating obstructions of the small intestine.[4] However, experimentally, little morphologic mucosal damage occurred after creating extra-abdominal intraluminal pressures < 18 cm H_2O for 4 hours in the small intestine of horses.[5] The difficulty in demonstrating histologic mucosal alterations may indicate that the clinical adverse effects of intraluminal distention may not be as closely related to the morphologic mucosal injury seen with ischemia alone. In a recent study, intraluminal distention caused enough serosal injury to stimulate post operative peritoneal adhesions in foals.[6] The adhesions following bowel distention observed in this study suggests that pre-operative damage could account for some post operative complications following correction of small intestinal obstructions.

Studies in other species have documented the reduction of intestinal blood flow during intraluminal distention,[7-12] however there are few reports which describe the intramural distribution of blood flow.[10,11,12] Variations in the injection techniques has resulted in conflicting descriptions of the altered perfusion patterns during intraluminal distention.[10,11,12] Microangiography and light microscopy (LM) of specimens injected with a radiopaque solution and scanning electron microscopy (SEM) of vascular microcorrosion

casts has recently been used to provde a description of the microvascular architecture of the normal equine jejunum, ascending colon, cecum. [13-15] Similar methods were used to identify the vascular injury associated with strangulating obstructions of the ascending colon in horses.[16] The purpose of this study was to identify the altered vascular patterns during distention and reperfusion following decompression of the equine small intestine using microangiography and light microscopy of perfused specimens and scanning electron microscopy of vascular corrosion casts.

MATERIALS AND METHODS

Seven healthy adult horses of mixed breeding and sex, 3-12 years old (mean age, 5 years) were used. The horses were sedated with xylazine (0.5 mg/kg IV) and anesthesia was induced with guaifenesin (5%) and thiamayl sodium (0.3%) IV to effect. The horse was placed in dorsal recumbency and anesthesia was maintained with halothane and oxygen using intermittent positive pressure ventilation. Lactated ringers solution was administered at 5-10 ml/kg/hr IV. Mean blood pressure measured by direct arterial catherization was maintained $\geq$ 70 mm Hg.

The ventral abdomen was aseptically prepared and covered with a smooth plastic drape. A 35-cm ventral midline

incision was made cranially from the umbilicus. Beginning at the distal jejunum, just proximal to the large ileal vascular arcade, four 25-cm intestinal segments were identified. Two segments were designated as experimental and two alternating interposed segments were designated as controls.

Intraluminal distention was created in the two experimental segments as previously described by occluding the lumen at each end of the experimental segments with circumferential latex rubber penrose drains.[6] The drains penetrated the mesentery without occluding extramural vessels. A #10 french polyethylene catheter was inserted into an adjacent segment of normal intestine and passed into the experimental segment prior to lumen occlusion. Sterile lactated Ringer's solution was infused into the segment to produce an intramural pressure of 25 cm H_2O (18.4 mmHg) after the bowel had been returned to the abdomen. Intraluminal pressure was monitored using a pressure transducer and pressures in each segment were maintained by infusion of fluid as required for 120 minutes.

After 120 minutes, one distended segment and the adjacent control segment were resected. The second distended segment was decompressed and remained in the abdomen for a 60-minute reperfusion period after which it and the adjacent control segment were also resected. Simultaneous to this

experiment, other jejunal segments were used to describe the effects of ischemia on the microcirculation.[17]

Immediately following resection, the segments were placed in 37°-C isotonic NaCl. The distended segments were maintained in the distended state throughout the perfusion procedures. The jejunal arteries were cannulated with blunt 20 gauge needles and blood was removed by infusion with NaCl. Perfusion pressures were periodically monitored with a mercury manometer connected to the injection syringe and did not exceed 140 mm Hg. In each horse, the four segments (2 experimental and 2 control) were divided in half, each supplied by an artery and vein from the mesenteric arcade.

Microangiography

In one half of each segment, the jejunal artery was perfused with a modified radiopaque barium mass as described by Schlesinger[18] and Snyder et al.[14] Adequate vascular filling was determined by observing an even distribution of the solution throughout the segment and observing the solution flowing from the mesenteric vein. After perfusion the segments were fixed in 10% formalin for 24 hours.

Complete (circular) cross sections and partial (flat) full-thickness sections and flat sections including only the submucosa-mucosa and muscularis-serosa layers were taken

from each segment as previously described by Snyder.[14] All were placed in non screened cassettes and radiographed in a cabinet x-ray unit[d] using panchromatic black and white microfine grain film[e]. Eight microangiographs, taken from the antimesenteric border or full circumference for cross sections, were evaluated for each intestinal segment. All microangiographs were evaluated blindly by three of the authors and the experimental sections were described as having an increased, decreased or equal number of filled vessels as compared to the corresponding control sections. Comparisons were between all distended segments and corresponding controls, reperfused segments and controls and distended and reperfused segments. The full thickness sections were trimmed, embedded in paraffin, sectioned at 4*um* and stained with hematoxylin and eosin (H&E) for histologic evaluation.

Microvascular Casting

The remaining half of each segment was used to create vascular microcorrosion casts by infusion with a methylmethacrylate mixture.[a] The resin was combined with a methylmethacrylate monomer[b] in an 80% dilution. After the catalyst[c] was added, the casts were allowed to polymerize in a 48-hour water bath after which the segments were immersed

in 10% potassium hydroxide (KOH) for tissue digestion. The KOH solution was changed daily until all the tissue was digested in 2-3 days.

After tissue maceration, the vascular casts were rinsed with deionized water and dehydrated in sequential alcohol solutions, air-dried, and sectioned under a dissecting microscope. Selected specimens were mounted on aluminum stubs, sputter coated with gold and viewed with a scanning electron microscope[c] at an accelerating voltage of 10 kV.

RESULTS

The gross observation of a thinned and more transparent bowel wall following distention is consistent with other reports of experimentally induced bowel distention[20,21,22,23] and with our clinical observations in naturally occurring obstructions. All control segments had normal bowel wall thickness and a pink serosal color. Perfusion of the jejunal mesenteric artery with the blue-dyed barium solution resulted in an even distribution of the blue color throughout all layers of the distended and control segments; however dilated vessels were observed branching from the mesenteric arcade and surrounding the circumference of the dis-

tended segment. After 60 minutes of decompression the bowel wall thickness returned to normal, but scattered serosal ecchymoses were present on the experimental segments. The distended segments required repeated infusions of lactated Ringer's solution to maintain a constant intraluminal pressure at 25 cm H_2O.

Microangiography and Histologic Evaluation

Comparison of all cross and flat-section microangiographs between distended and control segments, distended segments following 60 minutes of reperfusion and controls, and distended and reperfused experimental segments are listed in Table 1. In the distended full-thickness flat sections, there was a decreased vascular density in all microangiographs compared to the control sections. Although complete villus perfusion was apparent, the number of filled vessels observed in the distended submucosa-mucosa sections bowel was less than in the controls. The intervillus space was increased in all experimental sections. There was a consistent decrease in the vascular density in the microangiographs of the muscularis-serosa sections in the distended segments when compared to controls. Flat section microangiographs of the seromuscular layer revealed a rectangular vascular pattern in the control segments. There was

a marked reduction in the number of perfused vessels and alteration of the rectangular vascular pattern in the distended sections (Figure 1A,1B). In the cross section microangiographs, the vascular plexus in the submucosal layer was readily observed and appeared enlarged in the experimental segments. The mucosa had a decreased vascular density, although perfusion of the extensive villus capillary network was evident. The vascular plexus located between the circular and longitudinal muscle layers was perfused; however the smaller arterioles that normally branch from this plexus to supply the longitudinal muscle and serosa were absent (Figure 2A,2B).

In paraffin sections, the injected blue-colored barium solution was identified in all layers of the control segments. In the distended sections, the perfusion solution was located within the mucosa, including the subepithelial capillaries of the villus, the submucosa, and the vascular plexus within the muscularis. The perfused barium was observed throughout the circular muscle, but only sparsely seen in the longitudinal muscle layer, and no barium was located in the serosa (Figure 3A,3B).

There was no change in the microscopic morphology of the control segments except for serosal mesothelial cell loss and edema. In the distended segments slight edema formation was observed in the lamina propria and central

lacteals of the villi and the submucosal vessels appeared enlarged (Figure 4). There was also mesothelial cell loss, serosal edema, mild PMN infiltration and erythrocyte leakage within serosa. Occasionally, erythrocytes were seen within the circular and longitudinal muscle layers.

The full-thickness microangiographs of the distended segments following reperfusion had either a decreased (4) or equal (2) vascular density when compared to the control segments. A decreased (3) or equal (3) vascular density was observed in the microangiographs of the submucosa-mucosa flat sections and 4 of the muscularis-serosa flat sections had a decreased number of filled vessels with the remaining 2 having equal vascular filling as listed in Table 1. Experimental cross section microangiographs revealed filling of the vessels in the mucosa, submucosa, and muscularis; however scattered extravasation of the barium (ecchymosis) was seen at the junction of the circular and longitudinal muscle layers (Figure 5). The serosa remained devoid of filled vessels.

All microangiographs of both cross and flat sections revealed a decreased vascular density in the distended segments when compared to the distended reperfused segments. The jejunal wall thickness increased after decompression and the intervillus space returned to a pre-distention state. Previous unapparent capillaries in the seromuscular layer

became evident upon decompression with the rectangular vascular pattern of the circular and longitudinal muscle layers becoming re-established.

Histologic examination revealed perfusate in all layers of the control segments. The control segments had no change in morphology of the mucosa or submucosa but serosal mesothelial cell loss, mild PMN infiltration and edema were present. In the distended reperfused sections, the barium was located in the mucosa, submucosa and both circular and longitudinal muscle layers, with no perfusate identified in the serosa layer. A grade 1 mucosal lesion occurred in the reperfused segments where the surface epithelial cells separated from the basement membrane forming Gruenhagen's space at the villus tip (Figure 6).[19] There was also slight edema formation in the villus lamina propria and central lacteals. PMN infiltration and hemorrhage occurred within the muscle layers and extensive hemorrhage, increased PMN infiltration, edema and mesothelial cell loss was observed in the reperfused serosa (Figure 7).

Vascular Replicas

The microvasculature of all control specimens had similar morphology when compared to the normal equine jejunum as described by Dart et al.[13] The microvascular changes

observed in the experimental distended and reperfused segments were consistent in all specimens. The arterial and venous circulations were distinguished by their respective elliptical or circular endothelial cell nuclear impressions as described by Snyder et al.[14] In the experimental specimens, the central arteriole and subepithelial capillary network of the villus and the capillaries surrounding the mucosal gland crypts were filled with the injected methylmethacrylate. The villus height was reduced and there was a marked increase in the intervillus space compared to controls (Figure 8A & 8B). The submucosal vessels appeared enlarged and the network of smaller arteries and arterioles arising from the submucosal vascular plexus which supplied the seromuscular layer in the control specimens was not present in the experimental specimens.

In the experimental reperfused specimens, the villus height and intervillus space returned to normal (Figure 9). Following decompression of the experimental segments, perfusion improved in all layers; however, the overall number of perfused vessels was less than seen in the control specimens. The submucosal vessels remained slightly enlarged.

DISCUSSION

Recently, intraluminal distention has received attention as a potential cause of post-operative complications previously attributed to surgical manipulation. In one report, distention alone caused enough serosal injury to stimulate post operative peritoneal adhesions in foals[6] which suggests that bowel distention contributes to a disturbance in serosal healing.

Numerous studies have reported diminished intramural blood flow during luminal distention, although the intraluminal pressure required to compromise the vasculature has varied.[7,8,9,20,21,22,23] Ohman observed no circulatory impairment following intraluminal pressures of 20 mm Hg for 60 minutes in denervated feline small intestine.[20] Boley and then Tunik reported that luminal pressures greater than 30 mm Hg were necessary to disrupt the microcirculation in canine intestine.[10,11] However, recent experiments using microspheres or venous outflow detection methods reported impaired capillary flow and increased vascular resistance at 15-20 mm Hg intraluminal pressure.[12,24,25] These in vivo studies were performed within the closed abdomen of piglets which probably increased the extrinsic pressure exerted on the bowel wall and may account for the circulatory impairment at these lower intraluminal pressures. These previous reports sup-

port our results of altered intramural perfusion using an intraluminal pressure of 25 cm H_2O (18.4 mm Hg) in this study.

Microangiography revealed significant diminished vascular density to the seromuscular layer and, to a lesser degree, the mucosa with an intraluminal pressure of 25 cm H_2O. The seromuscular rectangular vascular pattern observed on flat sections results from the vessel distribution in the circular and longitudinal muscle layers crossing at right angles to each other.[14] The rectangular vascular pattern was not present in the experimental sections indicating lack of perfusion in one of the muscle layers and/or in the serosa. These observations differ from previous reports describing selective ischemia of the mucosa and muscle layers with maintenance of submucosal and serosal perfusion during distention.[10,11] This difference may be due to the silicone injection technique used in those studies; the silicone viscosity is 3-6X that of the medium used in this study and blood.[26] The particle size and viscosity of the injected solutions in this study allowed filling of all vessels in the control segments as observed in the normal microvascular circulation of the equine jejunum,[13] but demonstrated altered patterns of vascular perfusion in the experimental segments.

The vascular pattern of the normal equine jejunum is

composed of branches of the mesenteric arcade which penetrate the serosa to form an extensive submucosal vascular plexus.[13] Arterioles arising from this plexus compose 2 mucosal vascular networks. An arteriole ascends to the mucosa and branches to form an extensive capillary network surrounding the mucosal crypt glands as a second arteriole continues to ascend the villus eccentrically before arborizing into a subepithelial capillary network; both drain back into the submucosal vein. The muscularis is supplied by branches from the submucosal plexus which form a smaller parallel plexus between the circular and longitudinal muscle layers. Smaller right angle branches from this plexus supply the serosa. The peripheral location, smaller size and sharp angle of divergence may render these serosal vessels more susceptible to collapse.

Although the experimental mucosal vascular density was decreased compared to controls, complete filling of the villous subepithelial capillary network was seen. If these vessels were not perfused, mucosal degeneration similiar to that reported after ischemic strangulation obstruction would be expected.[27,28] Our findings of villus lamina propria edema without mucosal necrosis are consistent with previous reports on the morhologic effects of distention of equine small intestine.[5] Previous intestinal studies suggest that

increased intraluminal pressure causes mural compression which collapse the thin-walled venules.[29,30] Venous obstruction results in increased capillary hydrostatic pressure which forces fluid into the villous interstitium resulting in interstium edema.[30] Changes in lymph pressure and flow reflect capillary filtration in the small intestine.[7] Granger reported increased lymph pressure and flow rate with a concomitant decrease in blood flow over physiologic ranges of distention pressures (0-20 mmHg) in the feline small intestine supporting this view.[30] Once the fluid accumulation in the interstitium exceeds the volume capacity of the lymphatics, the net effect is secretion of fluid through the mucosa into the intestinal lumen which exacerbates the distention.[31] Swabb *et al* demonstrated that 15-30 cm H_2O intraluminal pressure induced secretion of water and electroltyes in the ileum, decreased absorption in the ileum, and produced no changes in fluid transport in the rabbit colon.[31] Intraluminal pressures of 15 mmHg caused decreased absorption in the ileum of ponies; whereas higher pressures resulted in the secretion of water, sodium and potassium.[32]

The serosal mesothelial cell loss and edema observed in the control segments and similar changes of neutrophil infiltration and hemorrhage in the experimental segments in

this study has been previously reported in distention studies in foals.[6] Intraluminal distention may induce a serosal lesion similiar to that observed in the mucosa in which arterial perfusion continues in the presence of venous occlusion (collapse with distention) resulting in edema, hemorrhage and cellular infiltration as the tissue becomes compromised.[28]

The microvascular corrosion casts supported the microangiographic and light microscopic findings of decreased vascular filling in the seromuscular layer. We also observed a reduction in villus height and marked increase in the intervillus space. Harris *et al* suggested that the increased villus space promotes access of the luminal contents to the intervillus transport sites thereby augmenting the absorptive surface area with distention.[32] However other studies have demonstrated that the small intestine functionally converts from absorption to secretion at 15 cm H2O intraluminal pressure.[29,31,32] The altered mucosal morphology is probably a mechanical separation due to increased luminal pressure. Using a similiar resin-casting technique, Shikata described an arteriovenous shunt at the villus base at 40 mmHg intraluminal pressure in the dog.[25] This was not observed in the vascular replicas in this study.

The decreased vascular density following 60 minutes of decompression in most of the reperfused segments observed in this study has been reported in previous work, although the timing of vascular injection and blood flow measurement appears to be important to the response observed. Many studies report an acute hyperemic response after deflation of distended bowel followed by a more prolonged reduction in blood flow.[21,23,34-37] Reactive hyperemia has been described following brief periods of arterial occlusion in ischemic intestine in which the intensity of the response was related to the extent and duration of the arterial occlusion.[37] Using microspheres, Ruf quantified the reduction in flow 30 minutes after decompressing bowel distended to 15-60 mm Hg and reported that mucosal blood flow decreased to less than half but muscularis flow greatly increased.[12] Ohman described an initial increase in blood flow following deflation of cat intestine; however, the flow rate never attained pre-distention levels and vascular resistance remained above control values.[20] Tunik showed a 45% reduction in blood flow in all layers measured 2 hours after deflation of canine jejunum distended to 45 cm H2O.[11] Two reperfused segments in this study demonstrated equal vascular density to the control segments. This may be the result of individual variation to the effects of increased luminal pressure at the

specific time of vascular injection. Additional studies recording the vascular response at specific time intervals following decompression are needed.

After reperfusion, extravasation of the perfused barium was observed at the junction of the circular and longitudinal muscle layers which coincides with our clinical observation of serosal ecchymosis following decompression and reperfusion of distended bowel. This may result from damage to the vessels in this area at the time of distention and is supported by the extensive hemorrhage surrounding the vessels in the serosa and longitudinal muscle layer in the histologic sections that we observed in this study (Figure 8). The mechanism of this injury was not determined by this study.

In this study, we observed an increased number of capillaries in all intestinal layers following decompression of distended bowel which may cause a reperfusion injury in the post-operative period after surgical correction of small intestinal obstructions. Reperfusion injury is a term used to describe continued tissue damage resulting from the reintroduction of oxygen into hypoxic tissue.[38-41] The progressive mucosal and serosal lesions seen histologically and the neutrophil infiltration in the decompressed segments supports the role of reperfusion in the equine small intes-

tine but does not confirm this mechanism as the cause of a progressive lesion.

Serosal ischemia during distention could contribute to other post-operative complications seen following surgical relief of small intestine obstructions such as adhesion formation. Lundin *et al* found that bowel to bowel adhesions formed in foals having distended or ischemic intestine which could not be distinguished on gross or histologic evaluation.[6] Ischemia has been reported as a potent stimulus for adhesion formation in many species including the horse.[6,42-46] This study demonstrates serosal ischemia during distention which may explain the post-operative peritoneal adhesions commonly seen clinically with distended small bowel.

Autoregulation and oxygen uptake are terms used to describe the intrinsic ability of an organ to maintain a constant blood flow and tissue oxygenation in the face of a fluctuating arterial pressures and oxygen demands. This phenomenon can be demonstrated in isolated organs and appears to be independent of extrinsic innervation or circulatory hormones. Autoregulation of the mesenteric circulation has been documented in both the dog and cat[47] and are diminished or abolished with increased intraluminal pressures.[10] After decompression the autoregulatory function and oxygen uptake of the intestine returns[7] which may be beneficial

during this low flow state. A similar mechanism may exist in the horse which emphasizes the importance of decompression of any distended bowel at the time of surgical relief of intestinal obstructions

Additional increments of fluid was required to maintain a constant intraluminal pressure of 25 cm H_2O in this study. This has been reported in many intestinal distention studies[5-8,12] and is described as "delayed compliance" or "stress relaxation".[7,8,20] Each increment of added fluid causes a steep rise in intraluminal pressure and decrease in blood flow, but the bowel wall responds by stretching to accommodate the increased volume thus reducing the intraluminal pressure.[7,8] This relaxation is followed by an immediate recovery of blood flow which is less complete as the distention progresses.[20]

In conclusion, this study identifies altered intramural vascular patterns during distention and reperfusion of small intestinal segments at intra-abdominal luminal pressures of 25 cm H_2O. We observed decreased seromuscular vascular perfusion during distention followed by an increased vascular perfusion with continued tissue injury and inflammation after decompression. This suggests that chronic increases in intraluminal pressure may contribute to post-operative complications such as bowel necrosis and adhesion formation

following the surgical relief of small intestinal obstructions.

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FIGURE LEGENDS

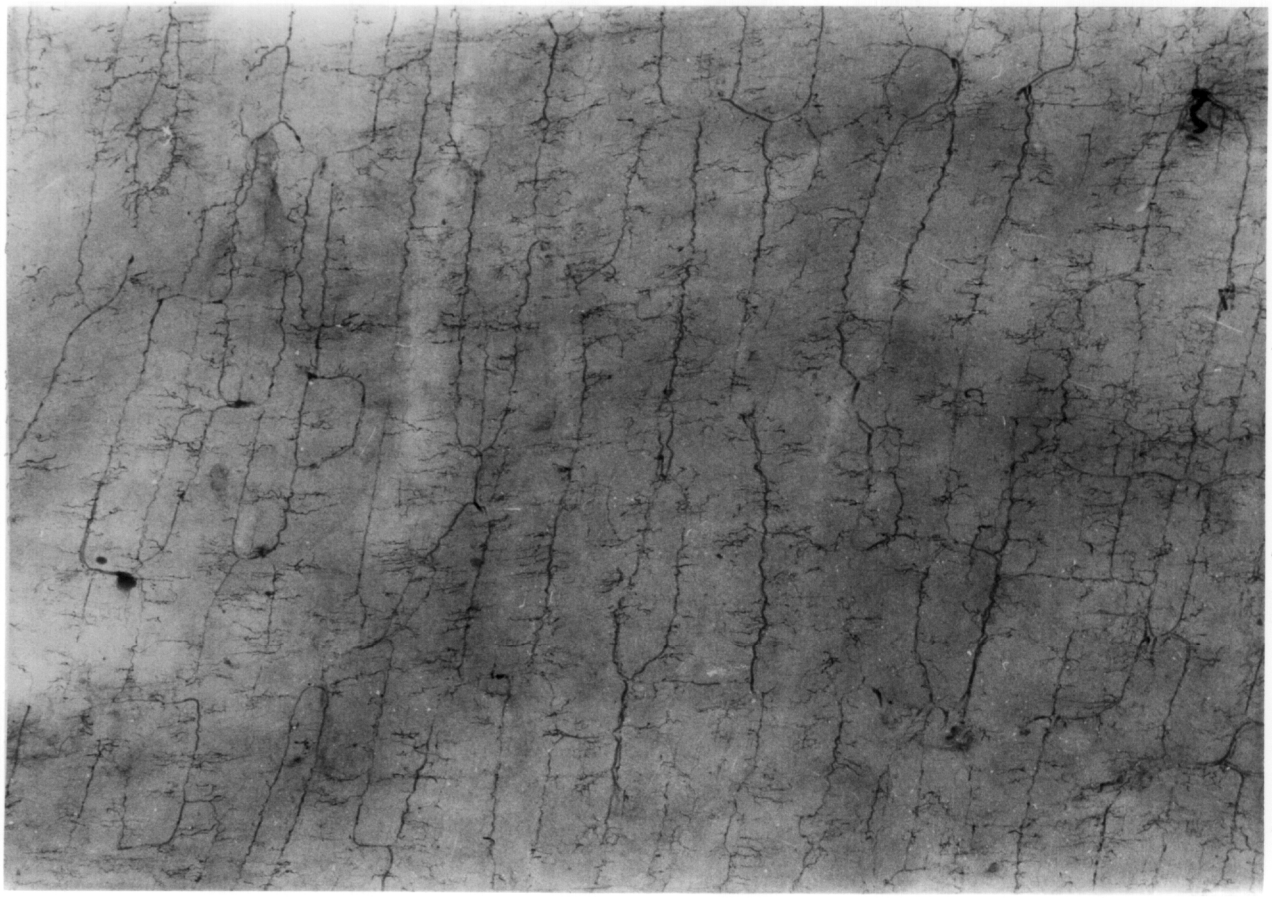

Figure 1A Microangiographs of flat sections of the muscularis-serosa layer. A. Control segments have a rectangular vascular network from the vessel distribution in the circular and longitudinal muscle layers crossing at right angles to each other.

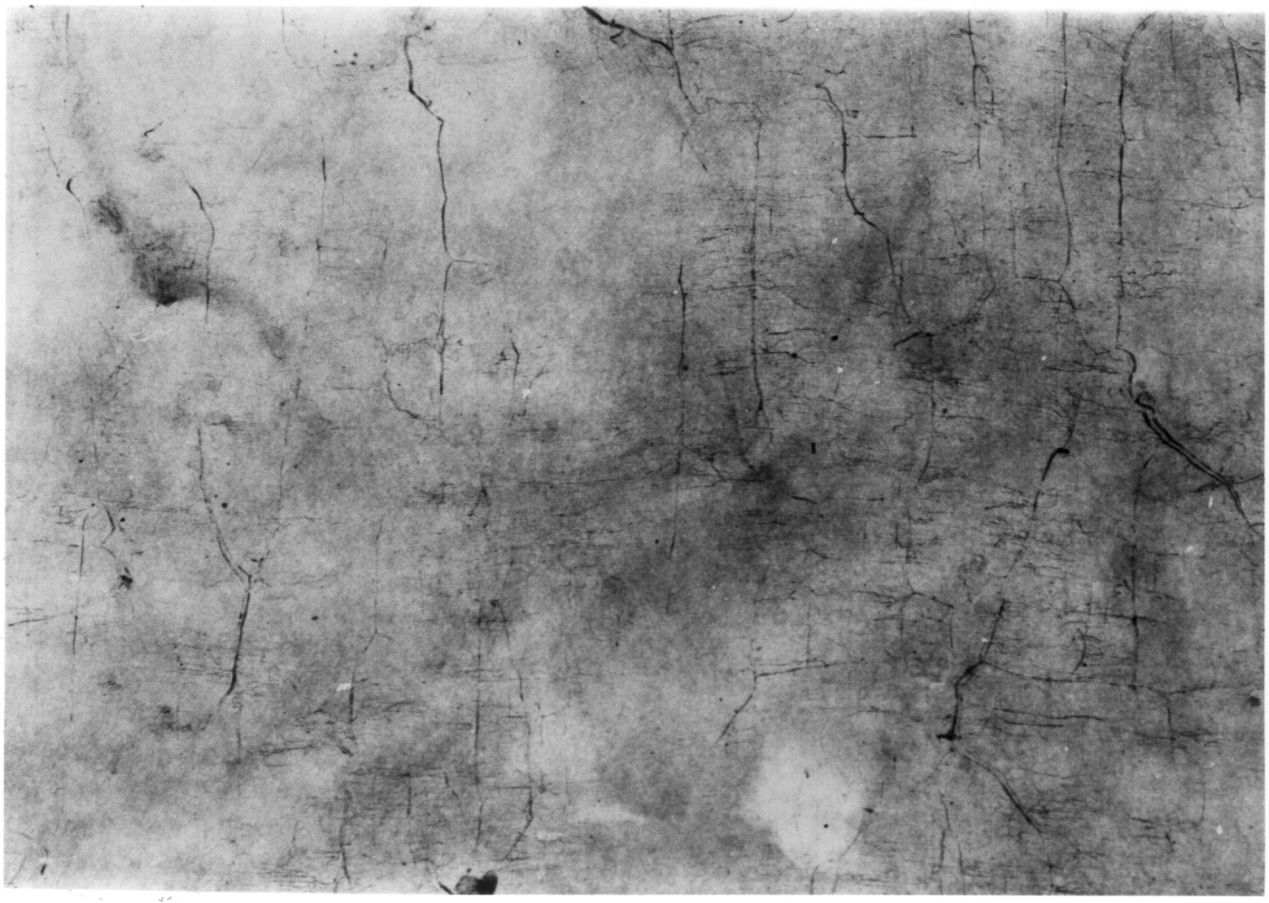

Figure 1B. Distended segments have areas devoid of vessels and the rectangular vascular pattern is not evident.

Figure 2A Microangiographs of cross section of jejunum. A. Control segment: vessels perfused in the mucosa (M), submucosa (SM), circular muscle (CM), longitudinal muscle (LM), and serosa (S).

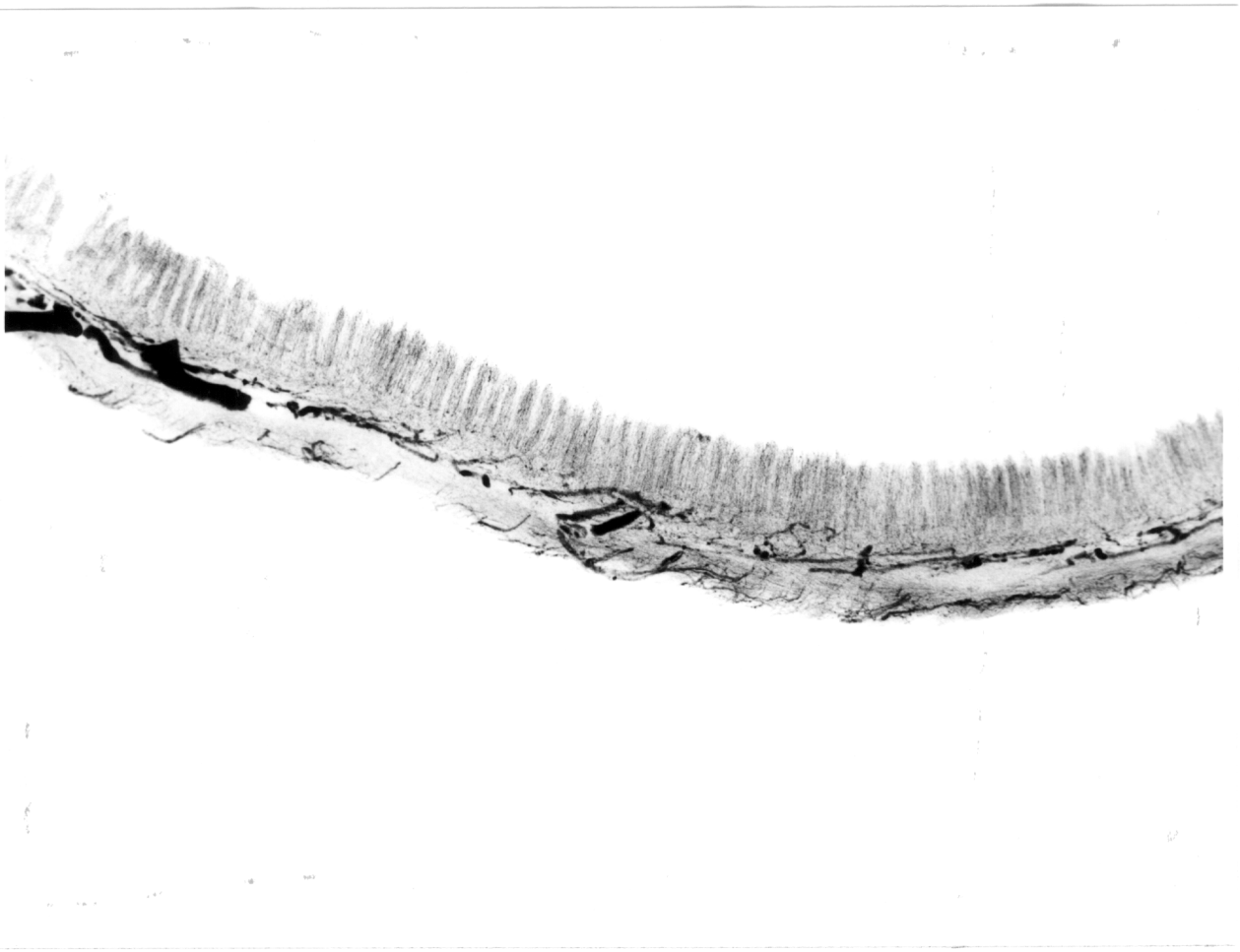

Figure 2B. Distended segment: vessels perfused in the mucosa (M), submucosa (SM), but incomplete vessel perfusion in the muscle layers and the serosa.

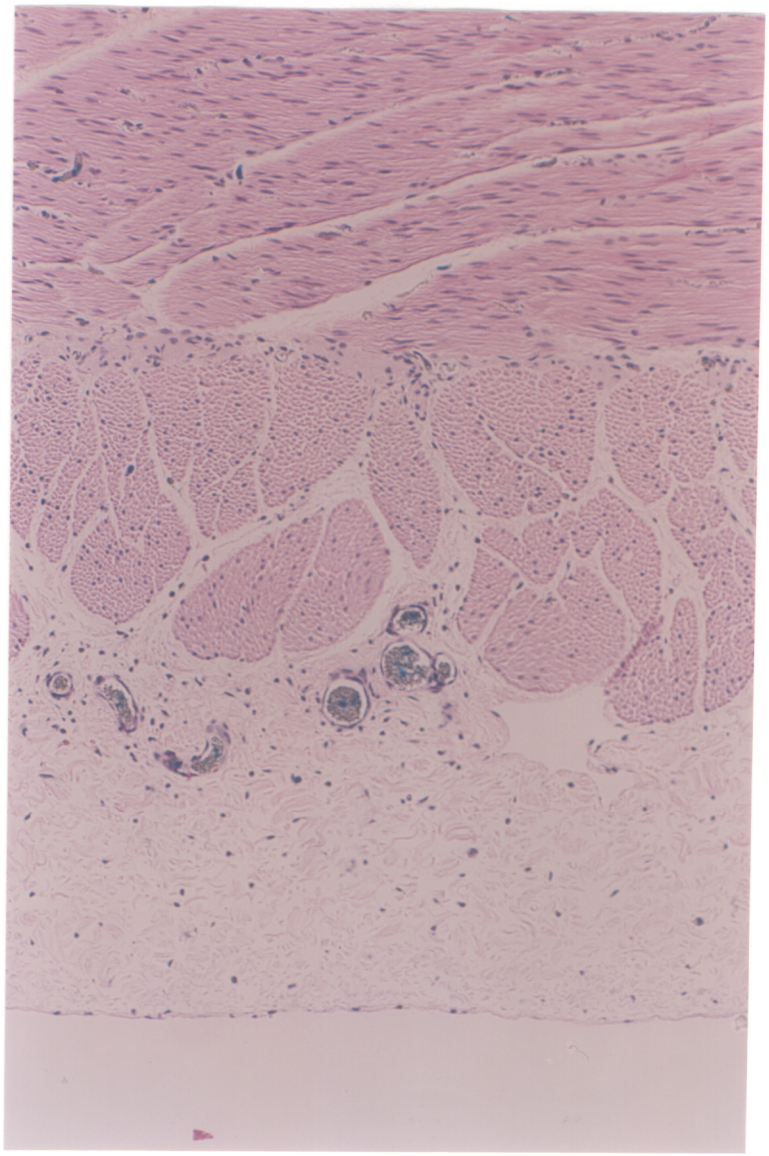

Figure 3A Photomicrographs of the seromuscular layer of the full-thickness microangiograph sections. A. Control segments have the perfused blue-colored barium located in both muscle layers and serosa.(H&E) (100x)

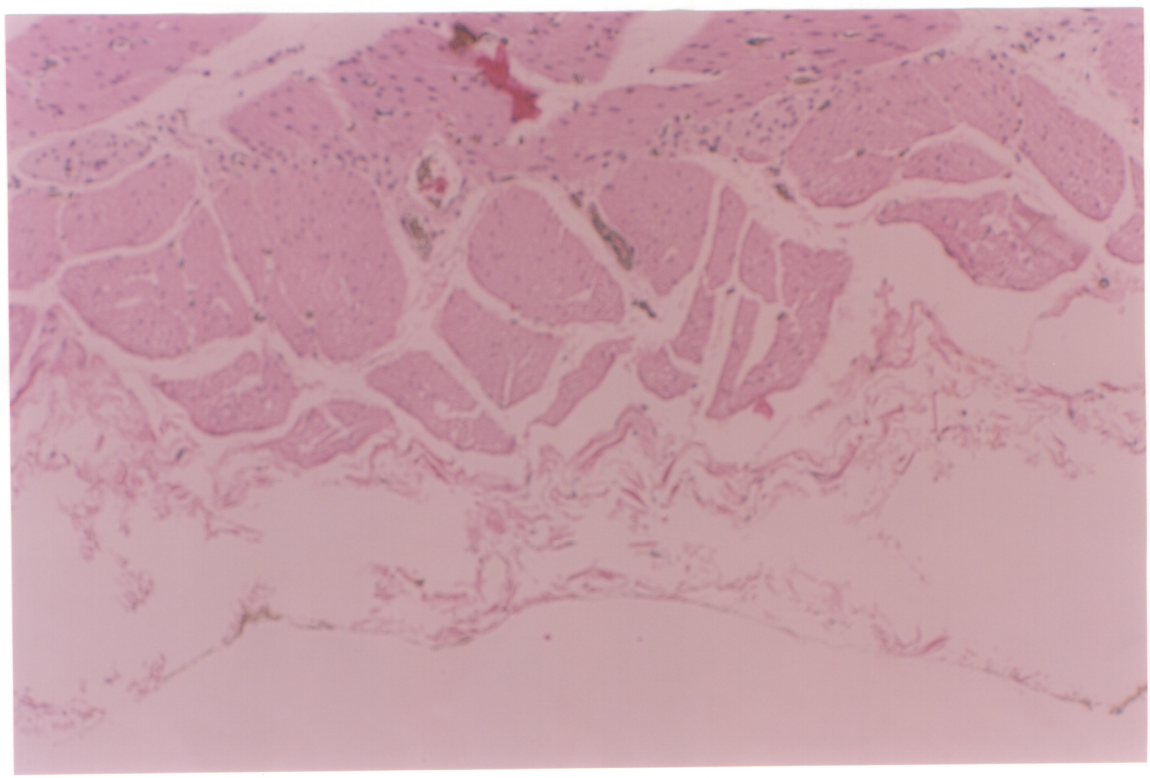

Figure 3B. Distended segments have the barium dye identified in the circular muscle layer, rarely in the longitudinal muscle layers and none in the serosa.(H&E) (100x)

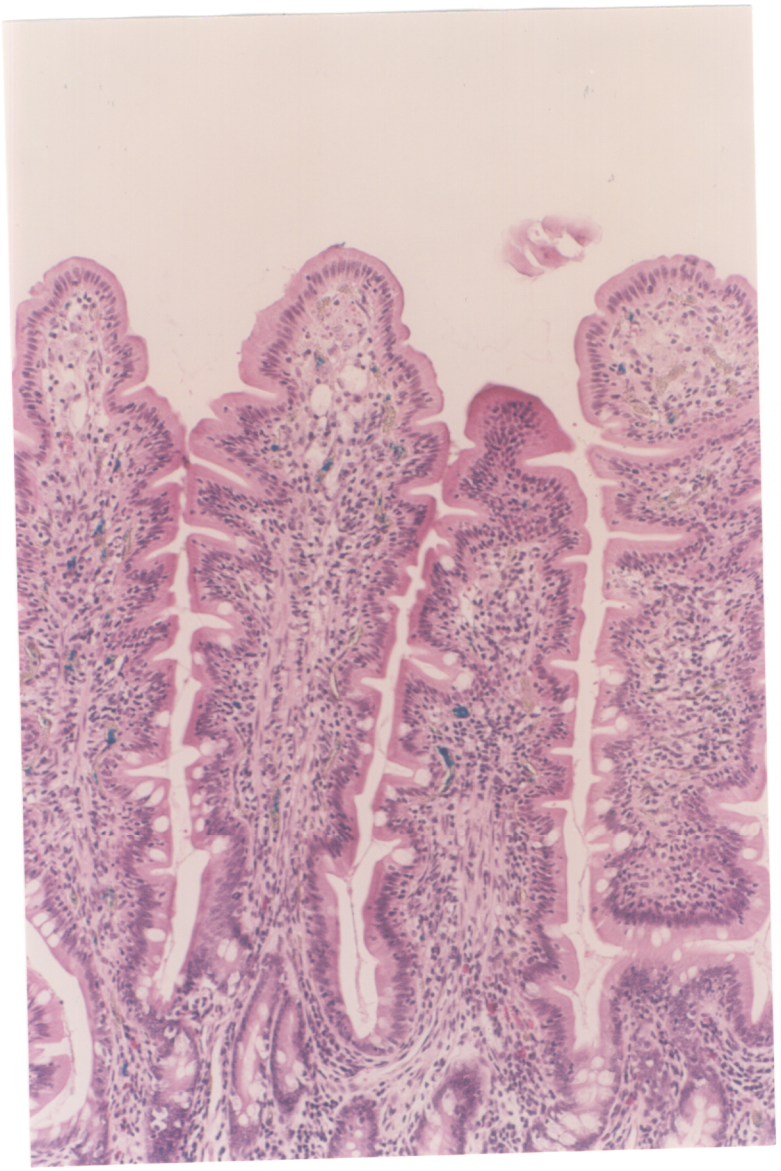

Figure 4 Photomicrograph of the villus in the distended segment. The barium dye is located in the subepithelial capillaries (arrow) and edema formation is seen in the lamina propria and central lacteals.

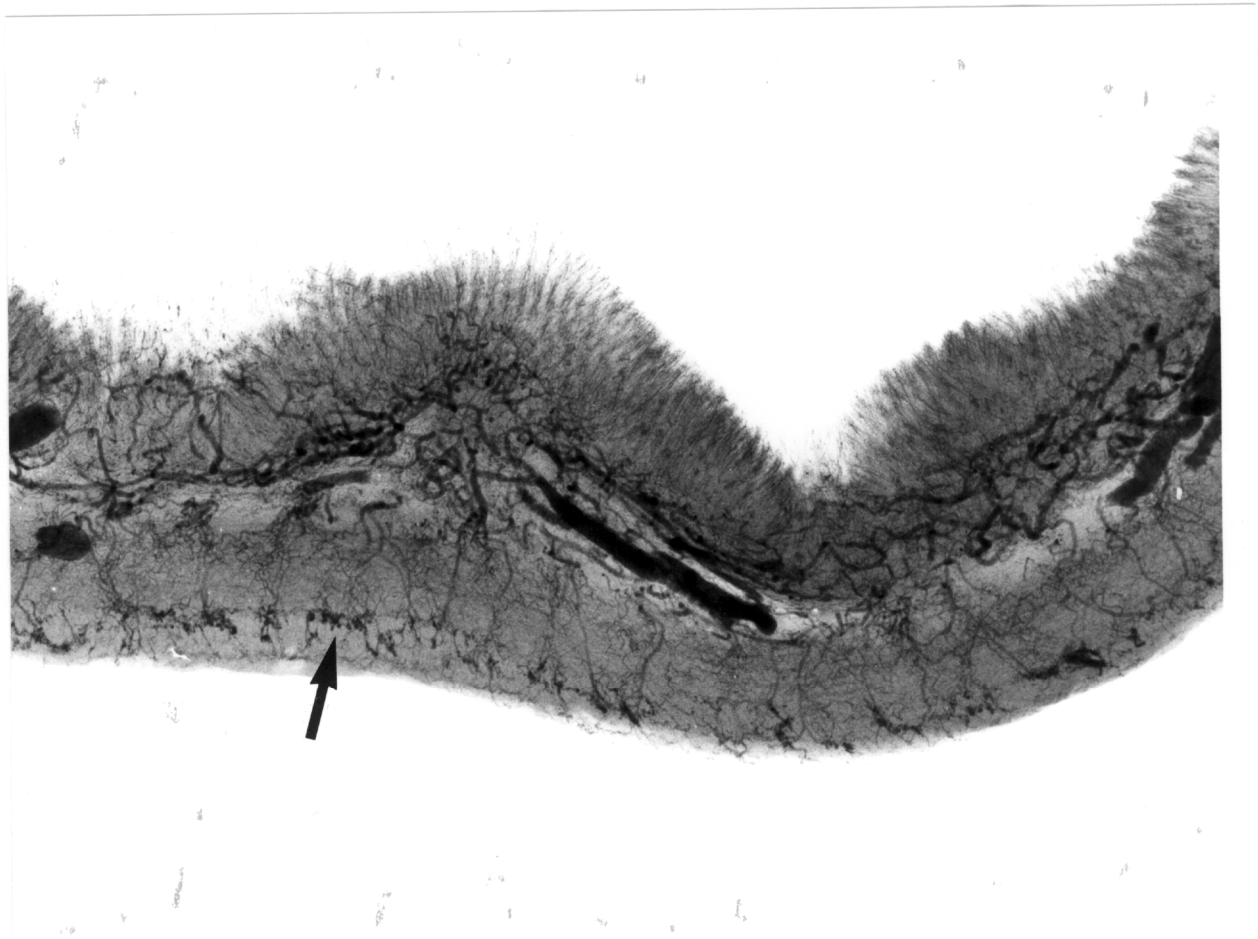

Figure 5 Microangiograph of cross section of the decompressed segment after 60 minutes of reperfusion. Perfused vessels are located in all bowel layers except the serosa. Extravasation of the barium solution is seen at the junction of the circular and longitudinal muscle layers (arrow).

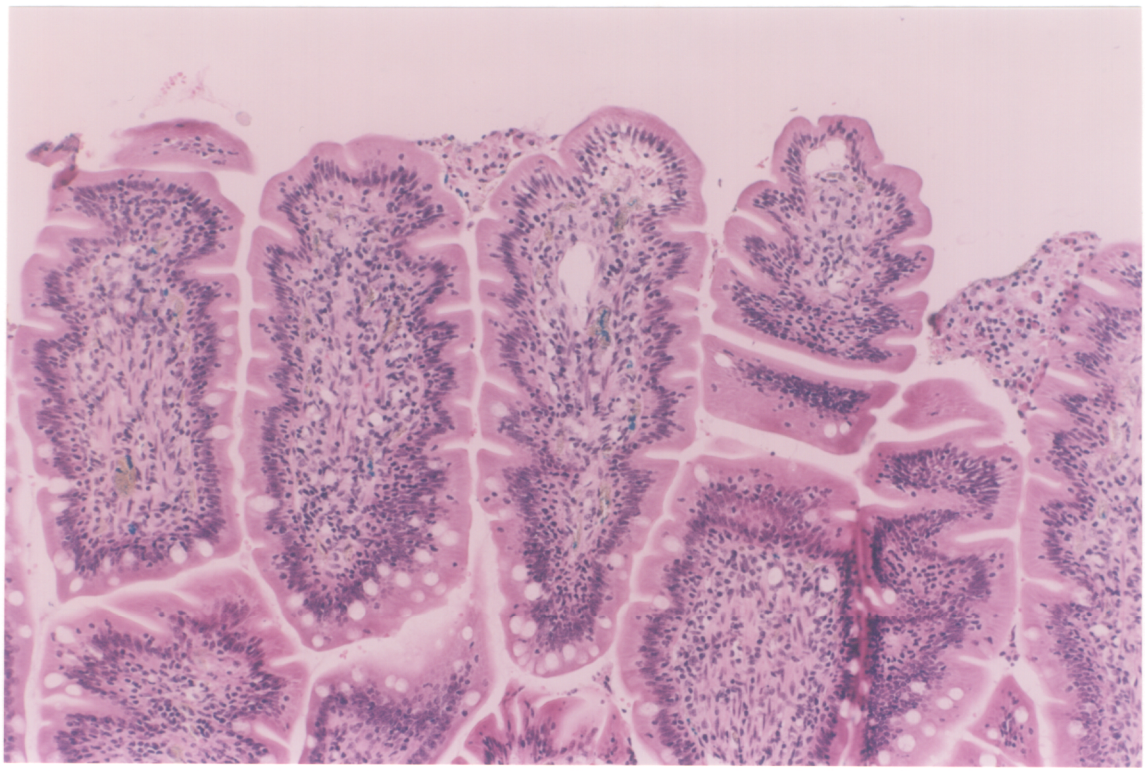

Figure 6 Photomicrograph of mucosa in the reperfused segment. A grade 1 mucosal lesion is present with the seperation of the surface epithelial cells from the basement membrane forming Gruenhagen's space. The subepithelial capillaries are perfused with the barium solution. (H&E) (100x)

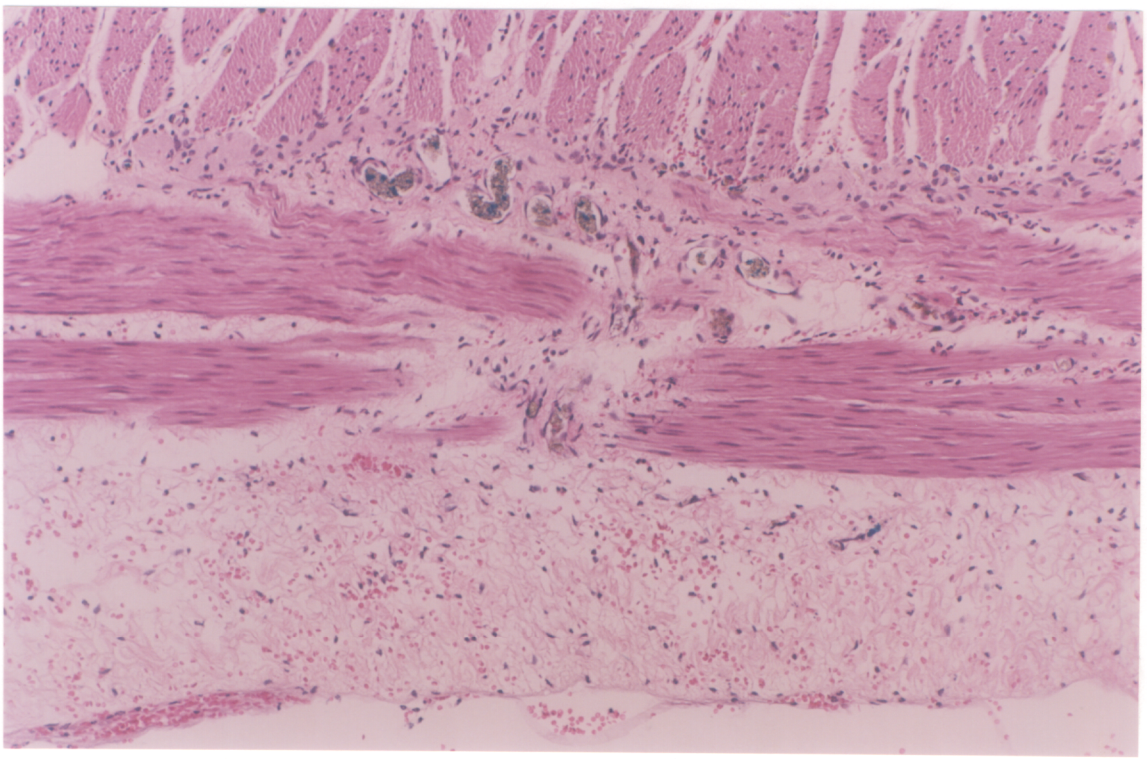

Figure 7 Photomicrograph of seromuscular layer after reperfusion showing cellular infiltrate and hemorrhage in the muscle layers and extensive hemorrhage, cellular infiltrate, edema and mesothelial cell loss in the serosa. Note absence of perfusion medium in the serosa. (H&E) (100x)

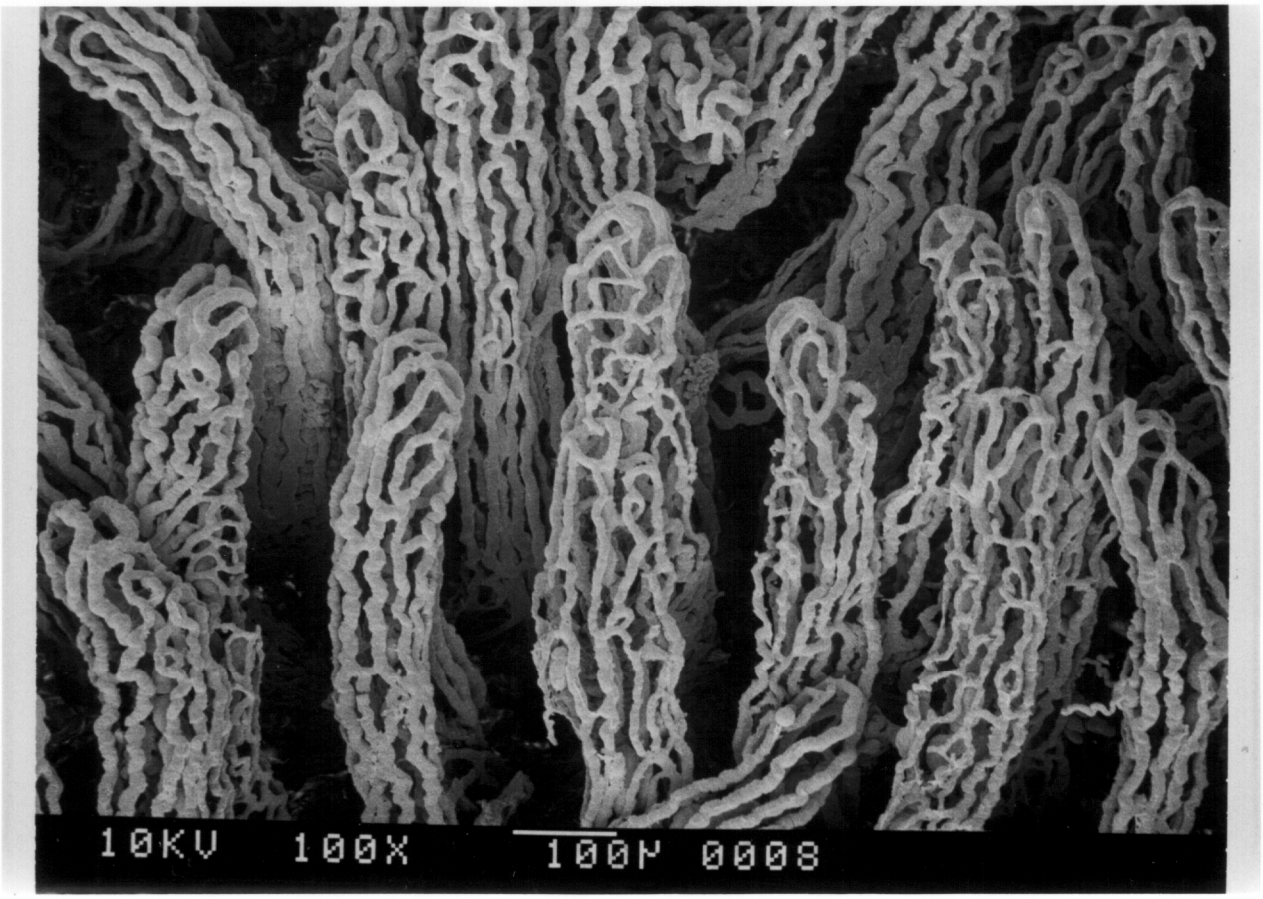

Figure 8A Vascular replica of the control villus (A) with long, slender villi and complete perfusion of the villus capillary network.

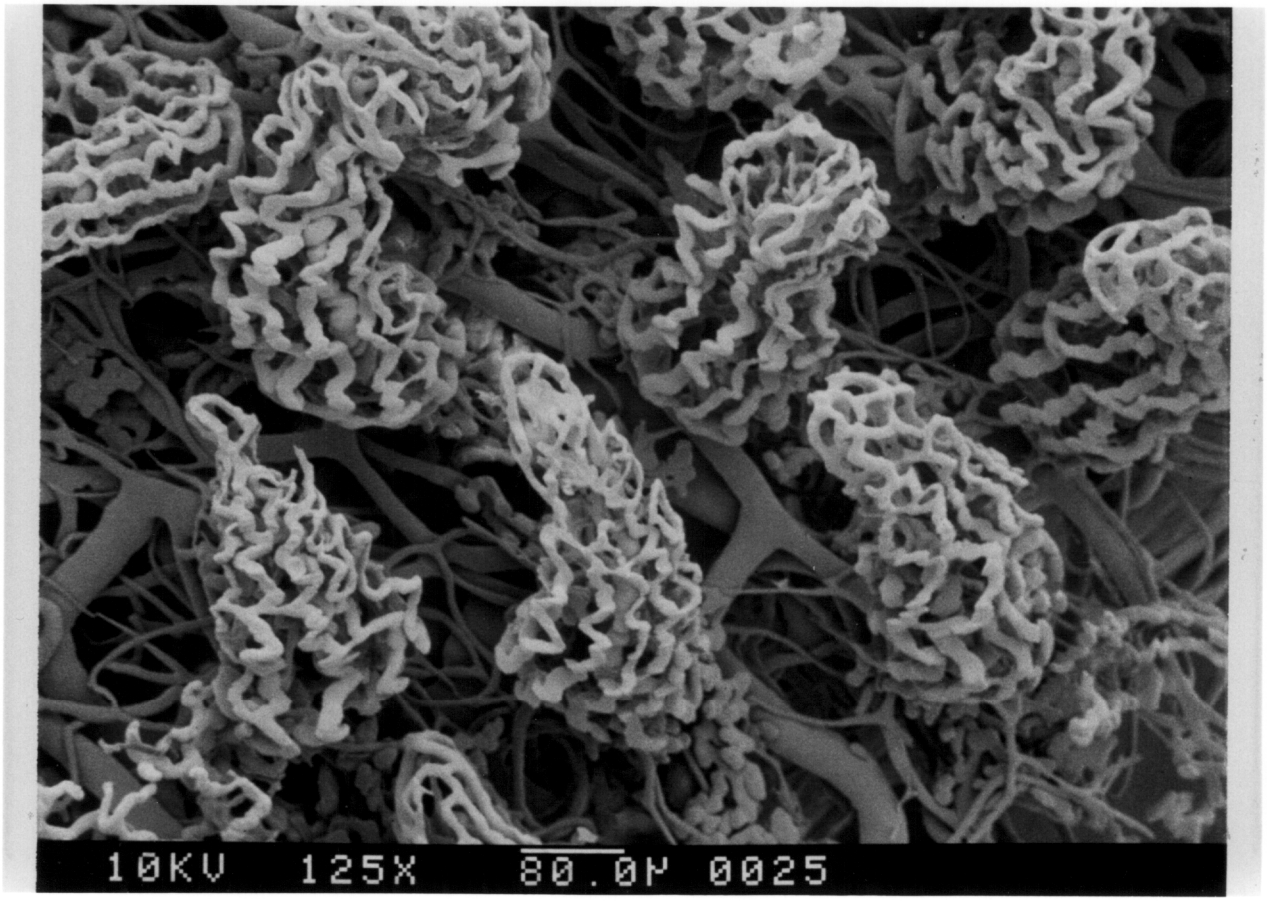

Figure 8B. Distended villi which are short and thickened with an increased width to the intervillus space although the villus capillary network is evident.

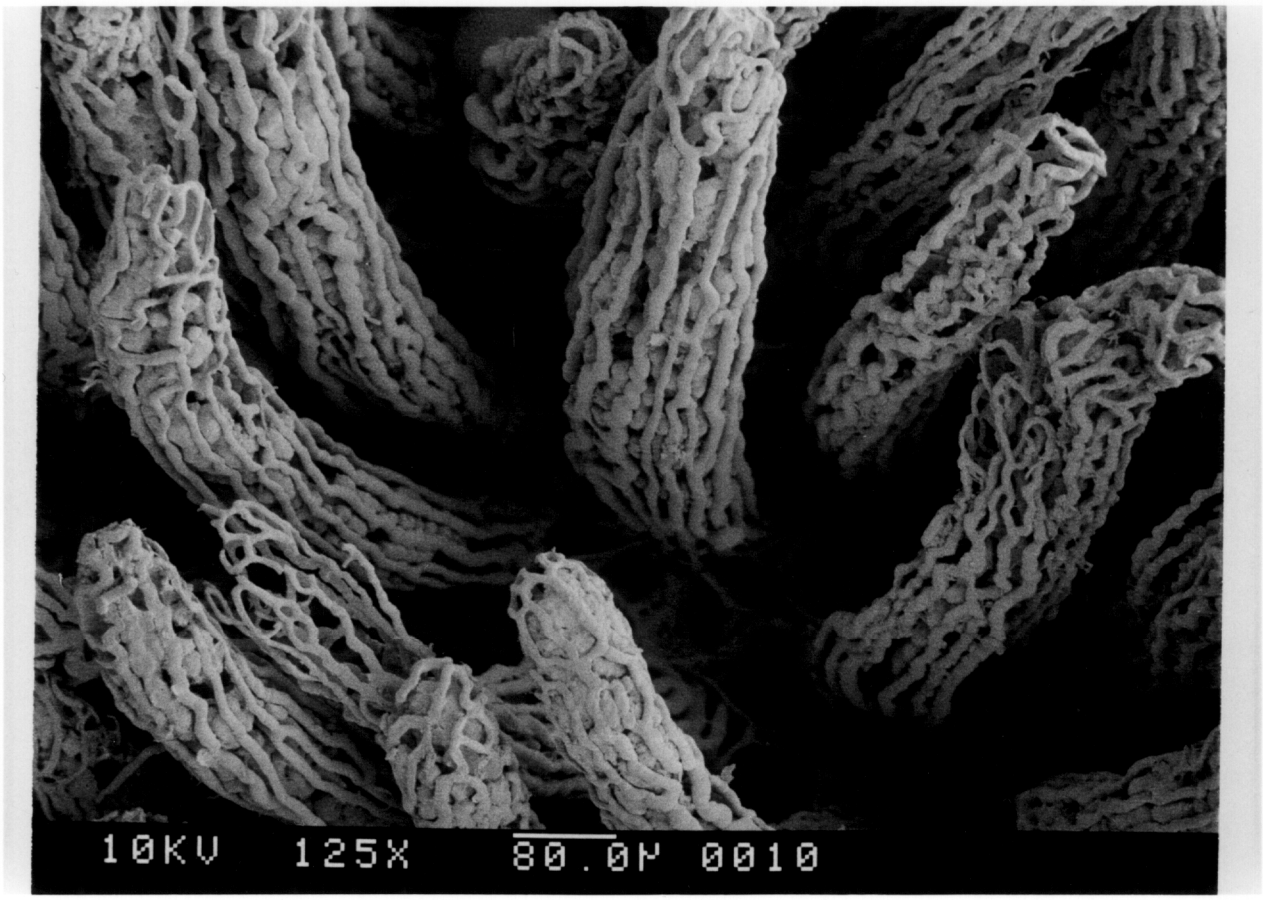

Figure 9 Vascular replica after decompression and reperfusion of the distended segment where the villi have returned to a pre-distention state.

SUBSCRIPTS

a. Japan Vilene Hospital and Ink, Toyoko, Japan
b. Ted Pella Inc., Tustin, Calif.
c. Japan Vilene Hospital and Ink, Toyoko, Japan
d. Hewlett Packard, Mcmimville, Ore.
e. Eastman Kodak, Rochester, NY

Table 1: Comparison of all microangiographs between experimental and control segments.

	Full-thickness sections	Submucosa-mucosa flat sections	Muscularis-serosa flat sections	Cross-sectionss
Distended compared to control segments	6/6 decreased * vascular density	6/6 decreased vascular density	6/6 decreased vascular density	6/6 decreased vascular density to all layers
Reperfused compared to control segments	4/6 decreased vascular density; 2/6 equal vascular density	3/3 decreased vascular density; 3/3 equal vascular density	4/6 decreased vascular density; 2/6 equal vascular density	4/6 decreased vascular density; 2/6 equal vascular density
Distended compared to reperfused segments	6/6 decreased vascular density	6/6 decreased vascular density	6/6 decreased vascular density	6/6 decreased vascular density

* Vascular density defined as the number of perfused vessels observed.

CONCLUSIONS

An intra-abdominal intraluminal pressure of 25 cm H_2O for 120 minutes resulted in altered intramural vascular patterns in the equine small intestine. Decreased vascular filling in the seromuscular layer was evident by microangiography and methylmethacrylate injection techniques. The most severe microvascular injury occurred in the longitudinal muscle layer and serosa. Submucosal vessels were congested and, although the mucosal vascular structures were evident, there was a decreased vascular density when compared to control segment microangiographs. Histologic examination demonstrated mucosal edema with serosal mesothelial cell loss, edema and neutrophil infiltration.

Reperfusion for 60 minutes resulted in an increased number of observable vessels in all bowel layers; however, the vascular density did not attain the pre-distention state and mucosal and serosal damage progresed.

It appears that increased intraluminal pressure causes ischemic injury to the seromuscular layer of the equine small intestine with a lesser insult to the mucosal epithelium. Decompression results in progressive tissue damage and inflammation despite increased vascular perfusion which

probably is a reperfusion injury mechanism.

The intraluminal distention reduced serosal perfusion which is in agreement with the experimental hypothesis. This serosal ischemia probably contributes to post-operative complications such as continued tissue injury, ileus and adhesion formation often seen following the surgical relief of small intestinal obstructions in the horse.

Further studies are needed to predict bowel viability and survival when distended small intestine is encountered during abdominal surgery. A clinical study measuring the intraluminal pressure of distended small intestine and correlating luminal pressure with histologic appearance and seromuscular layer vascularity would assist the surgeon in predicting horse survival.

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