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THE EFFECTS OF ISCHEMIA - REPERFUSION AND DIMETHYL SULFOXIDE ON THE EQUINE JEJUNUM

By

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ABSTRACT

THE EFFECTS OF ISCHEMIA - REPERFUSION AND DIMETHYL SULFOXIDE ON EQUINE JEJUNUM

By

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The effects of ischemia and reperfusion, with and without dimethyl sulfoxied (DMSO) pretreatment (1gm/kg.bwtIV), on intestinal vascular resistance(R), oxygen consumption(VO_2), motility, wall compliance(C), arteriovenous potassium concentration difference($\Delta AV[K^+]$) and mucosal morphology were determined in eighteen ponies using neurally intact jejunal segments, perfused at constant flow with heparinized blood. Ischemia caused: 1)An increase in R immediately upon reperfusion, then a decrease to levels below pre-ischemic values for the remainder of the study. 2)A decrease in VO_2 during the entire reperfusion period. 3)An increase in amplitude of contractions immediately upon reperfusion. 4)A decrease in frequency of contractions during ischemia and 5)An increase in $\Delta AV[K^+]$ immediately upon reperfusion. DMSO administration prevented the decrease in R during reperfusion. DMSO treatment did not improve the morphologic appearance of the mucosa. Although the action of DMSO in modulating post-ischemia vascular reactivity may warrant further investigation, its failure to maintain tissue oxygen consumption, prevent tissue potassium loss or improve the morphologic appearance of the mucosa, suggest that DMSO was not effective in preventing injury to the equine jejunum caused by one hour of arterial occlusion followed by reperfusion.

This thesis is dedicated to my parents,

Robert Keith and Shirley Jean Arden,

for their love, patience and encouragement.

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LIST OF ABBREVIATIONS

Blood Flow	BF
Intestinal Blood Flow	IBF
Intestinal Vascular Resistance	R
Intestinal Oxygen Consumption	VO_2
Intestinal Arterial - Venous Oxygen Content Difference	AVOX
Potassium Concentration	$[K^+]$
Intestinal Potassium Loss	$\Delta AV[K^+]$
Intraluminal Pressure	ILP
Intestinal Wall Compliance	C
Light Microscopy	LM
Scanning Electron Microscopy	SEM
Transmission Electron Microscopy	TEM
Oxygen Derived Free Radical	ODFR
Dimethyl Sulfoxide	DMSO
Superoxide Dismutase	SOD
Adenosine Triphosphate	ATP
Adenosine Monophosphate	AMP
Xanthine Dehydrogenase	XD
Xanthine Oxidase	XO

I. INTRODUCTION

The acute abdominal crisis remains an important cause of morbidity and mortality in the domesticated horse.[1-4] Although overall mortality rates among cases of abdominal pain attended by veterinarians in the field are relatively low (3%), mortality rates rapidly rise in animals having recurrent pain (28.6%).[1] Of 2,385 horses referred to 12 United States university veterinary hospitals for acute abdominal pain in the period 1982-1984, the overall mortality rate was 38.1%.[2] In those undergoing exploratory laparotomy, mortality was 52.2%.[2]

Although abdominal pain may arise from a variety of mechanical, chemical or thermal stimuli to any visceral organ or the peritoneum[5], the majority of episodes involve bowel and may be grouped into one of four pathophysiologic categories; simple obstruction, strangulating obstruction, non-strangulating infarction and inflammatory.[6,7] In simple obstruction, there is obstruction of the digestive tract without significant compromise of vascular integrity. Obstruction may be partial or complete and physical or functional. In strangulating obstruction, luminal obstruction is combined with variable degrees of compromise of vascular integrity. The obstruction is predominantly physical and usually extraluminal, although theoretically, a functional component exists due to ischemic interruption of neuromuscular mechanisms.[8-12] Nonstrangulating infarction occurs when vascular compromise is not accompanied by physical bowel obstruction. Mesenteric thromboembolism, such as that resulting from parasitism, is most commonly blamed for the disorder[13-15]; however, in some cases physical obstruction of the

major mesenteric vessels is not apparent at necropsy, and some consider that a functional or spastic vascular obstruction may occur.[16,17] In these cases parallels may be drawn with the syndrome of nonocclusive mesenteric infarction seen in man.[18-20] Abdominal pain may also result from primary mucosal, mural or peritoneal inflammation. Common examples include parenchymal abscess, localized peritonitis, enteritis and colitis. Of these four categories, the highest mortality rates are associated with strangulating obstruction and non-strangulating infarction, where damage to intestinal tissue results from severe or prolonged reduction in tissue blood flow. In the study cited above[2], diseases in these two categories accounted for 21.9% of hospital colic admissions and carried a mortality rate of 79.9% and 85.8% respectively. This investigation is directed toward these two clinical groups, where intestinal ischemia is associated with very high mortality.

There exist numerous descriptions of the morphologic and ultrastructural changes that result from controlled jejunal ischemia in the rat, dog and cat[21-39], however such descriptions are limited for the horse.[40-43] Ischemic injury to the intestine causes lesions which progress centrifugally from the mucosal epithelium toward the serosa.[19] The intestinal epithelium not only performs absorptive and secretory functions[44], but forms a vital functional barrier between the body and the intestinal chyme.[21,45-47] Interest in the study of ischemic intestinal injury stems not only from concern for local intestinal viability following regional disruption of blood flow[19], i.e. strangulating obstruction and non-strangulating infarction, but also from the knowledge that in many species general circulatory failure is temporally correlated with

development of generalized intestinal mucosal lesions.[28,45,48,49] This relationship represents a positive feedback situation where in the first instance systemic circulatory inadequacy leads to splanchnic hypoperfusion and intestinal mucosal injury[28,32,49-52] and in the second instance, disruption of mucosal integrity contributes to circulatory deterioration, both by direct liberation of intestinal origin cardiocirculatory depressant mediators[45,46,48,53-56] and by disruption of epithelial barrier function allowing access of toxic materials (eg, endotoxin) from the chyme to the general circulation.[47,57-60] Thus, although the vulnerable mucosa has a high regenerative capacity[23], the ability to limit mucosal injury during regional or generalized splanchnic hypoperfusion has far reaching implications.

Although tissue damage in ischemic states may involve inadequate supply to the tissues of diffusible nutrients other than oxygen and inadequate removal of accumulated products of cellular metabolism[61], traditionally cellular injury and tissue disruption has been predominantly attributed to an inadequate supply of oxygen.[62-79] According to this "anoxic theory" of cellular injury, an inadequate supply of molecular oxygen forces cells to rely on temporary and somewhat inefficient means of energy provision, such as anaerobic glycolysis. If such a state is prolonged, production of high energy intermediates such as adenosine triphosphate (ATP), cannot keep pace with utilization, cellular energy charge falls and endergonic processes responsible for cellular homeostasis slow or cease. Metabolic disruption is exacerbated by accumulation of the by-products of anaerobic metabolism, such as lactic acid, altering optimal cytoplasmic pH.

Cellular membrane function is an early casualty, as failure of sodium-potassium ATPase activity leads to disturbed regulation of cellular sodium, potassium and calcium concentrations and distributions. Inappropriate fluid accumulations occur secondary to ionic shifts. Binding of phorbols of ionized calcium to calmodulin occurs, forming calcium-calmodulin complexes which have been implicated in a number of degenerative processes including mitochondrial toxicity, separation of cell junctions, alteration of microtubules and activation of phospholipases. Terminally, hydrolysis of membrane phospholipids occur, including those in the plasma membrane and those in membranes surrounding intracytoplasmic organelles such as mitochondria, golgi and lysosomes. Liberation of lysosomal hydrolases enhances membrane degredation and autolysis ensues.

Although few researchers doubt that the above sequence of events are important in cellular injury where oxygen deficit results from complete and permanent cessation of blood flow, several observations have led numerous investigators to pose an alternate theory of tissue injury associated with ischemic states. First, many ischemic states are characterized by intermittent or low blood flow (hypoperfusion) rather than permanent cessation of blood flow. Under such circumstances, it has been demonstrated that much of the observed injury occurs during the periods of increased flow (reperfusion).[33,46,80,81] Indeed, in some models, injury after a period of no flow or low flow was not as severe as that occurring after an equivalent period including a shorter duration of no or low flow, followed by a period of reperfusion.[37] Secondly, it has been found that administration of a number of

compounds during or following reduced flow, but prior to reperfusion, can significantly attenuate the tissue injury normally observed. These compounds have in common the ability to inhibit the formation or action of reactive oxygen intermediates, known as oxygen derived free radicals (ODFR).[29-34,36-39,82-89] Although the pathobiology of free radical formation and mechanisms of free radical mediated injury will be covered in more detail in the review to follow, advocates of this "reperfusion theory" of ischemic tissue injury, suggest that deprivation of oxygen from the tissue creates a biochemical environment that is primed for free radical generation upon reintroduction of molecular oxygen. Subsequent tissue damage is a result of free radical interaction with and disruption of cellular membrane lipids and depolymerization of intercellular ground substances.[90] The exciting corollary of the "reperfusion theory" of ischemic tissue injury is that, under some circumstances, therapy may be instituted well after the onset of the initiating pathogenic mechanism, i.e. tissue hypoxia, and yet reduce or remove the deleterious functional and structural consequences.

The "reperfusion theory" of ischemic tissue injury has been investigated in a number of tissues including brain[91-94], myocardium[95-98], kidney[99], skeletal muscle[100], skin[101], and intestine.[29-34,36-39,82-89,102-106] Over the period 1981-1988 Parks, Granger et al[29,34,37,38,82-89,102-105], using a feline model of regional, jejunal hypoperfusion, have been able to document functional and morphologic protection using a number of free radical inhibiting compounds including allopurinol, superoxide dismutase, catalase, manitol, iron chelators, protease inhibitors and dimethyl sulfoxide. The ability of dimethyl sulfoxide to limit

ischemic intestinal injury has also been documented by a number of other investigators utilizing murine and canine models.[85,87] Dimethyl sulfoxide (DMSO) is an aprotic solvent with numerous pharmacologic actions[107], including the ability to irreversibly bind the highly reactive hydroxyl radical ($OH^{\bullet}$).[108] It has for many years been utilized in equine medicine as a topical anti-inflammatory and analgesic drug.[109] It has more recently been employed systemically in horses with acute central neurologic conditions.[109] It is therefore possible that systemic administration of DMSO to horses with confirmed ischemic intestinal disease may limit intestinal tissue injury and, in combination with accepted surgical and medical intervention, decrease the high mortality currently associated with this condition. Thus, as DMSO is widely available, inexpensive and currently used systemically in horses, I chose to investigate the ability of this drug to attenuate the physiologic and morphologic changes induced in equine jejunum by ischemia and reperfusion.

II. GENERAL REVIEW OF LITERATURE

A. PATHOPHYSIOLOGY OF INTESTINAL ISCHEMIA

It has been claimed that man's interest in the intestine and its circulation may be traced back at least 30,000 years, where a cave painting in Lascaux, France depicts red loops of bowel protruding from a bison's abdomen.[110] Man's early concepts of the general circulation placed great importance on the role of the splanchnic circulation. Phlebotomy, the practice of removing blood from the peripheral veins to relieve evil humors from the abdominal and other viscera, developed from the works of Diogenes and Hippocrates describing vascular communications between the veins of the forearm and those of the splanchnic circulation.[110] The Greek theory of sanguification, the process by which blood was formed from food, proposed that blood is formed in the liver, following suction of food from the intestinal circulation through the portal vein. In his essays on vascular anatomy and physiology, Galen (AD 129-199), furthered the theory of sanguification, suggesting the bi-directional flow of portal venous blood depending on nutritional needs.[110] It is interesting to note that the concepts of sanguification persisted until the works of Harvey[111] and Leeuwenhoek[112] in the 17th century, who described the importance of the heart and the circulatory nature of blood flow (Harvey) and the details of microvascular anatomy (Leeuwenhoek). Hales[113], in 1733, perfused dog intestine with water of varying temperatures and developed the concepts of blood flow regulation by vasoconstriction and vasodilation. It is of particular interest to the author to note the work of Poiseuille in 1813[114], who in experiments

perfusing equine jejunum, developed the currently held principles of vascular hydraulics, and the instrument that was to become central to arterial and venous pressure investigation, the mercury manometer. Progress since this time has largely paralleled the development of advanced histologic and physiologic techniques and has included as milestones, advanced descriptions of vascular anatomy[115-118], elucidation of the relationships between intestinal blood flow, oxygen consumption and the functions of secretion and absorption[119-122], the relationship between motility and blood flow[123-125], permeability characteristics of the intestinal microvasculature[126-129], extrinsic control of splanchnic blood flow[7,30,35,130-132], concepts of blood flow autoregulation and autoregulatory escape [133-136], and the role of the intestinal circulation in general circulatory homeostasis.[137-140]

Historically, interest in the pathophysiology of intestinal ischemia stems not so much from an interest in the fate of bowel deprived of its blood supply by regional physical displacement, i.e. strangulating obstruction (eg. volvulus, torsion, intussusception, strangulating hernia) or even regional vascular accidents,(eg. thromboembolism, verminous mesenteric arteritis), but rather from an interest in elucidating the relationships between general circulatory inadequacy and intestinal mucosal lesions. Perhaps the first recognition of this relationship was a report in 1823 by Cumin[141] on the occurrence of hemorrhagic necrosis of the intestinal mucosa in humans with severe burn injury. In the century and a half to follow, such observations were made with increasing frequency, first in association with burn injury[142-145], then hemorrhage and trauma[146-147], cardiac failure[148-150] and enteritis and endotoxemia.[151,152] Indeed,

intestinal lesions became recognized in association with almost any cause of generalized circulatory embarrassment[153-155] or chronic debility.[156-157] The severity of intestinal lesions varied greatly, from incidental necropsy findings involving only the mucosa[145], to fulminant transmural necrosis involving a considerable length of the bowel.[156,158] In the latter case the literature often becomes confusing because of application of diverse terminology including: massive intestinal infarction, intestinal gangrene, postoperative enterocolitis, pseudomembranous enterocolitis, intestinal apoplexy, necrotizing jejunitis and colitis, acute necrotizing enterocolitis, acute hemorrhagic necrosis, endogenous gangrene[156], and most recently non-occlusive mesenteric ischemia/infarction.[154] The common denominator of these conditions was a lack of physical intraluminal obstruction of the mesenteric vasculature, hence the term "non-occlusive." To this list may be added a large number of conditions in which diminished intestinal mucosal blood flow, with or without vascular luminal compromise, is thought to play a role, eg. Staphylococcal and Clostridial enterocolitis, radiation enterocolitis, uremic colitis, potassium-induced stenotic ulcer, stress ulceration[159] and focal mucosal necrosis associated with systemic lupus erythematosis, dermatomyositis, rheumatoid arthritis, malignant atropic papulosis, diabetes, scleroderma[19] and transient ischemic colitis associated with oral contraceptive use.[160]

Whilst the concepts of ischemic intestinal injury resulting from circulatory shock or generalized debility were developing, another group of investigators emerged whose focus was defining the role of circulating humoral factors in the perpetuation of circulatory shock. The earliest reports were

those of Blalock[161] and Katzenstein[162], describing humoral factors resulting from traumatic crush injury and experimental tourniquet application respectively. In 1945, Wiggers and Ingraham[163], attempted from an experimental basis to standardize definitions of hemorrhagic shock and consolidated the concept of "irreversible" shock. They also noted an inverse relationship between the development of post-hypotensive hemorrhagic diarrhea in experimental dogs and survivability. It was not until 1957, however, that Richard Lillehei, in the classic paper "The intestinal factor in irreversible hemorrhagic shock"[164], suggested the importance of the ischemic intestinal lesions formed during systemic hypotension in the perpetuation and "irreversibility" of shock. This laid the ground work for a considerable body of work in the past 30 years documenting the role of intestinal origin biogenic amines, prostenoids, endotoxins, and cardiodepressant peptides in circulatory shock.[47,56,59,60,165,166] Thus, investigators concerned predominantly with the intestinal consequences of shock and debility, and those concerned predominantly with the systemic consequences of intestinal lesions, became united in their desire to elucidate mechanisms in the pathogenesis of ischemic intestinal disease. It should be noted at this point that this historical perspective explains some of the diversity in experimental models used to study intestinal ischemia. Models employed in a variety of species have included total regional occlusion[21] (from small segments of bowel to superior mesenteric artery occlusion), regional hypoperfusion[21,102], regional hypoperfusion plus regional sympathetic stimulation[24], hemorrhagic[164] and endotoxic[50] shock.

The splanchnic circulation receives about 28% of the resting cardiac output and contains 20% of the total blood volume, 65% of this being distributed to the mucosa.[167] It is well established that the earliest structural and functional changes seen in intestine subjected to ischemia are centered upon the mucosa.[21,28,167-169] Here, the earliest changes noted are epithelial loss from the villi beginning at the tips and progressing to the villus base in more advanced lesions.[21] With progression, the lamina propria and eventually muscularis and serosal layers become involved in the inflammatory and necrotizing process.[169] In the latter stages, bacterial invasion and dense leukocyte accumulations are prominent.[169] The apparent selective vulnerability of the mucosa may be related to many factors including its higher metabolic rate and hence nutrient requirements[170,171], the high relative sensitivity of the mucosal vasculature to sympathetic stimulation, and hence diminished flow in regional hypotensive states[167], intraluminal factors[28], mucosal intravascular coagulation[49,172], the architecture of the microvasculature[173] and the localization of enzymes required for free radical generation.[174,175] As mucosal integrity has been shown to play a vital barrier function between intestinal chyme, deeper intestinal layers and the intestinal circulation[47,57-60] and, as there is little doubt that transmural infarction necessitates early surgical resection rather than medical management alone[176], most investigations have centered on the pathogenesis of the early mucosal lesions.

The exact mechanism by which enterocytes are shed from ischemic mucosal villi has been the subject of considerable investigation and debate. Traditionally it was held that enterocyte lactate accumulation and the

resultant drop in intracytoplasmic pH produced lysosomal disruption with liberation of acid hydrolases, which subsequently disrupted plasma membrane integrity.[60,168] Black-Schaffer et al[158] suggested that ischemic depression of enterocyte membrane function was sufficient to allow overhydration of enterocytes leading to osmotic explosion with fragmentation and loss into the lumen. Since 1963, Bounous, in a series of publications[28,177-187], has developed the theory of "trypic enteritis." The theory essentially states that during low flow, hypoxic conditions, the energy depleted enterocytes cannot maintain luminal brush border glycoprotein production. The action of pancreatic elastase and bile salts in removing border glycoproteins in the face of decreased production renders the underlying structures accessible to the digestive action of pancreatic endopeptidases, including trypsin.

None of these theories are consistent with the histologic findings of Chiu[21], Brown[22], and Wagner[26] that ischemic villus injury is characterized by lifting of sheets of intact enterocytes away from the basement membrane and lamina propria, a process which progresses from the villus tip to the base. These groups propose that the basic lesion is the interposition of accumulated fluid between the enterocyte and the basement membrane and lamina propria. This fluid accumulation at the villus tip had for some time been recognized by microscopists as Gruenhagen's space.[21] Brown et al[22] extended this proposal to suggest that the fluid wedge not only acts to mechanically separate the epithelium from the lamina propria, but adds insult to the injury of hypoperfusion by decreasing the diffusion of oxygen and nutrients from capillaries to the enterocytes. Both Brown[22] and Wagner[26] have suggested that

the fluid arises from expulsion of cytoplasmic blebs of unwanted water from the enterocytes at their basilar portions. Wagner[26] contends that this is a preservative mechanism that cells employ in energy deficient states where the rapid fall in cellular ATP disables the sodium-potassium pump. An alternative mechanism may involve failure to remove fluids normally transported inward by the enterocyte due to lymphatic obstruction and capillary stasis.[21] A third proposal is that much of this fluid may arise from injured villus capillaries. This was originally suggested to reflect increased capillary permeability caused by endothelial anoxia[21,35,173], but has more recently been ascribed to the action of oxygen derived free radicals on intestinal capillary endothelia and basement membranes.[29,34,37,38,88,102-105] Indeed, the free radical mechanism of membrane damage may be extended to the enterocyte plasma membrane and basement membrane themselves, and thus may play a role in epithelial lifting and fluid accumulation, whether it be of enterocyte or capillary origin.

Whether one ascribes to the lysosomal disruption, osmotic explosion, tryptic digestion or subepithelial fluid accumulation theories of early mucosal injury, it is clear that currently most investigators attempt to explain their findings by reliance upon one of two basic mechanisms of ischemic injury. The first of these is the traditional anoxic theory, where falling cellular (predominantly epithelial and endothelial) energy charge results in membrane destabilization and eventual disruption. The second is the reperfusion theory, whereupon tissue disruption results from membrane lipid peroxidation and ground substance denaturation induced by oxygen derived free radicals. This latter theory will be discussed in more detail in the second part of this review.

Investigation of the responses of the equine jejunum to ischemia have been largely limited to the past 20 years. In 1968, Nelson and Collier[188] examined the systemic cardiovascular, hematologic and biochemical changes in horses with experimental colonic infarction, and compared the response in untreated horses to those treated with oral neomycin, repetitive endotoxin challenge and Clostridium perfringens toxoid. Typical histological lesions, progressing from the epithelium toward the serosa were briefly described. No attenuation of mucosal injury by treatments was described; however, oral antibiotic treatment was claimed to attenuate systemic changes. Hjortkjaer and Svendsen[40] studied the systemic responses in 7 horses, 4 of which had experimental intestinal volvulus simulated by mesenteric venous ligation combined with luminal occlusion of 17 hours duration. In all cases, the descriptions were consistent with transmural infarction, the mucosa being described as "black, edematous and swollen." White et al[41] examined jejunal mucosal morphology in 5 ponies subjected to 30-180 minutes of experimental strangulation obstruction, induced by mesenteric arterial and venous ligation and luminal obstruction. Their findings confirmed the character and development of villus lesions as described in dogs by Chiu[21] and the progression of structural damage following ligature removal. Sullins et al[42] differentiated between ischemic (arteriovenous occlusion) and hemorrhagic (venous occlusion alone) models of strangulation obstruction and suggested that hemorrhagic occlusion models more closely approximate the clinical situation of strangulating obstruction where mesenteric pressure is more likely to cause venous than arterial compression. With the exception that venous occlusion

caused increased congestion, edema and hemorrhage throughout the bowel wall, mucosal lesions were graded the same after 1 to 3 hours of either form of occlusion.

In a histologic assessment of the gastrointestinal lesions from 30 horses with a naturally occurring colic, Meschter el al[7] described mucosal lesions similar to those resulting from experimentally induced ischemia, not only at the primary lesion site, but also at sites proximal and distal to the primary lesion. The changes reported included epithelial sloughing, diffuse multifocal subsurface aggregates of necrotic debris, subepithelial cleft formation, vascular engorgement and neutrophilic infiltration. Although splanchnic ischemia and/or endotoxemia was suggested as causing these secondary lesions, their origin was not concluded. This study also suggested a correlation between histologic mucosal lesion severity and survivability. This latter finding was reinforced by the study of Allen, White and Tyler[189], where correlation was also found between mucosal lesion severity, intraluminal hydrostatic pressure, peritoneal fluid protein concentration and survivability in 20 horses with naturally occurring obstructive small intestinal disease. In a subsequent study, Allen et al[190] was unable to correlate the mucosal lesions found in distended bowel anterior to the primary lesion with the presence of increased intraluminal hydrostatic pressure.

Freeman et al[43] used venous and arterial-venous occlusion models similar to those of Sullins et al[42] to examine mucosal healing and chronic changes in equine jejunum following 2 and 3 hours of vascular occlusion. Their data suggests that in both models, epithelial regeneration is well progressed by 12

hours post-ischemia. Chronic sequella of both forms of injury were minimal, and limited to mesenteric contraction in the venous occlusion group.

Morphologic changes in equine large colon subjected to experimental ischemia have recently been described by Snyder, Orlander and Pascoe el al.[191] Using both arterial-venous and venous occlusion models, they described mucosal changes that were characterized by epithelial cellular necrosis prior to sloughing. These findings differ considerably from that reported for small intestinal lesions in the horse and other species.

There has been very little work on therapeutic intervention directed at limiting early mucosal lesions in ischemic equine bowel. Nelson et al[188] suggested that oral antibiotic adminstration may ameliorate some of the systemic consequences of colonic infarction, but histologic improvement was not noted. This therapy has not gained favor in the past 20 years. Moore, White and Trim et al[192] found that administration of intraluminal oxygen prior to reperfusion helped to preserve mucosal integrity in ponies with experimental strangulation obstruction. In the past 8 years, however, this has not become a common practice perhaps because of technical limitations. Parker, Fubini and Carr[193] evaluated the use of low dose heparin therapy for prevention of intraabdominal adhesions in ponies with ischemic intestinal lesions. Although their report suggested a beneficial effect, the number of experimental animals involved were rather limited. To date there has been no investigation of the role of oxygen derived free radicals in the pathogenesis of ischemic intestinal injury in the horse.

B. PATHOBIOLOGY OF OXYGEN DERIVED FREE RADICAL MEDIATED TISSUE INJURY

It was about 500 years ago that Leonardo da Vinci observed that only part of air is consumed during combustion and respiration[194]; however, it was only about 200 years ago that Lavoisier recognized oxygen as an element and its necessity for respiration.[194] Since that time, oxygen has generally come to be held as an element central to the survival of animal species. Only relatively recently has the potential "toxicity" of oxygen been recognized in biologic processes. Elemental or molecular oxygen exists naturally as 2 atoms (O_2) which possess interesting physical attributes. Molecular oxygen possesses 2 unpaired electrons, of parallel spin in its outer orbits. This configuration tends to "seek" electrons and thus, thermodynamically, molecular oxygen tends to act as an electron acceptor, i.e. oxidizing agent. Kinetically, however, molecular oxygen is slow to react in spontaneous oxidizing reactions, as one of its outer electrons must undergo spin reversal before divalent (2 electron) or quadravalent (4 electron) oxidation can occur.[195] This spin restriction, however, makes oxygen suitable for enzymatic reduction, as in those reactions, time for spin reversal can occur.[195] The electron seeking (oxidizing) character of molecular oxygen is harnessed as the thermodynamic "pull" behind mitrochondrial electron transfer and coupled reactions (eg. ATP production), which are the focus of cellular aerobic energy provision.[195]

It has been hypothesized, however, that the "beneficial" effect of oxygen is the result of evolutionary adaption to the "toxicity" of oxygen. The earliest life forms resulting from the interactions of hydrogen, ammonia, methane,

water and radiation were presumably anaerobic organisms.[196] The development of blue-green algae led to the release of molecular oxygen into the biosphere.[197,198] The presence of oxygen tends to oxidize carbon to carbon dioxide, hydrogen to water, and nitrogen to nitrous oxide[197] and increasing concentrations of atmospheric oxygen applied severe evolutionary pressure to organisms dependent upon anaerobic photoreduction and fermentation to evolve mechanisms for the biological control of oxygen.[197] The mechanism so evolved, is the tetravalent reduction of O_2 to H_2O[197,199], i.e. the mitochondrial cytochrome chain. It is thus speculated that the cytochrome system might have evolved as a mechanism for oxygen detoxification, to later further evolve and be exploited for its efficient production of energy from metabolism of scarce organic substrates.[196,198,199]

A category of related molecular species are the oxygen derived free radicals. By definition a "free radical" is any atom, group of atoms or molecule with one unpaired electron occupying an outer orbit.[197] This configuration leads to high reactivity as there is a strong tendency to gain (oxidize) or lose (reduce) electrons from or to another molecule. Thus, by definition, molecular oxygen itself may be described as a "bi-radical", because of its two unpaired outer electrons. Free radicals derived from oxygen include the superoxide radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), the hydroxyl radical ($OH^{\cdot}$) and singlet oxygen (O_2^1).[197] The latter is a form of molecular oxygen where one of the outer electrons has been raised to a higher and thus more reactive energy state. The involvement of free radicals in biological systems was first described by Haber and Willstatter in 1931[200,201] observing the oxidation of

organic compounds and enzymes by hydroxyl radicals. In the early 1960s singlet oxygen was recognized[202] and its role in photosensitized oxidations discovered.[203] It was not until the late '60s, however, that McCord and Fridovich discovered the production of the superoxide free radical in biological reactions[204] and described the enzymatic role of superoxide dismutase (SOD).[205] The latter discovery, in particular, led to the concept that oxygen derived free radicals were regularly produced in biologic processes and that evolution had led to development of a number of cellular mechanisms to "detoxify" these potentially damaging species. If we return to the concept that molecular oxygen is safely handled by its tetravalent reduction by the mitochondral cytochrome system, Fridovich[206] recognized that up to 5% of the oxygen consumed escapes tetravalent reduction and undergoes sequential univalent reduction, with concommitant production of the oxygen derived free radicals $O_2^{\cdot-}$, $OH^{\cdot}$, H_2O_2. Indeed, the protective cellular enzymes SOD, catalase and some peroxidases, probably developed to protect the cell from this "univalent leak."[197,198,206] Following the discoveries of McCord and Fridovich in the late 1960s[204,205], there was an explosion of interest in the roles of oxygen derived free radicals in biologic and, indeed, pathologic processes. Work in the 1970s particularly emphasized their role in phagocyte bactericidal activity[207,208], phagocyte mediated inflammatory reactions[209,210], mechanisms of radiobiological damage[211] and pulmonary oxygen toxicity. [212] McCord has estimated that in the period 1969-1983 some 6,000 published reports dealt with the cytotoxicity of the superoxide radical.[195]

In general, oxygen derived free radicals (ODFR) directly mediate tissue damage, both at extracellular and intracellular locations. Extracellularly, glycosaminoglycans, including hyaluronic acid, which form part of basement membranes, intercellular matrices and specialized fluids such as the vitreous and synovial fluid, have been shown to undergo degradation when exposed to radical generating systems.[197] Such degradation has been termed oxidative-reductive depolymerization.[197] Collagen has also been shown to undergo structural alteration when exposed to similar systems.[213] The major destructive effects however, are cellular and involve biomembranes. Peroxidations of polyunsaturated fatty acids (PUFA) within biomembranes results in a chain reaction effect, with sequential PUFA peroxidation and membrane destabilization.[197] Both the plasmalemma and membranes enveloping cellular organelles, such as lysosomes and mitochondria, may be involved. Lastly, DNA damaged by radical flux has been demonstrated[214] and this, as well as the action of lipid peroxidation products, may play a role in carcinogenesis.[197] In addition to primary actions of ODFR, many secondary actions may lead to tissue damage. Among the many documented are release of lysosomal hydrolases[198], leukocyte chemotaxis[215], arachadonic acid release via alteration of phospholipase activity[197] and stimulation of mast cell histamine release.[216]

Our knowledge of the role of ODFR in ischemia-reperfusion injury has come predominantly from studies conducted in this decade, many of them in intestinal ischemia models. It has been known for some time that tissue damage was often more pronounced during the reintroduction of blood flow

rather than during the ischemic phase per se.[197,217] Thus, the terms "post-ischemic tissue injury" and "reperfusion injury" came to be used.[217] In 1980[197], DelMaestro postulated that post-ischemic injury may represent an increase in "univalent leak" during incomplete ischemia, coupled with decreased oxy-radical scavenging mechanisms. In 1981, Granger, Rutili and McCord[218], utilizing determination of osmotic reflection coefficient in a feline intestinal ischemia model, demonstrated the involvement of the superoxide radical in ischemic injury. Osmotic reflection coefficient, an index of microvascular permeability, had already been utilized as an indicator of early tissue injury.[219-221] In their model, pretreatment with superoxide dismutase (SOD), but not with benadryl and cimetidine, indomethacin or methylprednisolone, significantly attenuated the permeability increase induced by ischemia. In a further study[29], pretreatment with SOD or allopurinol were able to attenuate morphologic changes in feline intestine subjected to hypoperfusion. Further, they proposed a biochemical cascade to explain the role of free radical mediated ischemia-reperfusion injury. According to this theory, low tissue oxygen availability and hence, cellular energy charge, precipitated two biochemical conversions. The first was the catabolism of adenosine triphosphate (ATP) to adenosine monophosphate (AMP) and then adenosine, inosine, and hypoxanthine, i.e. the normal pathway for purine catabolism during energy deprivation. The second was the conversion of the cellular enzyme xanthine dehydrogenase (XD) to xanthine oxidase (XO). It is the action of XD upon hypoxanthine and xanthine which form uric acid, the usual excretory product of purine metabolism. However, the action of XO upon

hypoxanthine, in the presence of molecular oxygen, leads to a different reaction, and ODFR formation. Thus, it was suggested that low oxygen availability leads to cellular hypoxanthine and XO accumulation, whilst the reintroduction of oxygen during reperfusion triggers the sudden generation of ODFR. They were able to piece together this theory based on knowledge that 1) xanthine oxidase had been the first documented biologic source of superoxide[204], 2) intestine and liver were known to be the richest sources of xanthine oxidase[222,223], 3) conversion of xanthine dehydrogenase to xanthine oxidase had been documented in vitro under conditions of low oxygen tension or proteolysis.[222,223], 4) DelMaestro[224] had already demonstrated superoxide induced vascular leakage following application of xanthine oxidase and hypoxanthine to a hamster cheek pouch preparation, and 5) the drug allopurinol, a specific xanthine oxidase inhibitor, had been shown to provide protection in models of hemorrhagic shock[225] and myocardial ischemia.[226] It is interesting to note that the protective effects of allopurinol had previously been attributed to its ability to inhibit purine base loss from energy poor cells by xanthine oxidase inhibition.[222,223] It remained to be shown that conversion of xanthine dehydrogenase to xanthine oxidase (D→O conversion) occurred in vivo triggered by ischemia. McCord and Roy were able to provide this information in 1982[217] by assaying for XD/XO in rat ileal tissue frozen in liquid nitrogen at varying intervals following excision. Using this method, they demonstrated that D→O conversion occurred very rapidly, the majority within 5 to 10 seconds of excision and almost complete conversion within 40 seconds. The irreversibility of the D→O conversion by thiol reductants

suggested proteolytic conversion. The time course was probably too short for proteases derived from lysosomal disruption and as rapid calcium influx associated with cellular energy deprivation was known to occur[227], calcium modulated protease activity was suggested.[217] More recently, Parks et al[228] have been able to demonstrate that treatment with protease inhibitors attenuate vascular permeability increases and mucosal lesions associated with feline intestinal ischemia. Further work of this group has also focused on identifying the radical species most involved with ischemic intestinal injury. Both DMSO[82], a hydroxyl radical scavenger and iron chelators deferoxamine and apotransferrin[229], which prevent iron catalyzed hydroxyl radical production (Haber-Weiss reaction), prevent ischemia produced microvascular permeability changes. Thus, the hydroxyl radical has been implicated as the principle pathogenic species. A diagramatic summary of the biochemical processes leading to ODFR mediated ischemia-reperfusion injury, and potential therapeutic interventions is presented in Figure 1.

Although the pioneering work of McCord, Granger and Parks in elucidating the role of ODFR in ischemic processes has been reinforced by a large number of investigators working on intestine[30,33,36,39,83-85,87,88,106], muscle[100], heart[95-98], kidney[99], brain[91-94], and skin[101] models of ischemic injury, their theories are by no means universally accepted. Among the greatest proponents of the "anoxic theory" of ischemic intestinal injury are the group of Lundgren, Haglund et al.[35,41] During intestinal low flow states, it is argued, total villus blood flow is largely unchanged.[230,231] Increased blood transit time allows extravascular shunting of oxygen via the villus counter-current exchanger[232],

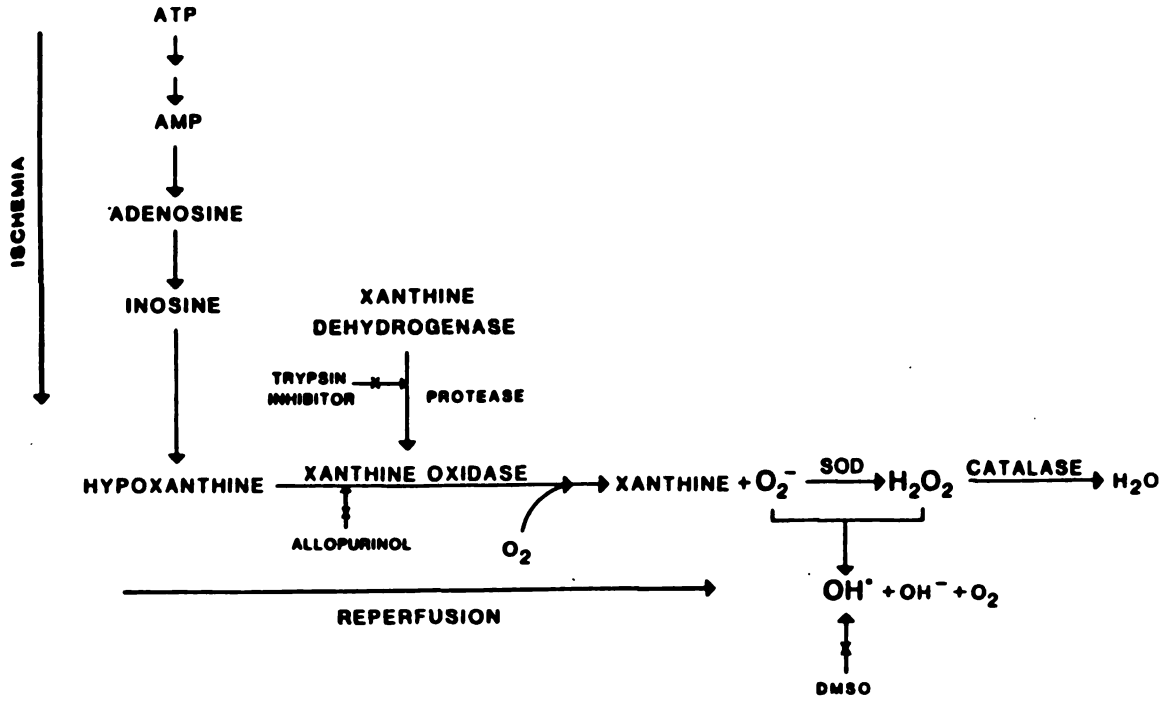

Figure 1. Proposed biochemical cascade for production of oxygen derived free radicals in ischemia-reperfusion injury, and potential points of therapeutic intervention. (From Granger D.N. et al [38])

making the tip relatively hypoxic and thus explaining the progression of intestinal lesions from the villus tip toward the base.[35] This group maintains that the most important tissue damage occurs during the ischemic phase[35] and vigorous debate within the literature continues.[35,37,38,41,169]

The specific objectives of the investigation here undertaken were; 1. To establish a model of equine jejunal ischemia-reperfusion injury, 2. To examine the effects of ischemia and reperfusion on equine jejunal vascular resistance, oxygen consumption, motility, tonus (compliance) and potassium loss, 3. To extend current knowledge regarding the morphologic and ultrastructural changes in equine jejunum following ischemia and reperfusion and 4. To determine the ability of a systemically administered hydroxyl radical scavenging agent, dimethyl sulfoxide, to attenuate the above physiologic and pathologic changed induced in equine jejunum by ischemia and reperfusion.

III. EFFECTS OF ISCHEMIA AND DIMETHYL SULFOXIDE ON EQUINE JEJUNAL VASCULAR RESISTANCE, OXYGEN CONSUMPTION, INTRALUMINAL PRESSURE AND POTASSIUM LOSS.

A. MATERIALS AND METHODS

Experimental Preparation: Eighteen healthy, mixed-breed ponies were studied. All ponies were vaccinated against equine influenza, encephalomyelitis and tetanus,[a] treated with an anthelmintic (pyrantel pamoate, 6 mg/kg of body weight, per os),[b] and fed a diet of mixed hay and oats for one month prior to beginning the study. Food but not water was withheld for 12 hours prior to beginning the study. All experiments were terminal and conducted under general anesthesia. Ponies were anesthetized with an intravenous bolus of 10% thiamylal[c] administered to effect (6.6-11 mg/kg), placed in dorsal recumbency and mechanically ventilated[d] with 100% O_2 and halothane.[e] Arterial blood gas tensions[f] were measured hourly to permit adjustments in ventilation and ensure normal blood pH, $PaCO_2$ and hemoglobin saturation throughout the procedure. Lactated Ringer's solution was administered intravenously at 10 ml/kg/hr. The facial artery was

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[a]Envac FC-4 with Havlogen, Haver-Lockhart Laboratories, Shawnee, Kan.

[b]Strongid Paste, Pfizer Inc., New York, NY.

[c]Bio-tal, Boehringer-Ingelheim Animal Health Inc., St. Joseph, Mo.

[d]Mark 9 Aereo Respirator and Mark 4 Anesthesia Assistor/Controller, Bird Corporation, Palm Springs, Calif.

[e]Fluothane, Ayerst Laboratories Co., Cleveland, Ohio.

[f]Radiometer (ABL-1); The London Co., Cleveland, Ohio.

cannulated (PE-240 tubing) and connected to a pressure transducer[g] and a four channel direct writing oscillograph[h] to continuously measure mean arterial blood pressure (MAP, mmHg). Through a ventral midline laparotomy, a 10-15 cm segment of distal jejunum, supplied by a single artery and drained by a single vein was isolated, such that its mesentery and neural supply remained intact. Both ends of the segment were tied and divided from adjacent segments to exclude collateral blood flow. Following administration of heparin[i] (500 IU/kg/hr IV), an extracorporeal circuit was established between the femoral artery and the single artery perfusing the intestinal segment. This was achieved by interposition of a pulsatile pump[j] between the above arteries using PE-360 tubing and a 14-gauge needle (Figure 2). A 20-gauge needle, which was connected to PE-90 tubing and a pressure transducer[g], was inserted into the perfusion artery catheter to monitor perfusion pressure continuously. By regulating the pulsatile pump, blood flow was adjusted until the perfusion pressure equalled the systemic arterial pressure. Blood flow(BF) was held constant throughout the experiment, and flow determined at the end of each experiment, using a graduated cyclinder and stopwatch. Following evacuation of the luminal contents, a thin rubber balloon connected to a rubber tube was secured within the segment lumen, partially filled with 37° C

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[g]Model P-2306, Statham Instruments Inc., Cleveland, Ohio.

[h]Model SP-2000, Gould Inc., Cleveland, Ohio.

[i]Hypo-Med Inc., Chicago, Ill.

[j]Model T8SH, Sigmamotor Inc, Middleport, NY.

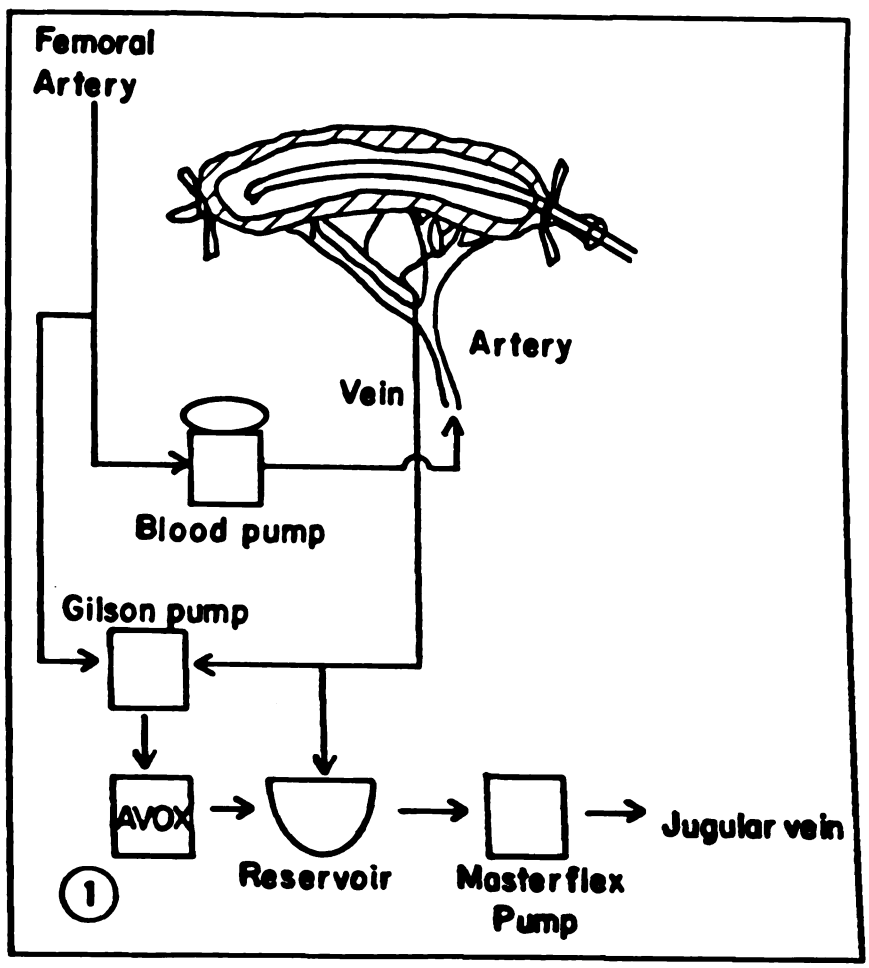

Figure 2. Schematic drawing of the intraluminal balloon and extracorporeal circuits for measuring intestinal vascular resistance, motility, and arteriovenous oxygen content difference under constant blood flow conditions. AVOX=arteriovenous oxygen content analyzer.

water and connected to a pressure transducer[g] by a 3-way stopcock to continuously record changes in intraluminal pressure (ILP, mmHg). A thermocouple was placed within the bowel wall and a heat lamp adjusted such that the bowel temperature remained at 37°C. The bowel was moistened with saline and covered with a plastic film. Paired arterial and venous blood from the bowel segment was collected into 10 cc serum tubes, centrifuged and the serum assayed for potassium content by an ionspecific electrode technique.[k] The intestinal segment was trimmed of mesentery and weight (K, gm) recorded.[l]

Intestinal blood flow (IBF) was calculated by normalizing flow to 100 gm of tissue: IBF (ml/min/100 gm) = BF.100 / K. Intestinal vascular resistance (R) was calculated by dividing perfusion pressure (P) by intestinal blood flow (IBF): R (mmHg/ml/min/100 gm) = P / IBF. Oxygen consumption (VO_2) was calculated as the product of oxygen content difference ($\Delta AVO2$) and intestinal blood flow (IBF): VO_2 (ml/min/100 gm) = ΔAVO_2.IBF. Intestinal motility was assessed as frequency and amplitude of rhythmic (2-5 seconds duration) changes in luminal pressure. To better assess bowel tone, intestinal wall compliance (C) was determined by addition of three 60 ml increments of warm (37°C) saline to the luminal balloon via the stopcock. Intestinal compliance (C) was calculated by dividing the highest volume introduced into the balloon (180 ml) by the difference between intraluminal pressures at 0 and 180 ml balloon volume: C(ml/mmHg) = ΔV / ΔILP where V=180 ml.

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[k]Beckman System E4A; Bechman Instruments Inc., Clinical Instruments Division, Brea, Calif.

[l]Mettler p163; Mettler Instrument Corp., Hightstown, NJ.

Arteriovenous potassium concentration difference ($\Delta AV[K^+]$, mEq/L) was determined at the end of the baseline recording phase, immediately upon reperfusion and at 30 and 60 minutes of reperfusion. In the groups subject to ischemia (III and IV), a series of fifteen consecutive paired 10 cc aliquots were collected immediately upon reperfusion, so that a tissue K^+ washout curve could be generated. The total quantity of K^+ lost into the first 150 ml of reperfusion blood was used to calculate a potassium loss index: K^+ lost mEq/100 gm = $\Delta AV[K^+]_{1\text{-}15}$.

Experimental Design: The 18 ponies were divided into four groups: Group I, Control (n=3); Group II, DMSO (n=3); Group III, Ischemia (n=6); Group IV, Ischemia, DMSO (n=6). In all four groups, the measured variables were allowed to stabilize following instrumentation. This was followed by a 30-minute recording phase to establish steady baseline values (baseline phase). In Groups I and II perfusion continued uninterrupted for a further 120 minutes. In Groups III and IV, flow was completely halted for 60 minutes by turning off all pumps (ischemia phase). Flow was then reintroduced at the previous rate for a further 60 minutes (reperfusion phase). In Groups II and IV dimethyl sulfoxide[m] (1 gm/kg body weight) diluted in 1 L of lactated Ringer's solution was administered intrajugularly over 10 minutes immediately prior to the reperfusion period. Groups I and III received an equal volume of lactated Ringer's.

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[m]Methyl sulfoxide, 99+%; Aldrich Chemical Co., Inc., Milwaukee, Wis.

In all 4 groups, MAP and ILP were recorded throughout the 150 minute protocol. R and ΔAVO_2 were recorded in all phases in Groups I and II and during baseline and reperfusion phases in Groups III and IV. Compliance (C) measurements were made 15 minutes into the baseline phase and at 30 minute intervals thereafter. Potassium samples were collected as described above.

Analysis of Data: Changes in R, VO_2, frequency and amplitude of rhythmic contractions, C and $\Delta AV[K^+]$ were compared to baseline values within groups by a repeated measures analysis of variance, with differences between means evaluated by the Student-Newman-Keuls procedure ($p < 0.05$). Regression of tissue potassium loss against preischemic tissue oxygen consumption was analyzed by the least squares regression analysis.

B. RESULTS

In Groups I and II there were no significant changes in R, VO_2, C, $\Delta AV[K^+]$, amplitude or frequency of rhythmic contractions over time. In Group III, R was significantly increased immediately upon reperfusion ($p < 0.01$) and had returned to baseline value within 5 minutes. By 15 minutes of reperfusion, R had fallen to a level significantly below baseline and continued to fall for the remainder of the reperfusion period. A similar significant rise in R immediately upon reperfusion was seen in Group IV. Within 5 minutes, however, R had returned to baseline values and did not significantly change during the remainder of reperfusion (Figure 3). In Group III, VO_2 was significantly lower ($p < 0.01$) than baseline by 5 minutes of reperfusion. VO_2 continued to decline during the reperfusion period. Similarly, in Group IV,

VO_2 was significant below baseline from 5 minutes of reperfusion onward (Figure 4). In Groups III and IV, frequency of contractions steadily declined throughout ischemia to reach values significantly below baseline by 30 minutes in Group III ($p < 0.01$) and 45 minutes in Group IV ($p < 0.05$). Within 5 minutes of reperfusion, however, frequencies had returned to baseline values in both groups (Figure 5). In Group III and IV, there was an increase in amplitude of rhythmic contractions immediately upon induction of ischemia and reperfusion. In Group III this was significant only at reperfusion, but in Group IV was significant at both time periods. In each case this was a short-term effect lasting approximately 3-4 minutes (Figure 6). There were no significant changes in intestinal wall compliance over time in any group. In both Groups III and IV $\Delta AV[K^+]$ immediately upon reperfusion was significantly higher than baseline values. The first reperfusion value (R0) represents the third reperfusion aliquot, which was usually the peak $\Delta AV[K^+]$. Subsequent reperfusion values (R30, R60) were not significantly different from baseline (Figure 7). For ponies in Groups III and IV potassium reperfusion washout curves were constructed as described above, allowing generation of an index of intestinal tissue potassium loss ($\Delta AV[K^+]^{1\text{-}15}$). As the patterns of loss were not different between groups (Figure 7), the two groups were combined and potassium lost analyzed against baseline VO_2 for each intestinal segment. There was significant regression of $\Delta AV[K^+]_{1\text{-}15}$ upon VO_2 (base) (Figure 8). Mean and standard error values for R, VO_2, frequency and amplitude of rhythmic contractions, C and $\Delta AV[K^+]$ are presented in the appendix [Tables 3-8].

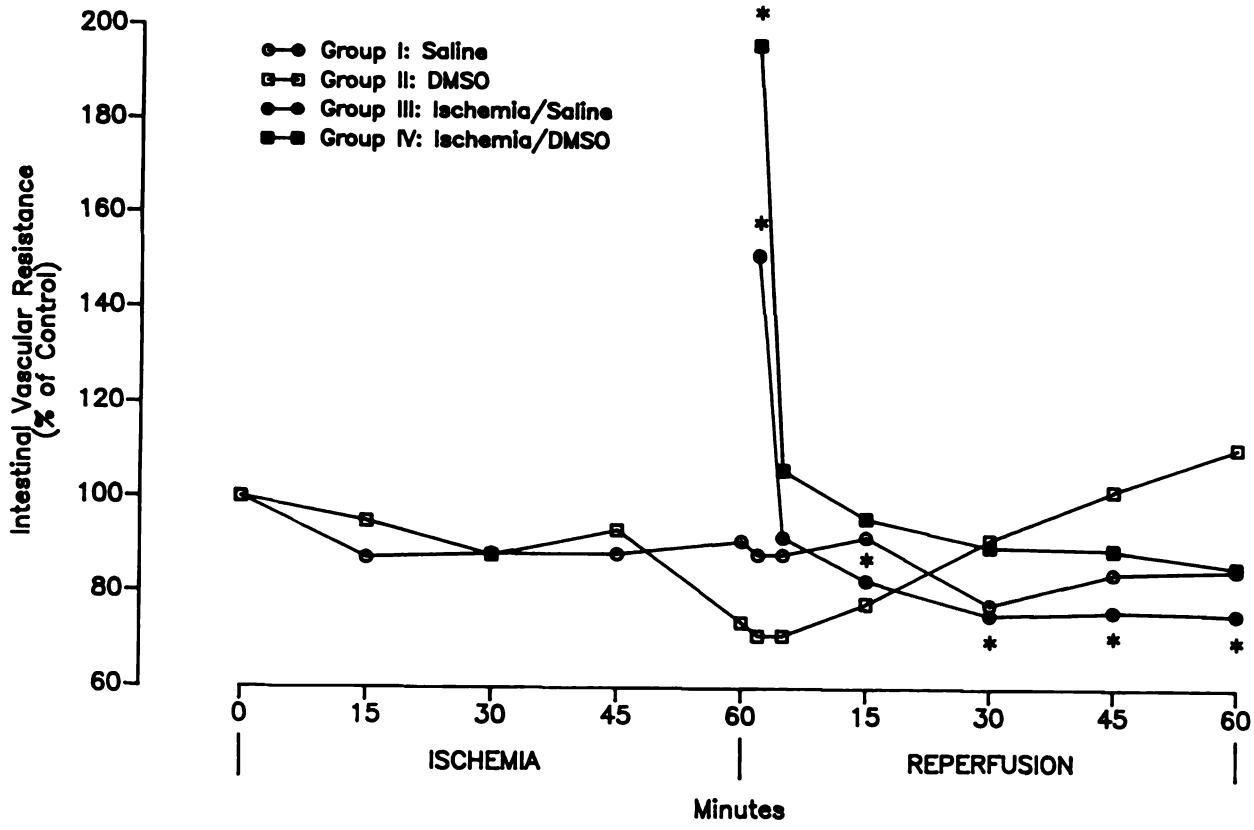

Figure 3. Changes in Intestinal Vascular Resistance (R) over time. R is expressed as a percentage of control (preischemic baseline) values. *Significantly different from preischemic value. ($P < 0.05$)

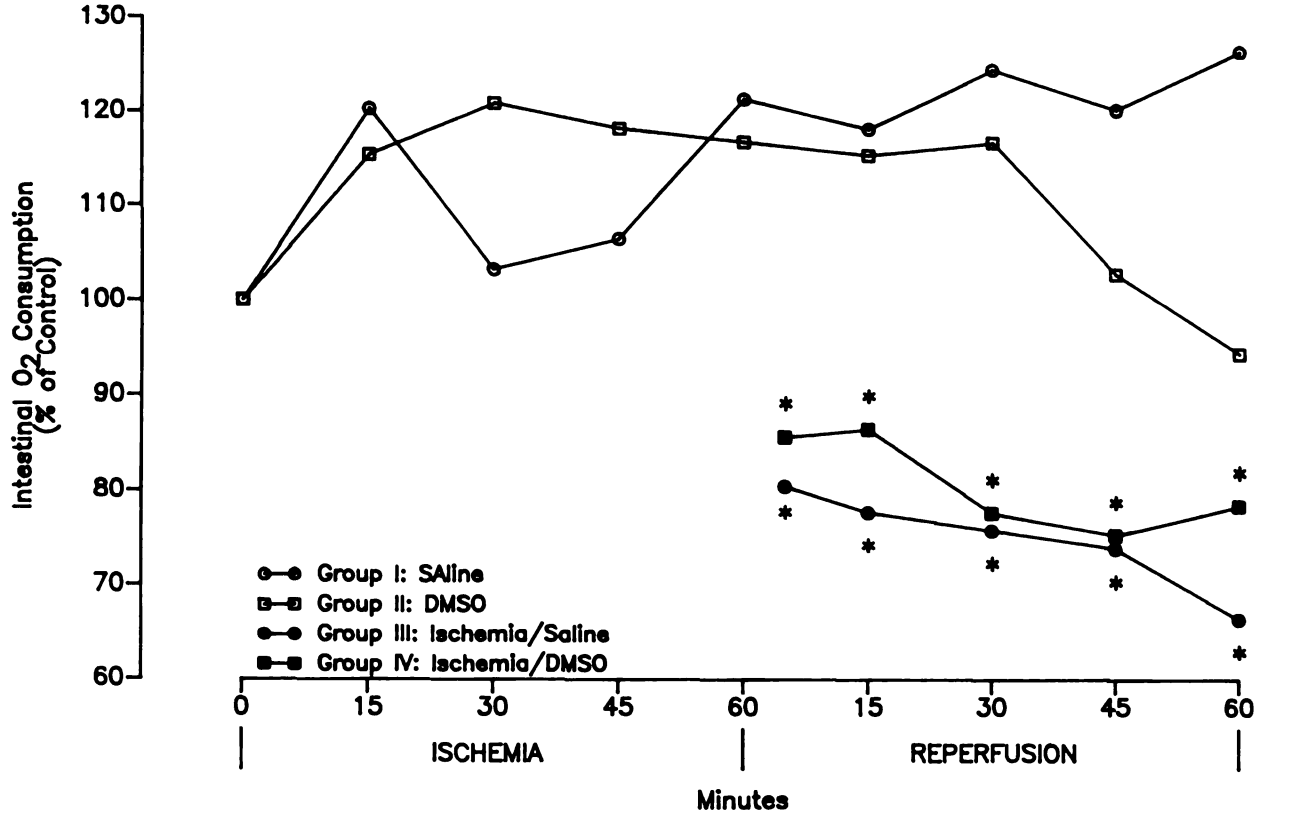

Figure 4. Changes in Intestinal Oxygen Consumption over time. VO_2 is expressed as a percentage of control (preischemic baseline) value. *Significantly different from preischemic value. ($P < 0.05$).

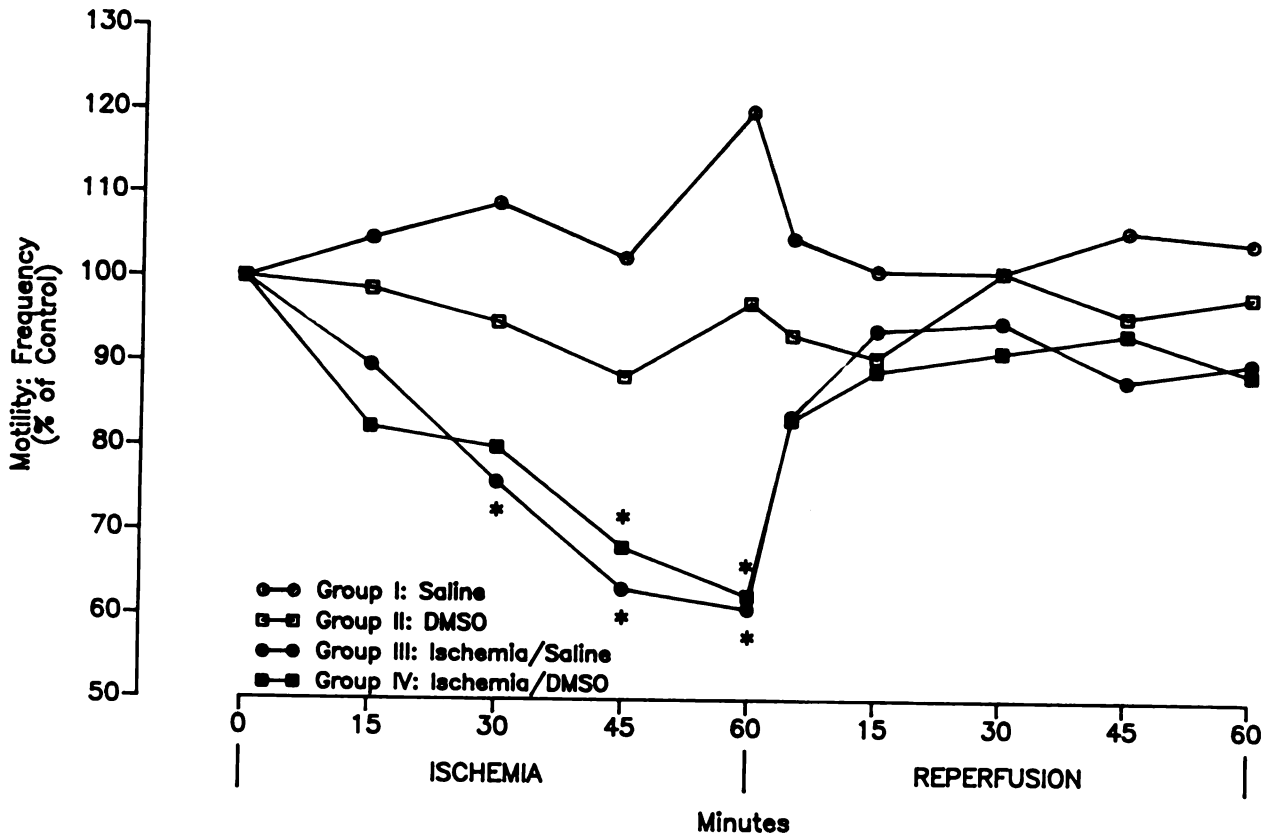

Figure 5. Motility: Changes in frequency of contractions over time. Frequency is expressed as a percentage of control (preischemic baseline) value. *Significantly different from preischemic value. ($P < 0.05$).

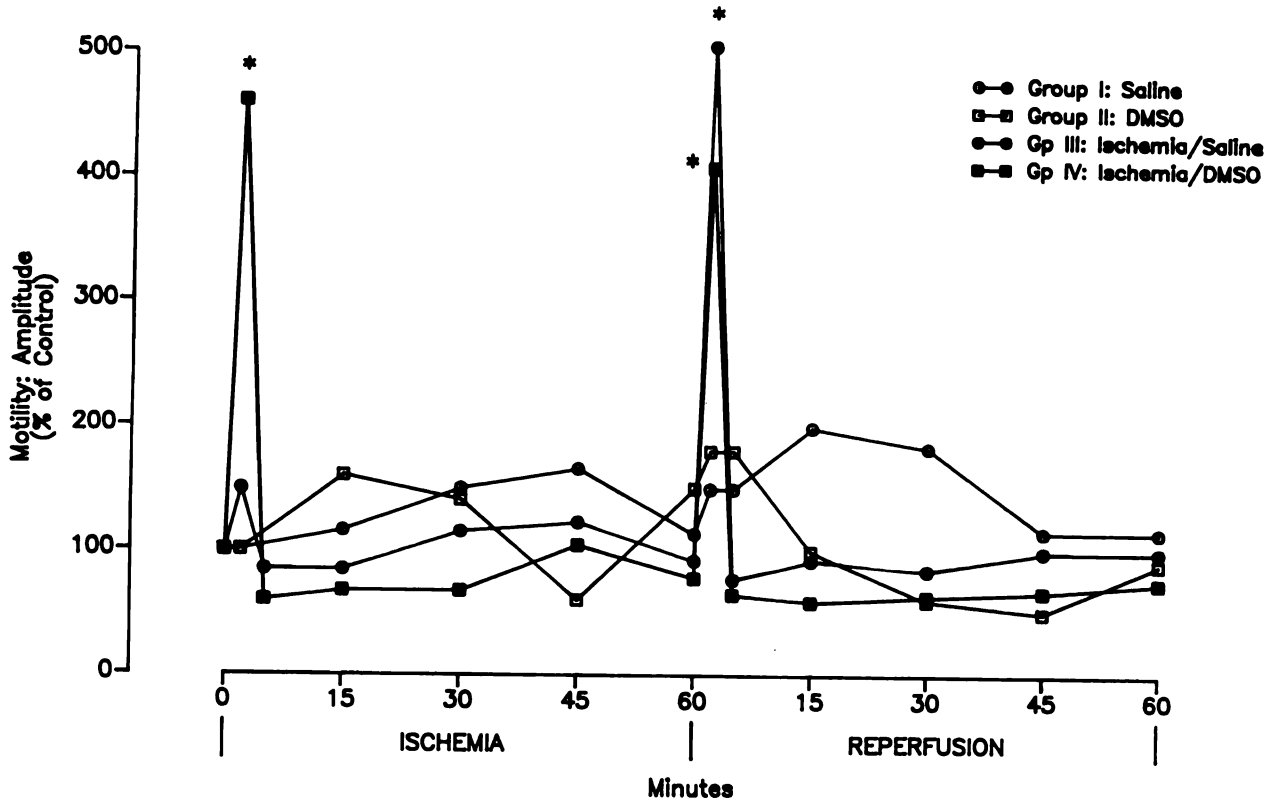

Figure 6. Motility: Changes in amplitude of contractions over time. Amplitude is expressed as a percentage of control (preischemic baseline) value. *Significantly different from preischemic value. ($P < 0.05$).

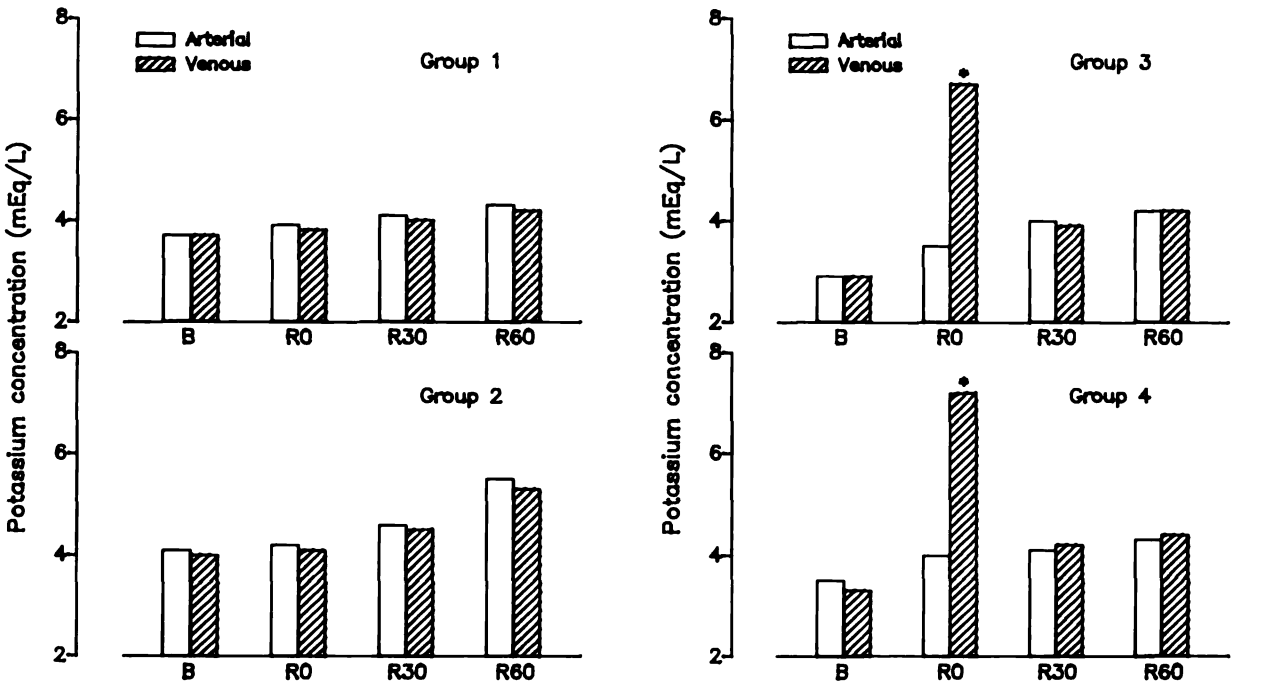

Figure 7. Potassium concentrations (mEq/L) in arterial and venous blood supplying the intestinal segment. B = preischemic baseline, RO = third 10cc aliquot immediately upon reperfusion, R30 = 30 minutes of reperfusion, R60 = 60 minutes of reperfusion. *Significantly different from preischemic value. ($P < 0.05$).

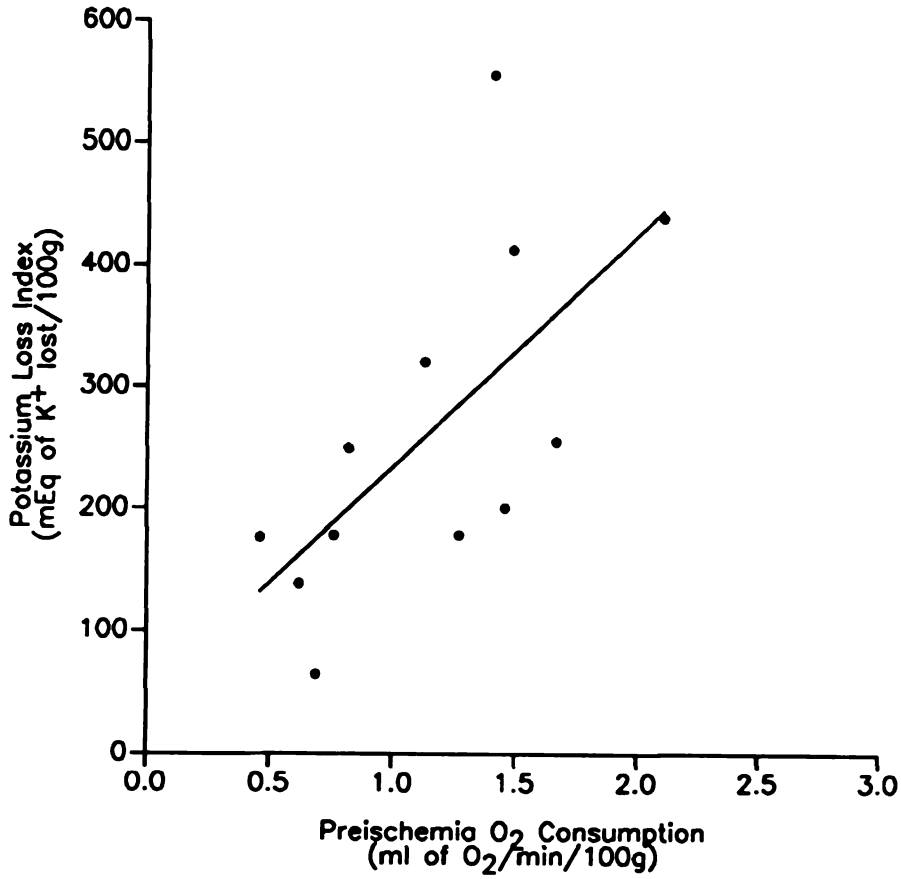

Figure 8. Least squares regression line for regression of potassium loss index ($mEqK^+$ lost/100gm) upon preischemic oxygen consumption (VO_2 base, mlO_2/min/100gm) for the 12 ponies in Gps III, IV.

C. DISCUSSION

In this study, I used a model of jejunal ischemia to demonstrate the changes that occur in rhythmic motility, wall compliance and potassium flux during and following one hour of ischemia and the changes in intestinal vascular resistance and oxygen consumption following one hour of ischemia (reperfusion).

Ischemia (Groups III and IV) caused a significant increase in vascular resistance immediately upon reperfusion (Figure 3). This is a brief effect lasting less than 5 minutes and is thought to result from compression of mural vessels by muscular activity initiated by the reintroduction of blood flow.[228-230] This can be appreciated from examination of Figure 6 (amplitude of rhythmic contractions), where intense contractions are seen to occupy the first 5 minutes of intestinal reperfusion. In Group III, this period is followed by a progressive decline in vascular resistance to levels which remain significantly below pre-ischemic baseline. There are two probable explanations for this decline. First, this may represent physiologic vasodilation to facilitate oxygen debt repayment (reactive hyperemia).[231-234] This is unlikely as in constant flow models of reactive hyperemia there is an increase in oxygen extraction [resulting in a temporary increase in oxygen consumption (extraction x flow)] as the principle mechanism of debt repayment.[234] In group III ponies, however, a decrease in oxygen consumption occurred, suggesting that the vasodilation seen in these ponies is not metabolically consistent with oxygen debt repayment. A second explanation is that the decreased vascular resistance

represents a vascular response to tissue injury (inflammatory response) or pathologic damage in the intestinal vasculature itself. Vasodilation[235] and increased capillary permeability[236] have both been recognized as early pathologic changes leading to tissue edema in various models of vascular injury. Further, in models of cerebral injury, such vasodilation has been in large part shown to result from generation of oxygen derived free radicals and is obtunded by administration of free radical scavenging agents such as SOD, catalase, DMSO and glycerol.[93,235,237,238] This is consistent with our finding that in Group IV ponies administered DMSO prior to reperfusion, no such decline in vascular resistance was observed. As vascular permeability and interstitial fluid accumulation were not quantitated however, it is not possible to conclude that maintenance of vascular tone necessarily represents a tissue protective effect.

Oxygen consumption by small intestine is an indicator of tissue activity and viability. In Groups I and II, there were no significant changes in intestinal oxygen consumption over time. In Group III, all values recorded during reperfusion were significantly below preischemic values (Figure 4). Oxygen is consumed by intestine both for metabolic functions of the mucosa and muscular activity of the intestinal wall. As bowel activity, assessed by amplitude and frequency of rhythmic contractions, was not below preischemic values during the reperfusion phase, it is more likely that decreased oxygen consumption reflects diminished mucosal rather than mural consumption. This thesis is supported by the accompanying histologic findings and reports from other studies in the dog,[21,103,239,240] cat,[29,32-34,82,102] rat,[35,241] horse,[41,42] and man[49];

where the mucosa has been shown to be the prime target for early ischemic injury. In Group IV ponies, administration of DMSO prior to reperfusion did not improve intestinal oxygen consumption during reperfusion, and therefore did not preserve metabolic activity of the intestinal segment.

There is considerable diversity within the literature as to the effects of ischemia on small intestinal motility. White et al,[41] in a subjective evaluation of motility in ponies undergoing experimental strangulation obstruction found that at 30 minutes of ischemia, bowel still responded to physical stimulation. There was return to "near normal" motility one hour following 50 minutes of ischemia but reduced motility after 2 hours. Davies and Gerring[242] found that deprivation of blood supply to small intestinal segments of horses led to significant reductions in frequency and amplitude of contractions from the first hour onward. Sullins et al[42] reported that in horses undergoing ischemic strangulation obstruction there was an initial increase in spontaneous motility as assessed visually, however specific time periods were not recorded. Our findings of a significant increase in amplitude of rhythmic contractions for less than 5 minutes at the onset of complete ischemia (Group IV) and again at reperfusion onset (Groups III and IV), are supported by a number of studies in other species.[8-12,229,242] The precipitating conditions for temporary hyperexcitability have not been established, but one of 3 mechanisms is usually suggested; hypopolarization of smooth muscle cells by potassium efflux,[243,244] humoral mediators of smooth muscle contraction such as seratonin,[245] histamine, catacholamines and polypeptides,[246] or a local neurally mediated reflex.[229] Although we found significant potassium loss from ischemic bowel

(Figure 7), the occurrence of increased activity at both onset of ischemia and reperfusion and the finding of Chou that local administration of tetradotoxin abolishes the response,[229] tend to support the latter theory. Our observations of a progressive decrease in frequency of contractions during the hour of ischemia, followed by return to normal within 5 minutes of reperfusion (Groups III and IV), are supported by other investigations.[8-12,229,242] As early as 1898, Bayliss and Starling[247] recognized that obstruction of the thoracic aorta in anesthetized dogs led to rapid cessation of rhythmic intestinal motility. With the interest in traumatology and shock research during World War II, several investigators found evidence of severe depression of gastrointestinal tonus and motility with hemorrhagic hypotension,[248-251] with return to normal following infusion of shed blood. More recent studies have indicated that the decrease in frequency of contractions is associated with a progressive decrease in Electrical Control Activity (ECA, slow wave) frequency with eventual disappearance of Electrical Response Activity (ERA, spiking activity) and in some models, disappearance of ECA.[8-12]

Intestinal wall compliance has been shown to be an accurate objective method of assessment of intestinal wall tone.[253] Sullins et al[42] reported observation of increased muscle tone and decreased intestinal diameter following one hour ofjejunal strangulation obstruction in ponies. Our findings of no significant change in intestinal wall compliance, either during ischemia or reperfusion, and those of other investigators,[8] do not support their observation. With regard to amplitude and frequency of contractions and bowel wall compliance, there were no major differences between Groups III

and IV during the reperfusion period, suggesting that DMSO administration had no effect on these variables.

Our observation of substantial potassium release from ischemic bowel upon reperfusion (Figure 5) support the finding of previous experimental investigations.[244,253,254] Potassium loss from a number of tissues subjected to ischemia or hypoxia is well documented;[255] however, the exact mechanisms are uncertain. It is unlikely to be a result of intracellular buffering of hydrogen ions associated with lowered tissue pH as exchange of cellular potassium is not associated with production of organic acids.[256] Most theories on potassium loss relate to decreased effectiveness of the sodium-potassium ATPase pump during energy deprivation, changes in the transmembrane electrochemical gradient for potassium or increased membrane permeability.[25] Cellular lysis leads to release of large quantities of potassium into the extracellular fluid. Therefore, at both a metabolic and structural level, cellular loss of potassium is a reflection of tissue injury. The majority of potassium loss occurred immediately upon reperfusion and was unaltered by administration of DMSO. The physiologic implications of excessive potassium release from ischemically injured bowel may include explanation, at least in part, of the decrease in vascular resistance noted in Group III during reperfusion, potassium being a potent vasodilator of the splanchnic bed.[253] As ponies that had received DMSO (Group IV) had a similar release of potassium but not decreased vascular resistance however, it is unlikely that extracellular potassium was a sole cause of the observed vasodilation. A more important consideration is the possible contribution of intestinal potassium to the fatal hyperkalemia reported following intestinal

revascularization and in the terminal stages of splanchnic ischemic shock.[253,254] At this time, it is unknown if hyperkalemia per se plays a critical role in the terminal cardiovascular deterioration of such shock in the horse.

Our finding of a significant regression of potassium loss upon preischemic oxygen consumption (Figure 8) demonstrates that the intestinal tissues with the highest oxygen demand is most likely to incur the greatest injury as a result of oxygen deprivation (ischemia or hypoxia). This finding adds weight to a prevous report that some analgesics used in the medical management of ischemic bowel disease, eg xylazine, may have deleterious effects on bowel viability by causing a simultaneous increase in intestinal oxygen consumption and vascular resistance (decreased blood flow at consant pressure).[258]

This study has utilized a model of jejunal ischemia-reperfusion to demonstrate the effects of one hour of ischemia and one hour of reperfusion on intestinal vascular resistance, oxygen consumption, motility, compliance and potassium loss. Further, the intravenous administration of dimethyl sulfoxide immediately prior to reperfusion abolished the decline in vascular resistance observed during reperfusion but otherwise had no effect on measured variables, in particular oxygen consumption or potassium loss. Whilst it may be argued that greater effect may have been seen if DMSO had been administered prior to the onset of ischemia, such findings would be of limited clinical value. Although the action of DMSO in modulating post-ischemic vascular reactivity may warrant further investigation, its failure to maintain tissue oxygen consumption or prevent tissue potassium loss suggests that

DMSO was not effective in preventing injury in this model of equine jejunal ischemia.

IV. THE EFFECTS OF ISCHEMIA - REPERFUSION AND DIMETHYL SULFOXIDE ON EQUINE JEJUNUM: A MORPHOLOGIC AND ULTRASTRUCTURAL EVALUATION.

A. MATERIALS AND METHODS

Experimental Preparation and Design: The experimental preparation and design employed in studying the effects of ischemia and reperfusion in jejunal segments from the eighteen ponies were described in the preceeding chapter. Following instrumentation of the experimental segment, a 2x4 cm full thickness section of tissue was removed from the antimesenteric border of a neighboring segment of jejunum (pre-ischemia). Following ischemia and reperfusion, a second 2x4 cm full thickness section (post-ischemia) was removed from the antimesenteric border of the experimental segment. Each section was divided equally between 10% formalin for light microscopy (LM) and Karnovsky's fixative for scanning (SEM) and transmission (TEM) electron microscopy. At 12 hours the Karnovsky's fixative was replaced with cacodylate buffer. Specimens intended for LM underwent routine preparation and staining with hematoxylin and eosin. Specimens for TEM underwent preparation with Zetterquist's osmium fixative prior to passage through increasing concentrations of alcohol and propylene oxide. Specimens for SEM underwent chemical dehydration utilizing passage through increasing concentrations of acetone and hexamethyldisalizine.

Specimen Evaluation: Light microscopy was used to evaluate the full thickness of each specimen. SEM examination concentrated on mucosal surface morphology. Both LM and SEM were used to grade villus lesions

from 0 (normal) to 5 (most severe) as described by Chiu and associates[21] (table 1). TEM examination centered on characteristics of the villus epithelium.

B. RESULTS

LM and SEM Evaluation:All pre-ischemia sections exhibited normal morphology. The majority of villi were from 800-1000 μm in length and 250-350 μm in width. Crypt diameters were 100-120 μm with a total mucosal depth of 1.2-1.6 mm (Figures 9A,B). Post-ischemia sections from Groups I and II were similar and ranged from normal morphology to mild changes characterized by mild submucosal edema, and petechial hemorrhage in the deeper layers of the lamina propria. Some villi showed shortening and thickening with superficial epithelial folding and vesicle formation of the villus tip. Most villi ranged in size from 500 to 650 μm in length and 300 to 400 μm in width. Crypt diameters remained 100-120 μm and total mucosal depth was 1.2-1.6 mm. Mucosal lesions were graded 0-1.5 (Figures 10;11A,B,C).

Post-ischemia sections from Group III revealed moderately severe changes. There were patchy areas of subserosal bleb formation but the muscularis layers appeared normal. There was extensive submucosal edema which stained eosinophilic suggesting a moderate protein content. The mucosa showed widespread shortening and widening of villi with diffuse villus tip epithelial folding, extensive subepithelial vesicle formation frequently accompanied by separation and in some cases sloughing of the villus epithelium, beginning at the villus tip and extending most of the way down the villi in the severely affected ones. The shortening of villi appeared to be

Table 1. Scoring system for grading mucosal morphology from LM and SEM examination (From Chiu et al[21]).

Grade	Description
0	Normal mucosal villi.
1	Development of subepithelial Gruenhagen's space, usually at the apex of the villus; often with capillary congestion.
2	Extension of the subepithelia space with moderate lifting of epithelial layer from the lamina propria.
3	Massive epithelial lifting down the sides of the villi. A few tips may be denuded.
4	Denuded villi with lamina propria and dilated capillaries exposed. Increased cellularity of lamina propria may be noted.
5	Digestion and disintegration of lamina propria; hemorrhage and ulceration.

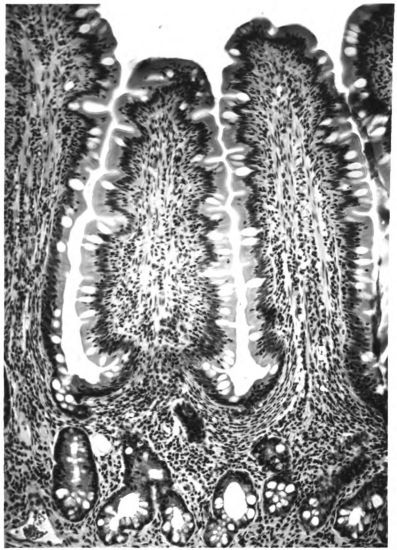

Figure 9A. Light microscopic appearance of normal villi from control sections taken prior to ischemia; H&E stain; x100 (A).

Figure 9B. Scanning electron microscopic appearance of normal villi from control sections taken prior to ischemia; x100 (B).

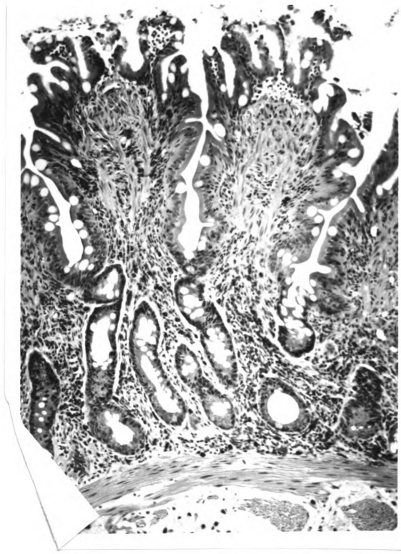

Figure 10. Light microscopic appearnace of "post-ischemia" villi from control groups I and II; H&E stain, x100. Note mild epithelial lifting toward the villus tip.

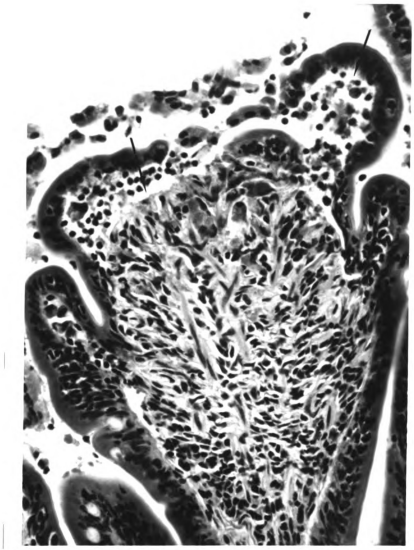

Figure 11A. Light microscopic appearance of mildest form of villus change following ischemia in groups III and IV; H&E stain: x250 (A). Arrows point to early subepithelial fluid accumulation at the villus tip.

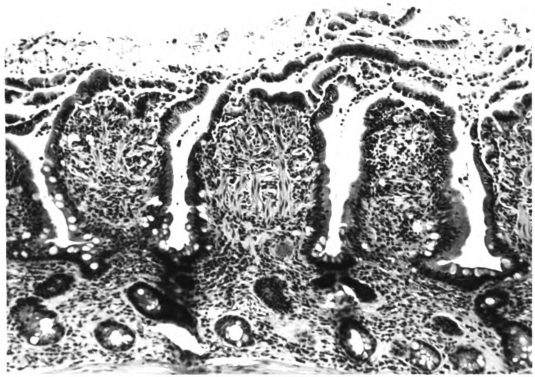

Figure 11B. Typical light microscopic appearance of post-ischemia villi from groups III and IV, H&E stain, x100 (B). Note shortening and thickening of villi and extensive denuding of villus epithelium toward tip.

Figure 11C. Typical scanning electron microscopic appearance of mucosal surface following ischemia in sections from groups III and IV; x150 (C). Note variety of lesion degree among villi, some villi showing tip folding while others (arrows) show extensive epithelial loss.

largely due to active villus contraction as well as epithelial loss from the villus tip. In many cases the villus tip was telescoped down into the villus forming a "collar" of epithelium around the tip (Figure 12A). SEM confirmed light microscopic observations that early enterocyte separation and lifting occurred from this epithelial "collar" as well as the villus tip (Figure 12B). Most villi ranged from 300-600 μm in length and 300-450 μm in width. Crypts remained 100-120 μm and total mucosal depth ranged from 0.6-1.5 mm. Mucosal lesions were graded 1.5-2.5.

Post-ischemia sections for Group IV demonstrated lesions of similar nature and severity to those in Group III. Villus, crypt and total mucosal dimensions were as for Group III . Mucosal lesions were graded between 1.5 and 2.5 in five of six ponies. One pony demonstrated more advanced changes with exposure of the entire villus lamina propria in some villi. Some of the small submucosal vessel walls demonstrated a granular and eosinophilic staining character suggestive of early fibrinoid necrosis. The lesions were graded at 4.0. A summary of mucosal lesion scores for all ponies is presented in Table 2.

TEM Evaluation: All Pre-ischemia sections exhibited normal structure characterized by close enterocyte cell to cell adhesion and uniform adhesion of enterocytes to the basement membrane. There were few intercellular spaces evident. The microvillus brush border was full and uniform while intracellular organelles (lysosomes, mitochondria, endoplasmic reticulum, golgi etc.) appeared within normal limits (Figure 13).

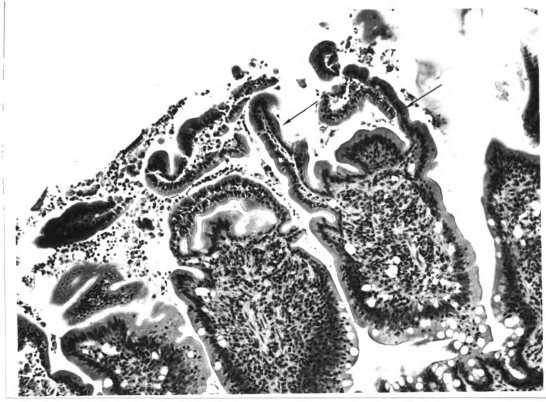

Figure 12A. Light microscopic appearance; H&E stain, x100 (A) and scanning electron microscopic appearance, x350 (B) of villi undergoing tip retraction following ischemia, groups III and IV. Arrows in both figures point to epithelial "collar" formed during tip retraction.

Figure 12B. Light microscopic appearance; H&E stain, x100 (A) and scanning electron microscopic appearance, x350 (B) of villi undergoing tip retraction following ischemia, groups III and IV. Arrows in both figures point to epithelial "collar" formed during tip retraction.

Table 2. Mucosal lesions scores ("pre"/"post" ischemic reperfusion)

Pony	Group I	Group II	Group III	Group IV
1	0/1.5	0/1.5	0/2.5	0/1.5
2	0/0	0/0	0/1.5	0/1.5
3	0/0	0/0.5	0/2.5	0/4.0
4			0/2.5	0/2.5
5			0/1.5	0/2.0
6			0/1.5	0/2.5
'Post' Mean ($\bar{X}$)	0.5	0.7	2.0	2.3
'Post' Range	0-1.5	0.5-1.5	1.5-2.5	1.5-4.0

Post-ischemia sections from the villus tip and sides in sections from Groups I and II displayed mild changes characterized by mild diffuse intracytoplasmic vacuolization. There did not appear to be swelling of mitochondria nor disruption of other intracytoplasmic organelles. Cell to cell adhesion and basement membrane integrity were maintained (Figure 14).

As the epithelium had largely sloughed from the villus tips in post-ischemia sections from groups III and IV, TEM examination was centered on the remaining epithelium of the villus sides (Figure 15A,B). Here, sheets of enterocytes were lifting away from the lamina propria. The basement membrane was frequently not identifiable. Enterocyte cell to cell adhesion was considerably disrupted, with the largest intercellular spaces evident at the cell bases. Cell to cell adhesion at the luminal surface was largely intact. Apical tight junctions and desmosomal junctions remained intact. There was some disruption of the microvillus brush border. In general, intracytoplasmic cisternal vacuolization was only slightly more marked than in post-ischemia sections from Groups I and II. Intracytoplasmic organelles maintained their structure and integrity. In particular, mitochondria did not appear swollen or disrupted and intramitochondrial cristae remained clearly visible.

C. DISCUSSION

This investigation demonstrates that in equine jejunum subjected to ischemia by one hour of total vascular occlusion followed by one hour of reperfusion, intact enterocytes are shed from villi in sheets following fluid accumulation in basilar intercellular spaces and between enterocytes and the

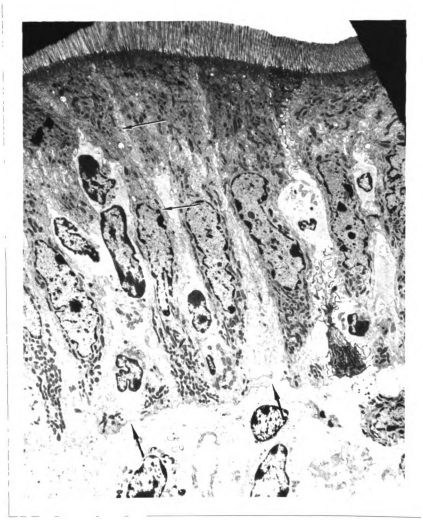

Figure 13. Transmission electron micrograph demonstrating healthy enterocytes in mucosal sections taken prior to ischemia; uranyl acetate-lead citrate; x1900. Lower arrows point to intact basement membrane, while upper horizontal arrows point to close cell to cell adhesion between enterocytes.

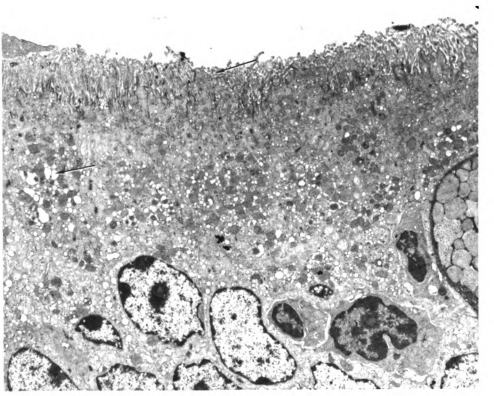

Figure 14. Transmission electron micrograph demonstrating mild enterocyte injury in "post-ischemia" sections from control groups I and II; uranyl acetate-lead citrate, x2000. Note damaged microvilli (top arrow) and diffuse intracytoplasmic vacuolization caused by dilation of cell cisternae (lower arrow).

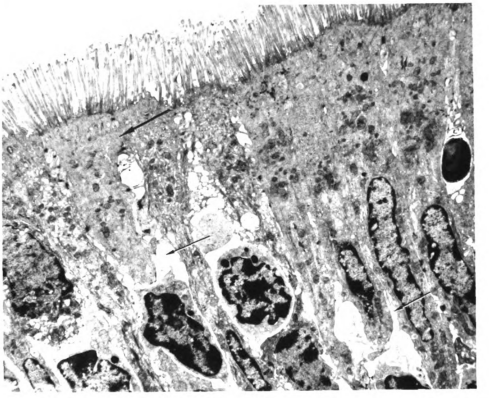

Figure 15A. Transmission electron micrographs of epithelium lifting from villus sides in post-ischemia sections from groups III and IV; uranyl acetate-lead citrate, x1900 (A,B). Lower small arrows in 7A point to intercellular fliud accumulation while apical cell to cell adhesion is maintained (large top arrows). Arrows in 7B show loss of attachment of enterocytes to basement membrane.

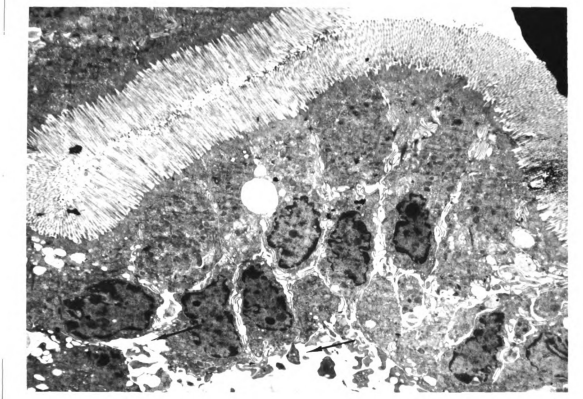

Figure 15B. Transmission electron micrographs of epithelium lifting from villus sides in post-ischemia sections from groups III and IV; uranyl acetate-lead citrate, x1900 (A,B). Lower small arrows in 7A point to intercellular fliud accumulation while apical cell to cell adhesion is maintained (large top arrows). Arrows in 7B show loss of attachment of enterocytes to basement membrane.

lamina propria. There is progressive detachment from the basement membrane. This finding is in agreement with those of numerous previous investigations.[21,22,26,41] Further, TEM examination of enterocytes separating from damaged villi do not identify major injury to intracytoplasmic organelles. Inparticular, mitochondria and lysosomes appear normal. Intracellular damage is predominantly limited to cisternal vacuolization suggestive of fluid accumulation. We have also identified a mechanical effect characterized by villus contraction and formation of an epithelial "collar" at the villus tip, exacerbating epithelial lifting. The systemic administration of dimethyl sulfoxide, a hydroxyl radical scavenging agent, immediately upon reperfusion, does not preserve mucosal morphology nor alter enterocyte ultrastructure.

Ischemic injury to the intestine causes lesions which progress centrifugally from the mucosal epithelium toward the serosa.[19] The exact mechanism, by which enterocytes are shed from ischemic villi, has been the subject of considerable investigation. Traditionally, it was held that enterocyte lactate accumulation and the resultant drop in intracytoplasmic pH produced disruption of lysosomes and liberation of acid hydrolases, which subsequently disrupted plasma membrane integrity.[60,168] Black-Schaffer et al[58] suggested that ischemic depression of enterocyte membrane function was sufficient to allow overhydration of enterocytes leading to osmotic explosion with fragmentation and loss into the lumen. Bounous[28] has advanced the theory that the principal insult is loss of brush border glycoproteins allowing access of pancreatic endopeptidases (eg trypsin) to the epithelium and underlying structures. None of these theories are consistent with the histologic findings

of Chiu[21], Brown[22], and Wagner[26] that ischemic villus injury is characterized by lifting of sheets of intact enterocytes away from the basement membrane and lamina propria, a process which progresses from the villus tip to the base. These groups propose that the basic lesion is the interpostion of accumulated fluid between the enterocyte and the basement membrane and lamina propria. This fluid accumulation at the villus tip had for some time been recognized by microscopists as Gruenhagen's space.[21] Brown et al[22] extended this proposal to suggest that the fluid wedge not only acts to mechanically separate the epithelium from the lamina propria, but adds insult to the injury of hypoperfusion by decreasing the diffusion of oxygen and nutrients from capillaries to the enterocytes. Our findings support those of the above authors, in that the predominant feature of epithelial injury was the breakdown of cell to cell adhesion in the basilar portion of the enterocytes and separation of enterocytes from the underlying basement membrane and lamina propria (Figures 11A;15A,B). This separation appeared to be associated with fluid accumulation in these regions. Intracytoplasmic changes included cisternal vacuolization and, in some sections, rarefaction of the apical terminal web and thinning and distortion of microvilli. Lysosomes and mitochondria did not appear different from those in normal pre-ischemia sections. Major organellar structural changes and enterocyte lysis are therefore not a prerequisite for the denuding of villus surfaces in ischemic injury.

A further mechanism which appeared to exacerbate villus injury in this model of ischemia was a mechanical effect initiated by marked villus contraction. On both LM and SEM examination of ischemic sections (Gps III

and IV), villi were seen to have their tip telescoped inward, forming a "collar" of epithelium around the villus tip (Figures 12A,B). This change was not recognized in pre ischemic sections and occasionally recognized in post sections from control groups I and II. Frequently, sheets of enterocytes were seen to lift from this "collar" along with, and occasionally prior to, lifting from the tip itself. In LM sections, exacerbation of the subepithelial space was seen in association with contraction of the villus core. Although the mechanism for this ischemia induced contraction is not clear, it is established that the villus core is well supplied with smooth muscle fibers oriented parallel to the long axis of the villus and basally associated with the muscularis mucosae.[44,258] In normal bowel, villi are known to undergo both swaying and contractile movements, influenced by the action of the local hormone villikinin.[44] Fibers of the muscularis mucosae are known to contract in response to both adrenergic and parasympathomimetic stimulation.[44] Sullins et al[42] noted villus contraction in a model of equine jejunal ischemia, but did not attribute to it any pathologic significance. As the physiologic study demonstrated significant contraction of the mucosal musculature in response to ischemia and reperfusion, it is not unreasonable to assume that ischemia, via neural or local humoral mechanisms, initiates contraction of individual villi, exacerbating epithelial lifting toward the villus tip, where fluid accumulation has began to separate enterocytes from their basal attachments.

As enterocytes broke away, they were frequently bound together in sheets by maintenance of tight junctions and desmosomal attachments at their apical extremities (Figure 15A). This suggests that the fluid accumulating in

the basilar and subepithelial regions did not arise by intercellular migration from the luminal surface. Both Brown[22] and Wagner[26] have suggested that the fluid arises from expulsion of cytoplasmic blebs of unwanted water from the enterocytes at their basilar positions. Wagner[26] contends that this is a preservative mechanism that cells employ in energy deficient states where the rapid fall in cellular ATP disables the sodium-potassium pump. An alternate mechanism may involve failure to remove fluids normally transported inward by the enterocyte due to lymphatic obstruction and capillary stasis.[21] A third proposal is that much of this fluid may arise from injured villus capillaries. This was originally suggested to reflect increased capillary permeability caused by endothelial anoxia[21,35,49,259] but more recently has been ascribed to the action of oxygen derived free radicals on intestinal capillary endothelia and basement membranes.[29,34,37,38,82,102-105] Indeed, the free radical mechanism of membrane damage may be extended to the enterocyte plasma membrane and basement membrane themselves, and thus may play a role in epithelial lifting and fluid accumulation, whether it be of enterocyte or capillary origin. Our results do not clarify the origin of the accumulated fluid; however, villus perivascular edema did not appear to be a prominent feature of the injury.

The failure of DMSO to limit epithelial lifting and preserve mucosal structure in this investigation (Figures 3B,C) is in disagreement with the findings of Ravid et al[85] and Demetriou et al[87] but in agreement with those of Dunn et al[83] and Itoh et al.[84] Further, Parks and Granger[82], Parks et al[86] and Perry et al[88] have previously demonstrated the ability of DMSO to limit post-ischemic intestinal vascular permeability. In explaining these differences,

several factors must be considered. First, it is clear that the model of injury may effect outcome. Parks, Granger and Perry[82,86,88] have used a hypoperfusion model rather than complete occlusion. Despite the findings of Ravid et al[85] and Demetriou et al[87], who found positive protection even in complete ischemia models, Parks[105] and McCord[90] have recently suggested that ODFR play a less important role following complete intestinal ischemia (total occlusion), than with hypoperfusion. This is in marked contrast to the myocardium, where ODFR scavengers appear to have equal benefit in both total and partial vascular occlusion models.[90] Recently, Carati et al[106] has demonstrated the ineffectiveness of DMSO in limiting post-ischemic intestinal microvascular permeability following total occlusion ischemia. Similarly, duration of ischemia may play a role. Linas, Wittenburg and Repine[261] have demonstrated that DMSO is effective in limiting reperfusion injury of the kidney following 20 and 30 minutes but not 45 minutes of ischemia. The effect of species differences also cannot be discounted. The work of Parks, Granger and Perry[82,86,88] was conducted in cats while the other investigations were performed in rats.[83,84,85,87] The occurrence, distribution and conversion of the enzymes required for ischemic generation of ODFR still awaits documentation in a large number of species. Although the ineffectiveness of DMSO in this model of jejunal ischemia-reperfusion injury may bear relevance to the clinical situation where total vascular occlusion causes regional ischemia, alternative models utilizing continuous low flow states may be a more appropriate method of evaluation of radical scavengers for the clinical situation of incomplete regional vascular occlusion or generalized splanchnic hypoperfusion.

In addition to the injury model, the timing of therapeutic intervention may also effect results. In the studies led by Parks[86], Perry[88], Dunn[83], Itoh[84], and Carati[106], DMSO treatment was administered prior to and/or during the ischemic episode. In our study and those of Ravid et al[85], Demetriou et al[87] and Linas et al[261], therapy was administered only upon reperfusion. For the clinical situation where regional vascular obstruction is suddenly removed (eg by intrasurgical manipulation) modelled here as total occlusion followed by reperfusion, we feel that only treatments effective when initiated at reperfusion are truly relevant. For the setting of incomplete regional occlusion or generalized hypoperfusion, experimental evaluation of therapies instituted during a low flow state bear clinical relevance. DMSO was not effective in this model of complete occlusion followed by reperfusion, suggesting that its use to limit injury to jejunum suffering complete regional vascular occlusion is not clinically warrented. It is suggested that further investigation of the use of free radical scavengers in equine intestinal ischemia be directed toward their use in limiting injury associated with low flow states.

V. SUMMARY AND CONCLUSIONS

Acute abdominal pain in the horse is frequently associated with intestinal ischemia. When a specific region of the bowel is affected (i.e. regional ischemia), the mechanisms most commonly involved are vascular compression (i.e. strangulating obstruction) or primary vascular insufficiency (non-strangulating infarction). In several species, it has been suggested that other areas of the bowel may also suffer injury due to hypoperfusion associated with circulatory shock states (i.e. generalized ischemia). Intestinal ischemia has been associated with disruption of mucosal barrier function, liberation of humeral mediators of circulatory shock, and increased mortality.

In the past 7 years, evidence has accumulated that suggests ischemic tissue damage may be largely prevented by systemic administration of agents which prevent the formation of, or scavenge, oxygen derived free radicals (ODFR) formed during post-ischemic reperfusion. Dimethyl sulfoxide (DMSO), a hydroxyl radical scavenging solvent, is one such agent. It has for some time been used as an anti-inflammatory agent, both topically and systemically, in the horse.

In this investigation, we established a model of equine jejunal ischemia-reperfusion injury, which allowed determination of indices of vascular reactivity (intestinal vascular resistance; R), oxidative tissue metabolism (intestinal oxygen consumption; VO_2), whole organ function (motility), cellular integrity (potassium leakage; $\Delta AV\ [K^+]$) and documentation of morphologic changes i.e. light (LM), scanning (SEM) and transmission (TEM) electron

microscopy. The model we employed was a constant flow, vascularly isolated, neurally intact jejunal preparation in heparinized, anesthetized ponies. Following a 30 minute baseline recording phase, ischemia was induced by complete vascular occlusion for 1 hour followed by flow reintroduction for 1 hour. It is thus felt that the preparation most closely models complete regional ischemia rather than regional or generalized hypoperfusion. In ponies receiving treatment, DMSO was delivered as an intrajugular bolus, diluted in saline immediately prior to intestinal reperfusion.

In the physiologic study, it was found that ischemia caused 1) an increase in R immediately upon reperfusion. Within 15 minutes R had decreased to a level below pre-ischemic values and remained at this level until the end of the study. 2) VO_2 was decreased during the entire reperfusion period. 3) Amplitude of rhythmic contractions increased immediately upon reperfusion. 4) Frequency of rhythmic contractions decreased during ischemia, and 5) ΔAV $[K^+]$ increased immediately upon reperfusion. DMSO administration prevented the decrease in R during reperfusion; however, it did not alter the trend in any other variable.

In the pathology study, it was found that combined ischemia and reperfusion of 2 hours duration produced moderately severe mucosal injury to the equine jejunum, characterized principally by the disruption of enterocyte attachment from the basement membrane and lamina propria. The process was most marked at villus tip and progressed down the villus sides. Enterocyte intracytoplasmic organelle changes were not a prominent feature of the injury. These findings support the importance of mechanisms leading to early

subepithelial fluid accumulation rather than direct severe enterocyte injury. Further, a pathomechanical effect caused by vigorous villus contraction appeared to exacerbate epithelial lifting at the villus tip. DMSO administration failed to improve the appearance of the mucosa.

Although the action of DMSO in modulating post-ischemic vascular reactivity may warrant further investigation, the failure of DMSO therapy to improve postischemic tissue oxygen consumption, limit tissue potassium loss upon reperfusion or improve the morphologic appearance of the damaged mucosa suggests it was ineffective in preventing this form of ischemia-reperfusion injury. These findings are consistent with emerging evidence that oxygen derived free radicals may play a limited role where intestinal ischemia is of long duration or caused by complete cessation of blood flow. This study suggests that the clinical use of DMSO to limit injury to equine jejunum suffering complete regional vascular occlusion is not warranted. Further, it is recommended that investigation of free radical scavenger use in equine intestinal ischemia be directed toward their use in limiting injury associated with low flow states.

APPENDIX

APPENDIX

Table 3. Intestinal Vascular Resistance (R; mm Hg/ml/min/100gm)

		Ischemia (minutes)					Reperfusion (minutes)					
		0	15	30	45	60	2	5	15	30	45	60
Group I (Control)	Mean	1.34	1.17	1.18	1.18	1.22	1.18	1.18	1.23	1.04	1.13	1.14
	Std error	0.17	0.17	0.19	0.08	0.03	0.04	0.04	0.10	0.05	0.15	0.10
Group II (DMSO)	Mean	2.21	2.10	1.94	2.06	1.63	1.57	1.57	1.72	2.02	2.25	2.45
	Std error	0.40	0.48	0.41	0.39	0.41	0.41	0.41	0.49	0.57	0.67	0.83
Group III (Ischemia)	Mean	2.07					3.13	1.90	1.71	1.56	1.58	1.57
	Std error	0.16					0.37	0.12	0.10	0.10	0.09	0.09
Group IV (Ischemia/DMSO)	Mean	1.68					3.30	1.78	1.61	1.51	1.50	1.44
	Std error	0.17					0.41	0.24	0.18	0.21	0.22	0.22

APPENDIX (CONT'D)

Table 4. Intestinal Oxygen Consumption (VO_2; ml O_2/min/100gm)

		Ischemia (minutes)					Reperfusion (minutes)				
		0	15	30	45	60	5	15	30	45	60
Group I	Mean	0.94	1.0	1.13	1.0	1.14	1.16	1.11	1.17	1.13	1.19
(Control)	Std error	0.18	0.30	0.33	0.20	0.11	0.10	0.08	0.11	0.12	0.08
Group II	Mean	0.72	0.83	0.87	0.85	0.84	0.87	0.83	0.84	0.74	0.68
(DMSO)	Std error	0.23	0.18	0.19	0.23	0.23	0.25	0.26	0.20	0.24	0.23
Group III	Mean	1.07					0.86	0.83	0.81	0.79	0.71
(Ischemia)	Std error	0.24					0.23	0.21	0.20	0.20	0.19
Group IV	Mean	1.25					1.07	1.08	0.97	0.94	0.98
(Ischemia/DMSO)	Std error	0.17					0.14	0.15	0.12	0.13	0.15

APPENDIX (CONT'D)

Table 5. Frequency of rhythmic contractions (Contractions/minutes)

		Ischemia (minutes)					Reperfusion (minutes)				
		0	15	30	45	60	5	15	30	45	60
Group I (Control)	Mean	7.8	8.17	8.5	8.0	9.4	8.2	7.9	7.9	8.3	8.2
	Std error	0.09	0.30	0.26	0.12	0.97	0.07	0.15	0.23	0.37	0.09
Group II (DMSO)	Mean	7.8	7.7	7.4	6.93	7.6	7.3	7.1	7.9	7.5	7.7
	Std error	0.48	0.27	0.75	0.64	0.66	0.56	0.93	0.86	0.70	0.93
Group III (Ischemia)	Mean	8.7	7.8	6.6	5.5	5.3	7.3	8.2	8.3	7.7	7.9
	Std error	0.47	0.48	0.27	0.36	0.63	0.28	0.44	0.37	0.41	0.36
Group IV (Ischemia/DMSO)	Mean	8.5	7.0	6.8	5.8	5.28	7.1	7.6	7.8	8.0	7.6
	Std error	0.73	0.16	0.30	0.28	0.71	0.32	0.18	0.40	0.45	0.51

APPENDIX (CONT'D)

Table 6. Amplitude of rhythmic contractions (mm Hg)

		Ischemia (minutes)							Reperfusion (minutes)					
		0	2	5	15	30	45	60	2	5	15	30	45	60
Group I (Control)	Mean	0.5	0.5	0.5	0.58	0.75	0.83	0.57	0.75	0.75	1.0	0.92	0.58	0.58
	Std error	0	0	0	0.08	0.14	0.33	0.16	0.25	0.25	0.50	0.42	0.08	0.08
Group II (DMSO)	Mean	0.83	0.83	0.83	1.33	1.17	0.5	1.25	1.5	1.5	0.83	0.5	0.42	0.75
	Std error	0.33	0.33	0.33	0.44	0.55	0.29	0.38	0.76	0.76	0.36	0	0.08	0.14
Group III (Ischemia)	Mean	1.3	1.94	1.1	1.1	1.5	1.6	1.2	6.6	1.0	1.2	1.1	1.3	1.3
	Std error	0.22	0.47	0.29	0.38	0.38	0.39	0.31	0.84	0.27	0.42	0.28	0.42	0.27
Group IV (Ischemia/DMSO)	Mean	1.05	4.84	0.63	0.71	0.71	1.1	0.82	4.3	0.68	0.62	0.67	0.71	0.79
	Std error	0.18	2.89	0.06	0.10	0.10	0.26	0.27	1.51	0.10	0.08	0.11	0.10	0.19

APPENDIX (CONT'D)

Table 7. Intestinal wall compliance at 180ml volume (C; ml/mm Hg)

			Ischemia (minutes)		Reperfusion (minutes)	
		Baseline	15	45	15	45
Group I (Control)	Mean	29.94	24.76	27.18	27.4	31.32
	Std error	8.13	5.29	5.62	8.81	7.55
Group II (DMSO)	Mean	26.03	25.61	26.38	22.67	26.05
	Std error	6.3	7.38	6.53	7.06	8.89
Group III (Ischemia)	Mean	21.98	25.35	18.99	21.26	24.35
	Std error	3.87	4.18	4.62	3.93	3.52
Group IV (Ischemia/DMSO)	Mean	23.29	23.96	23.29	25.7	23.66
	Std error	6.26	7.17	3.5	4.52	6.29

APPENDIX (CONT'D)

Table 8. Intestinal arterial and venous potassium concentrations ([K^+], meq/L)

		Baseline		Reperfusion (minutes) 0*		30		60	
		Arterial	Venous	Arterial	Venous	Arterial	Venous	Arterial	Venous
Group I (Control)	Mean	3.7	3.7	3.9	3.8	4.1	4.0	4.3	4.2
	Std error	0.10	0.15	0.20	0.23	0.12	0.09	0.09	0.09
Group II (DMSO)	Mean	4.1	4.0	4.2	4.1	4.6	4.5	5.5	5.3
	Std error	0.54	0.55	0.15	0.09	0.44	0.44	0.35	0.36
Group III (Ischemia)	Mean	2.9	2.9	3.5	6.7	4.0	3.9	4.2	4.2
	Std error	0.22	0.23	0.29	0.59	0.38	0.36	0.40	0.42
Group IV (Ischemia/DMSO)	Mean	3.5	3.3	4.0	7.2	4.1	4.2	4.3	4.4
	Std error	0.28	0.25	0.43	0.56	0.53	0.56	0.56	0.61

*0 Reperfusion value represents potassium concentration of third 10cc aliquot in washout series.

LIST OF REFERENCES

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1. Foreman JH, White NA: Incidence of equine colic in the University of Georgia Ambulatory Practice. In Moore JN, White NA, Becht JL (eds), Colic Research: Proceedings of the 2nd Symposium at the University of Georgia, Lawrenceville, Veterinary Learning Systems, 1986, pp. 30-31.

2. White NA, Moore JN, Cowgil LM, Brown J: Epizootiology and risk factors in equine colic at University Hospitals. In Moore JN, White NA, Becht JL (eds), Colic Research: Proceedings of the 2nd Symposiu m at the University of Georgia, Lawrenceville,Veterinary Learning Systems, 1986, pp. 26-31.

3. Huskamp B: The diagnosis and treatment of acute abdominal conditions in the horse: The various types and frequency as seen at the Animal Hospital in Hochmoor. In Byers TD, Moore JN, White NA (eds). Proceedings of the 1st Equine Colic Research Symposium at the University of Georgia, Athens, GA, 1982, pp. 261-272.

4. Pascoe PJ, McDonald WN, Trim CM, VanGorder J: Mortality rates and associated factors in equine colic operations - a retrospective study of 341 operations. Can Vet J 1983;24:76-85.

5. Guyton AC: Textbook of Medical Physiology, 6th Ed. Philadelphia, WB Saunders Co, 1981, pp 617-620.

6. Meschter CL, White NA, Tyler DE: Histopathologic lesions in the gastrointestinal tract of horses with natural occurring colic. In Byers TD, Moore NJ, White NA (eds). Proceedings of the 1st Equine Colic Research Symposium at the University of Georgia, Athens, GA, 1982, pp. 62-67.

7. Meschter CL, Tyler DE, White NA, Moore J: Histologic findings in the gastrointestinal tract of horses with colic. Am J Vet Res 1986;47:598-606.

8. Bean JW, Sidkey MM: Effects of low O_2 on intestinal blood flow, tonus and motility. Am J Physiol 1957;189:541-547.

9. Kowalewski K, Zajec S, Kolodej A: Effects of ischemic anoxia on myoelectrical and mechanical activity of the totally isolated canine stomach. Surg Gynecol Obstet 1975;140:187-192.

10. Kowalewski K, Zajec S, Kolodej A: Effects of ischemic anoxia on electrical and mechanical activity of the totally isolated porcine stomach. Eur Surg Res 1976;8:12-25.

11. Meissner A, Bowes KL, Sarna SK: Effects of ambient and stagnant hypoxia on the mechanical and electrical activity of the canine upper jejunum. Can J Surg 1976;19:316-321.

12. Cabot RM, Kohatsu S: The effects of ischemia on the electrical and contractile activity of the canine small intestine. Am J Surg 1978; 136:242-246.

13. Rooney JR: Autopsy of the horse. Baltimore, Williams and Wilkins Co, 1970, pp. 83-86.

14. Bennett DG: Predisposition to abdominal crisis in the horse. J Am Vet Med Assoc 1972;16(11):1189-1194.

15. Wright AI: Verminous arteritis as a cause of colic in the horse. Equine Vet J 1972;4:169-174.

16. White NA: Intestinal infarction associated with mesenteric vascular thrombotic disease in the horse. J Am Vet Med Assoc 1981; 178:259-262.

17. Davies JV, Gerring EL: Effect of experimental vascular occlusion on small intestinal motility in ponies. Equine Vet J 1985;17:219-224.

18. Renton CJC: Nonocclusive intestinal infarction. Clin Gastroenterol 1972;1:655-673.

19. Whitehead R: The pathology of intestinal ischemia. Clin Gastroenterol 1972;1:613-637.

20. Ottinger LW: Nonocclusive mesenteric infarction. Surg Clin North Am 1974;689-698.

21. Chiu CJ, McArdle AH, Brown R, Scott HJ, Gurd FN: Intestinal mucosal lesions in low-flow states I.A morphological, hemodynamic, and metabolic reappraisal. Arch Surg 1970;101:478-483.

22. Brown RA, Chiu CJ, Scott HJ, Gurd FN: Ultrastructural changes in the canine ileal mucosal cell after mesenteric arterial occlusion. Arch Surg 1970;101:290-297.

23. Robinson JWL, Mirkovitch J: The recovery of function and microcirculation in small intestinal loops following ischemia. Gut 1972;12:784-789.

24. Haglund U, Lundgren O: The effects of vasoconstricor fibre stimulation on the consecutive vascular sections of the small intestine of the cat during prolonged regional hypotension. Acta Physiol Scand 1972;85:547-558.

25. Aho AJ, Arstila AU, Ahonen J, Inberg MV, Scheinin TM: Ultrastructural alterations in ischemic lesions of small intestinal mucosa in experimental superior mesenteric artery occlusion. Effect of oxygen breathing. Scand J Gastroenterol 1973;8:439-447.

26. Wagner R, Gabbert H, Hohn P: The mechanism of epithelial shedding after ischemic damage to the small intestinal mucosa. A light and electron microscopic investigation. Virchows Arch 1979;30:25-31.

27. Gorey TF: The recovery of intestine after ischemic injury. Br J Surg 1980;67:699-702.

28. Bounous A: Acute necrosis of the intestinal mucosa. Gastroenterol 1982; 82:1457-1467.

29. Parks DA, Bulkley GB, Granger DN, Hamilton SR, McCord JM. Ischemic injury in the cat small intestine: Role of superoxide radicals. Gastroenterol 1982;82:9-15.

30. Dalsing MC, Sieber P, Grosfeld JL, Hasewinkel J, Hull M, Weber TR. Ischemic bowel: The protective effects of free-radical anion scavengers. J Ped Surg 1983;18(4):360-364.

31. Dalsing MC, Grosfeld JL, Schiffler MA, Van DW, Hull M, Buehner AL, Weber TR. Superoxide dismetase: A cellular protective enzyme in bowel ischemia. J Surg Res 1983;34:589-596.

32. Schoenberg MH, Muhl E, Selim D, Younes M, Schildberg FW, Haglund U. Post hypotensive generation of superoxide free radicals - possible role in the pathogenesis of intestinal mucosal damage. Acta Chir Scand 1984;150:301-309.

33. Younes M, Schoenberg MH, Jung H, Fredholm BB, Haglund U, Shildberg FW. Oxidative tissue damage following regional intestinal ischemia and reperfusion in the cat. Res Exp Med 1984;184:259-264.

34. Parks DA, Granger DN, Bulkley GB, Shah AK. Soybean trypsin inhibitor attenuates ischemic injury to the feline small intestine. Gastroenterol 1985;89:6-12.

35. Haglind E, Haglund U, Lundgren O, Stenberg B: Mucosal lesions of the small intestine after intestinal vascular obstruction in the rat. Acta Chir Scand 1985;151:147-150.

36. Schoenberg MH, Fredholm BB, Haglund U, Jung H, Selim D, Younes M, Schildberg FW: Studies on the oxygen radical mechanism involved in the small intestinal reperfusion damage. Acta Physiol Scand 1985, 124:581-589.

37. Parks DA, Granger DN: Contributions of ischemia and reperfusion to mucosal lesion formation. Am J Physiol 1986;250:G749-G753.

38. Granger DN, Hollworth ME, Parks DA: Ischemia-reperfusion injury: role of oxygen-derived free radicals. Acta Physiol Scand 1986; Suppl 548:47-63.

39. Younes M, Mohr A, Schoenberg MH, Schildberg FW: Inhibition of lipid peroxidation by superoxide dismutase following regional intestinal ischemia and reperfusion. Res Exp Med 1987;187:9-17.

40. Hjortkjaer RK, Svendsen CK: Simulated small intestinal volvulus in the anesthetized horse. Nord Vet Med 1979;31:466-483.

41. White NA, Moore JM, Trim CM: Mucosal alterations in experimentally induced small intestinal strangulation obstruction in ponies. Am J Vet Res 1980; 41:193-198.

42. Sullins KE, Stashak TS, Mero KH: Pathologic changes associated with induced small intestinal strangulation obstruction and nonstrangulating infarction in horses. Am J Vet Res 1985;46:913-916.

43. Freeman DE, Cimprich RE, Richardson DW, Gentile DA, Orsini JA, Tulleners EP, Fetrow JP: Early mucosal healing and chronic changes in pony jejunum after various types of strangulation obstruction. Am J Vet Res 1988;49:810-818.

44. Davenport HW: Physiology of the Digestive Tract. 5th Edition. Chicago Year Book Medical Publishers 1982, pp. 174-226.

45. Lillehei RC: The intestinal factor in irreversible shock. Surgery 1957;42:1043-1054.

46. Haglund U, Lundgren O: Intestinal ischemia and shock factors. Fed Proc 1978;37:2729-2733.

47. Gans H, Matsumoto K: The escape of endotoxin from the intestine. Surg Gynecol obstet 1979;139:395-402.

48. Falk A, Myrvold HE, Haglund U: Cardiopulmonary function as related to intestinal mucosal lesions in experimental septic shock. Circ Shock 1982;9:419-432.

49. Haglund U, Hulten L, Ahren C, Lundgren O: Mucosal lesions in the human small intestine in shock. Gut 1975;16:979-984.

50. Falk A, Myrvold HE, Lundgren O, Huglund U: Mucosal lesions in the feline small intestine in septic shock. Circ Shock 1982;9:27-35.

51. Bailey RW, Bulkley GB, Hamilton SR, Morris JB, Huglund UH: Protection of the small intestine from nonocclusive mesenteric ischemic injury due to cardiogenic shock. Am J Surg 1986;153:108-116.

52. Mittermayer C, Riede U: Human pathology of the gastrointestinal tract in shock, ischemia and hypoxemia. In Cowley RA, Trump BF(eds) Pathophysiology of shock, anoxia and ischemia, Baltimore, Williams and Wilkins, 1982;pp.301-308.

53. Lefer AM: Role of myocardial depressant factor in the pathogenesis of circulatory shock. Fed Proc 1970;29:1836-1847.

54. Pittman RP, Senko JT, Nyhof R, Chou CC: Release of cardiodepressants from the canine jejunum in irreversible hemorrhagic shock. Circ Shock 1983;11:149-158.

55. van der Meer C, Valkenburg PW, Snyder PM. Studies on hyperkalemia as a cause of death in intestinal ischemia shock in rats. Circ Shock 1986;19:329-345.

56. Alemayehu A, Sawmiller D, Chou BS Chou CC: Intestinal prostacycl in and thromboxane production in irreversible hemorrhagic shock. Circ Shock 1987;23:119-130.

57. Fine I. The intestinal circulation in shock. Gastroenterol 1967;52:454-458.

58. Black-Schaffer B, Gall EP, Shimizu RT, Esparza HS: The pathogenesis of the intestinal lesion of deep hypothermia and a proposed relationship to that of irreversible shock, including a note on a mechanism for the normal turnover of intestinal epithelium. Surgery 1967;61:904-914.

59. Tamakuma S, Rojas-Coroma R, Cuevas P, Fine J: Demonstration of a lethal endotoxemia of intestinal origin in refractory non-septic shock. Ann Surg 1971;173:219-224.

60. Abe H, Carballo J, Appert HE, Howard JM: The release and fate of the intestinal lysosomal enzymes after acute ischemic injury of the intestine. Surg Gynec Obstet 1972;135:581-585.

61. McCuskey RS: Microcirculation - basic considerations. In Cowley RA, Trump BF (eds) Pathophysiology of Shock, Anoxia and Ischemia, Baltimore, Williams & Wilkins 1982, pp. 156-164.

62. Trump BF, Berezesky IK, Cowley RA: The cellular and subcellular characteristics of acute and chronic injury with emphysis on the role of calcium. In Cowley RA, Trump BF (eds) Pathophysiology of Shock, Anoxia and Ischemia, Baltimore, Williams & Wilkins 1982, pp. 6-46.

63. Kahng MW: Intermediary metabolism. In Cowley RA, Trump BF (eds) Pathophysiology of Shock, Anoxia and Ischemia, Baltimore, Williams & Wilkins 1982, pp. 66-73.

64. Ozawa K: Energy metabolism. In Cowley RA, Trump BF (eds) Pathophysiology of Shock, Anoxia and Ischemia, Baltimore, Williams & Wilkins 1982, pp. 74-83.

65. Mela L: Mitochondrial function in shock, ischemia and hypoxia. In Cowley RA, Trump BF (eds) Pathophysiology of Shock, Anoxia and Ischemia, Baltimore, Williams & Wilkins 1982, pp. 84-94.

66. Sayeed MW: Membrane Na^+-K^+ transport and ancillary phenomena in circulatory shock. In Cowley RA, Trump BF (eds) Pathophysiology of Shock, Anoxia and Ischemia, Baltimore, Williams & Wilkins 1982, pp. 112-131.

67. Reimea KA, Jennings RB: Iron and water shifts, cellular. In Cowley RA, Trump BF (eds) Pathophysiology of Shock, Anoxia and Ischemia, Baltimore, Williams & Wilkins 1982, pp. 132-146.

68. Means AR, Dedman JR: Calmodulin - An intracellular calcium receptor. Nature 1980;285:73-77.

69. Chang YW: Calmodulin plays a pivotal role in cellular regulation. Science 1980;207:19-27.

70. Wildenthal K: Lysosomal alterations in ischemic myocardium. Result or cause of myocellular damage. J Mol Cell Cardiol 1978;10:595-603.

71. Elbrink J, Bihle I: Membrane transport - the relation to cellular metabolic rates. Science 1975;188:1177-1184.

72. Faber JL, Martin JT, Chien K: Irreversible ischemic cell injury. Am J Pathol 1978;92:713-732.

73. Hawkins HK, Ericsson JLE, Biberfeld P, Trump BF: Lysosome and phagosome stability in lethal cell injury. Am J Pathol 1972;68:265-287.

74. Paul RJ, Bauer M, Prease W: Vascular smooth muscle: Anaerobic glycolysis linked to sodium and potassium transport processes. Science 1979;206:1414-1416.

75. Trump BF, Laiho KU, Berezesky IK: The role of ion movements in cell injury and shock. Circ Shock 1979;6:182.

76. Schumer W, Erve PR: Cellular metabolism in shock. Circ Shock 1975;2:109.

77. Schumer W: Lactic acid as a factor in the production of irreversibility in oligemic shock. Nature 1966;212:1210.

78. Croker BP, Saladino AJ, Trump BF: Ion movements in cell injury: Relationship between energy metabolism and the pathogenesis of lethal injury in the toad bladder. Am J Pathol 1970;59:247.

79. Silver A: Ion fluxes in hypoxic tissues. Microvasc Res 1977;13:409-420.

80. Hearse DJ, Humphrey SM, Chain EB: Abrupt reoxygenation of the anoxic potassium-arrested perfused rat heart: The study of myocardial enzyme release. J Mol Cell Cardiol 1973;5:395-407.

81. Hearse DJ, Humphrey SM, Nayler WG, Slade A, Border D: Ultrastructural damage associated with reoxygenation of the anoxic myocardium. J Mol Cell Cardiol 1975;7:315-324.

82. Parks DA, Granger DN. Ischemia-induced vascular changes: Role of xanthine oxidase and hydroxyl radicals. Am J Physiol 1983;245:G285-G289.

83. Dunn SP, Gross KR, Dalsing M, Hom R, Grosfeld JL: Superoxide: A critical oxygen free radical in ischemic bowel injury. J Ped Surg 1984;19:740-744.

84. Itoh M, Guth PH: Role of oxygen-derived free radicals in hemorrhagic shockinduced gastric lesions in the rat. Gastroenterol 1985;88:1162-1167.

85. Ravid M, Van-Dyk D, Bernheim J, Kedar I: The protective effect of dimethyl sulfoxide in experimental ischemia of the intestine. Ann NY Acad Sci 1983;411:100-104.

86. Parks DA, Shah AK, Granger DN: Oxygen radicals: effects on intestinal vascular permeability. Am J Physiol 1984;247:G167-G170.

87. Demetriou AA, Kagoma PK, Kaiser S, Seiffer E, Niu XT, Levenson SM: Effect of dimethyl sulfoxide and glycerol on acute bowel ischemia in the rat. Am J Surg 1985;149:91-94.

88. Perry MA, Wadhwa S, Parks DA, Pickard W, Granger DN: Role of oxygen radicals in ischemia-induced lesions in the cat. Gastroenterol 1986;90:362-367.

89. Hernandez LA, Grisham, Shoals MB, Granger DN: A role for iron in oxidant mediated ischemic injury to intestinal microvasculature. Am J Physiol 1987;253:G49-G53.

90. McCord JM: Oxygen derived free radicals in post-ischemic tissue injury. New Eng J Med 1985;312:159-163.

91. Rosenblum W: Dimethyl sulfoxide effects on platelet aggregation and vascular reactivity in pial microcirculation. Ann Ny Acad Sci 1983;411:110-119.

92. Rosenblum WI, El-Sabban F: Dimethyl sulfoxide (DMSO) and glycerol, hydroxyl radical scavengers, in pair platelet aggregation within and eliminate the accompanying vasodilation of injured pial arterioles. Stroke 1982;13:35-39.

93. Wei EP, Cristman CW, Kontos HA, Povlishock JT: Effect of oxygen radicals on cerebral arterioles. Am J Physiol 1985;248:H157-H162.

94. Wei EP, Kontos HA, Cristman CW, Dewitt DJ, Povlishoch JT: Superoxide generation and reversal of acetylcholine - induced cerebral arteriolar dilation after acute hypertension. Circ Res 1985;57:781-787.

95. Guarnieri C, Flamigni F, Caldarera CM: Role of oxygen in the cellular damage induced by reoxygenation of hypoxic heart. J Mol Cell Cardiol 1980;12:797-808.

96. Hess ML, Manson NH, Okabe E: Involvement of free radicals in the pathophysiology of ischemic heart disease. Can J Physiol Pharmicol 1982;60:1382-1389.

97. Shlafer MS, Caine PF, Wiggins VY, Kirsch MM: Possible role for cytotoxic oxygen metabolites in the pathogenesis of cardiac ischemic injury. Circulation 1982;66(1):85-92.

98. Gardner TJ, Stewart JR, Casale AS, Downey JM, Chambers DE: Reduction of myocardial ischemic injury with oxygen-derived free radical scavengers. Surgery 1983;94:423-427.

99. Baker GL, Corey RJ, Autor AP: Oxygen derived free radical induced damage in kidneys subjected to warm ischemia and reperfusion. Ann Surg 1985;202:628-641.

100. Korthuis RJ, Granger DN, Townsley MI, Taylor AE: The role of oxygen derived free radicals in ischemia induced increases in canine skeletal muscle vascular permeability. Circ Res 1985;57:599-609.

101. Manson PN, Anthenelli RM, Im MJ, Bulkley GB, Hoopes JE: The role of oxygen-free radicals in ischemic tissue injury to island skin flaps. Ann Surg 1983;198:87-90.

102. Granger DN, Rutili G, McCord JM: Superoxide radicals in feline intestinal ischemia. Gastroenterol 1981;81:22-29.

103. Grogaard B, Parks DA, Granger DN, McCord JM, Forsberg O: Effects of ischemia and oxygen radicals on mucosal albumin clearance in intestine. Am J Physiol 1982;242(5):G448-454.

104. Granger DN, Parks DA: Role of oxygen radicals in the pathogenesis of intestinal ischemia. Physiologist 1983;26(3):159-164.

105. Parks DA, Bulkley GB: Role of oxygen-derived free radicals in digestive tract diseases. Surgery 1983;94(3):415-422.

106. Carati CJ, Rambaldo S, Gannon, Sholes BJ: Changes in macromolecular permeability of microvessels in rat small intestine after total occlusion ischemia-reperfusion. Microcirc Endothelium Lymphatics 1988;4:69-86.

107. DelaTorre JC (ed): Biological actions and medical applications of dimethylsulfoxide. N NY Acad Sci 1983;411.

108. Fox RB, Fox WK: Dimethyl sulfoxide prevents hydroxyl radical mediated depolymerization of hyaluronic acid. ANN NY Acad Sci 1983;411:14-18.

109. Alsup EM, Debowes RM: Dimethyl sulfoxide. J Am Vet Med Assoc 1984;185:1011-1014.

110. Granger DN, Shepherd AP: The intestinal circulation: A historical perspective. In Shepherd AP, Granger DN (eds) Physiology of the Intestinal Circulation. New York, Raven Press, 1984, pp. 1-8.

111. Harvey W: Movement of the heart and blood in animals. An anatomical essay (translated by KJ Franklin). Blackwell Oxford 1957, cited by Granger DN, Shepherd AP (eds) Physiology of the Intestinal Circulation. New York, Raven Press, 1984.

112. Leeuwenhoek AV: Anatomy of the slime within the guts and the use thereof. Phylos Trans R Soc Lond 1684, cited by Granger DN, Shepherd AP (eds) Physiology of the Intestinal Circulation. New York, Raven Press, 1984.

113. Hales S: Statistical essays: containing haemostaticks. Royal Society, Reprinted by Hafner Publishing Co, New York,1964, cited by Granger DN, Shepherd AP (eds) Physiology of the Intestinal Circulation. New York, Raven Press, 1984.

114. Poiseulle JLM: Recherches sur les causes du mouvement du sang les veins. J Univer Hebd Med Chir Prat 1830;1:289, cited by Granger DN, Shepherd AP (eds) Physiology of the Intestinal Circulation. New York, Raven Press, 1984.

115. Heller A: Uber die Blugefasse des Dunndarmes. Ber Sachs Ges Wiss 1872;24:265-171, cited by Granger DN, Shepherd AP (eds) Physiology of the Intestinal Circulation. New York, Raven Press, 1984.

116. Mall JP: Die Blut und Limph wege. In Dunn darm des Hundes. Abh Sachs Ges Wiss 1888;14:153-189. cited by Granger DN, Shepherd AP (eds) Physiology of the Intestinal Circulation. New York, Raven Press, 1984.

117. Lundgren O: Bloodflow distribution and countercurrent exchange in the small intestine. Acta Physiol Scand (Suppl) 1967;303:1-42.

118. Reynolds DG, Brim J, Sheehy TW: The vascular architecture of the small intestinal mucosa. Anat Rec 1967;159:211-218.

119. Brodie TG, Vogt H: The gaseous metabolism of the small intestine, Part I. The gaseous exchange during the absorption of water and dilute salt solutions. J Physiol 1910;40:135-172.

120. Wells HS, Johnson RG: The intestinal villi and their circulation in relation to absorption and secretion of fluid. Am J Physiol 1934;109:387-402.

121. Winne D: Formal kinetics of water and solute absorption with regard to intestinal blood flow. J Theor Biol 1970;27:1-18.

122. Fara JW, Rubenstien EH, Sonnenschein: Intestinal hormones in mesenteric vasodilation after intraduodenal agents. Am J Physiol 1972;223:1058-1067.

123. Larsen H, Chumley J: The effect of distention on blood flow through the intestines. Am J Physiol 1940;131:368-377.

124. Sidkey MM, Bean JW: Influence of rhythmic and tonic contractions of intestinal muscle on blood flow and blood reservoir function in dog intestine. Am J Physiol 1958;193:386-392.

125. Chou CC, Grassmick B: Motility and blood flow distribution within the wall of the gastrointestinal tract. Am J Physiol 1978;235:H34-H39.

126. Mayerson HS, Wolfrom CE, Shirley HH, Wasserman K: Regional difference in capillary permeability. Am J Physiol 1960;198:155-160.

127. Clementi F, Palade GE: The intestinal capillaries I. Permeability to peroxidase and ferritin. J Cell Biol 1969;41:33-58.

128. Mortillaro NA, Taylor AE: Interaction of capillary and tissue forces in the cat small intestine. Circ Res 1976;39:348-358.

129. Witte MH, Witte CL, Dumont AE: Estimates of net transcapillary water and protein flux in the liver and intestine of patients with portal hypertension from hepatic cirrhosis. Gastroenterol 1981;80:265-273.

130. Langley JN, Andersson HK: On the innervation of the pelvic and adjoining viscera. J Physiol (Lond.) 1895;18:67-105.

131. Burton-Opitz R: Ueber die stroemung des blutes in dem frebiete der pfortader. Arch f ges Physiol 1908;124:469-510 cited by Granger DN, Shepherd AP (eds) Physiology of the Intestinal Circulation. New York, Raven Press, 1984.

132. Hulten L: Extrinsic nervous control of colonic motility and blood flow. Acta Physiol Scand 1969;335:1-116.

133. Johnson PC: Myogenic nature of increased intestinal vascular resistance with a venous pressure elevation. Circ Res 1959;6:992.

134. Folkow B, Lewis BH, Lundgren O, Mellander S, Wallentin I: The effect of graded vasoconstrictor fiber stimulation on the intestinal resistance and compacitance vessels. Acta Physiol Scand 1964;6:445-457.

135. Selkurt EE, Rothe CF, Richardson D: Characteristics of reactive hyperemia in the canine intestine. Circ Res 1964;15:530-544.

136. Shepherd AP, Granger HJ: Autoregulatory escape in the gut: a systems analysis. Gastroenterol 1973;65:77-91.

137. Carmichael EA, Doupe J, Harper AA, McSwiney BA: Vasomotor reflexes in man following distension. J Physiol (Lond.) 1939;95:276-281.

138. Selkurt EE, Johnson PC: Effect of acute elevation of portal venous pressure on mesenteric blood volume, interstitial fluid volume and hemodynamics. Circ Res 1958;6:592-599.

139. Nishiyama K, Nishiyama A, Frohlich ED: Regional blood flow in normotensive and spontaneously hypertensive rats. Am J Physiol 1976;230:691-698.

140. Chou CC (ed): Contribution of splanchnic circulation to overall cardiovascular and metabolic homeostasis. Fed Proc 1983;42:1656-1693.

141. Cumen W: Cases of severe burns with dissections and remarks. Edinb Med Surg J 1823;19:337-344.

142. Swan J: Practical observations. Edinb Med Surg J 1823;19:344-347.

143. Long J: On the post-mortem appearance found after burns. London Med Gazette 1840;5:743-750.

144. Kirskey TD, Monkreef JA, Pruitt BA: Gastrointestinal complications in burns. Am J Surg 1968;116:627-633.

145. Law EJ, Day SB, MacMillan BG: Autopsy findings in the upper gastrointestinal tract of 81 burn patients. Arc Surg 1971;102:412-416.

146. Penner A, Burnheim AI: Acute postoperative enterocolitis. A study on the pathologic nature of shock. Arch Pathol 1939;27:966-983.

147. Pettet JD, Baggenstose AH, Dearing WH: Pseudomembraneous enterocolitis. Surg Gynecol Obstet 1954;98:546-552.

148. Hamilton M, Bonnet MJ, Lowe TE: Ischemic episodes in cardiac failure: Acute arterial insufficiency with and without structural blockage. Med J Aust 1955;42:93-98.

149. Ende N: Infarction of the bowel in cardiac failure. Eng J Med 1958;258:879-881.

150. Berger RL, Burn JJ: Intestinal gangrene associated with heart disease. Surg Gynecol Obstet 1961;112:529-533.

151. Shor-Pinsker E, Hernandez OA: Intestinal infarction: A complication of gastroenteritis and endotoxic shock in children. Arch Surg 1971;102:187-189.

152. Weil MH: Morphologic changes in dogs following the production of shock with endotoxin and their comparison to the morphologic changes occurring during shock associated with bacteremia in patients. J Clin Invest 1958;37:940-941.

153. Haglund U, Jodal M, Lundgren O: The small bowel in arterial hypotension and shock. In Shepard AP, Granger DN (eds). Physiology of the Intestinal Circulation. New York, Raven Press, 1984, pp. 305-320.

154. Bynum TE, Gallavan RH, Jacobson ED: The pathophysiology of nonocclusive intestinal ischemia. In Shepard AP, Granger DN (eds). Physiology of the Intestinal Circulation. New York, Raven Press, 1984, pp. 369-376.

155. Bailey RW, Bulkley GB: Role of the circulation in neonatal necrotizing enterocolitis. In Kvietys PR, Barrowman JA, Granger DN (eds). Pathophysiology of the Splanchnic Circulation. Vol. 1. Boca Raton, CRC Press Inc, 1987, pp. 141-164.

156. Marston A: The bowel in shock: the role of mesenteric arterial disease as a cause of death in the elderly. Lancet 1962;2:365-396.

157. Wilson R, Qualheim RE: A form of acute hemorrhagic enterocolitis aflicting chronically ill individuals. Gastroenterol 194;27:431-444.

158. Hoffman FG, Zimmerman SL, Cardwell ES: Massive intestinal infarction without vascular occlusion associated with aortic insufficie ncy. Eng J Med 1960;263:436-440.

159. Norris HT: Reexamination of the spectrum of ischemic bowel disease. In Norris HT (ed) Contemporary Issues in Surgical Pathology. Vol 2. Pathology of the Colon, Small Intestine and Anus. New York, Churchill Livingston, 1983, pp. 109-120.

160. Cotton PB, Lea Thomas M: Ischemic colitis and the contraceptive pill. Brit Med J 1971;3:27-28.

161. Blalock A: A study of thoracic duct lymph in experimental crush injury and injury produced by gross trauma. Johns Hopk Hosp Bull 1943;72:54-61.

162. Katzenstein R, Mylon E, Winternitz MC: The toxicity of thoracic duct fluid after release of tourniquets applied to the hind legs of dogs for the production of shock. Am J Physiol 1943;39:307-312.

163. Wiggers HC, Ingraham RC: Hemorrhagic shock: definition and criteria for its diagnosis. Am J Physiol 1945;1:30-36.

164. Lillehei RC: The intestinal factor in irreversible hemorrhagic shock. Surgery 1957;42:1043-1054.

165. Williams LF, Goldberg AH, Polanski BJ, Burn JJ: Myocardial effects of acute intestinal ischemia. Surgery 1969;66:138-144.

166. Lefer AM: Vascular mediators in ischemia and shock. In Cowley RA, Trump BF (eds), Pathophysiology of shock, anoxia and ischemia. Baltimore, Williams and Wilkins, 1982, pp. 165-180.

167. Watson DW, Sodeman WA. The small intestine. In Sodeman WA, Sodeman TM (eds). Pathologic Physiology - Mechanisms of Disease. 6th Edition Philadelphia, W.B. Saunders Co. 1979, pp. 824-859.

168. Robinson JWL, Mirkovitch V, Winistorfer B, Saegesser F: Response of the intestinal mucosa to ischemia. Gut 1981;22:512-527.

169. Parks DA, Jacobson ED: Intestinal ischemia. In Kvietys PR, Barrowman JA, Granger DN (eds). Pathophysiology of the Splanchnic Circulation. Vol 1. Boca Ratan, CRC Press Inc. 1987, pp. 125-140.

170. Shephard AP, Reidel GL: Differences in reactive hyperemia between the intestinal mucosa and muscularis. Am J Physiol 1984;247:G617-G622.

171. Martin AW, Fuhrman FA: The relationship between summated tissue respiration and metabolic rate in mouse and dog. Physiol Zool 1955;28:18-34.

172. Hardaway RM, McKay DG: Intravascular thrombi and the intestinal factor in irreversible shock. Ann Surg 1959;150:161-165.

173. Lundgren O, Haglund U: The pathophysiology of the intestinal countercurrent exchanger. Life Sci 1978;23:1411-1422.

174. Parks DA, Bulkley GB: Role of oxygen-derived free radicals in digestive tract diseases. Surgery 1983;94:415-422.

175. Auscher C, Amory W: The histochemical localization of xanthine oxidase in the rat liver. Biomedicine 1976;25:37-38.

176. Barnett WO, Petro AB, Williamson JW: A current apraisal of problems with gangrenous bowel. Ann Surg 1976;183:653-659.

177. Bounous G, Hampson LG, Gurd FN: Regional blood flow and oxygen consumption in experimental hemorrhagic shock. Arc Surg 1963;87:340-354.

178. Bounous G, Hampson LG, Gurd FN: Cellular nucleotides in hemorrhagic shock. Relationship of intestinal metabolic changes to hemorrhagic enteritis and the barrier function of intestinal mucosa. Ann Surg 1964;160:650-666.

179. Bounous G, McArdle AH, Hodges DM, et al: Biosynthesis of intestinal mucin in shock: relationship to trypic hemorrhagic enteritis and permeability to curare. Ann Surg 1966;164:13-22.

180. Bounous G: Role of the intestinal contents in the pathophysiology of acute intestinal ischemia. Am J Surg 1967;114:368-375.

181. Dekoos EB, Gibson WJ, Bounous G, et al: The influence of pancreatic duct ligation on the course of E. coli endotoxin shock in the dog. Can J Surg 1966;9:227-230.

182. Bounous G, Brown RA, Mulder DS, et al: Abolition of "tryptic enteritis" in the shocked dog. Arch Surg 1965;91:371-375.

183. Bounous G, Menard D, DeMedicis E: Role of pancreatic proteases in the pathogenesis of ischemic enteropathy. Gastroenterol 1977;73:102-108.

184. Bounous G, Proulx J, Konok G, et al: The role of bile and pancreatic proteases in the pathogenesis of ischemic enteropathy. Int J Clin Pharmacol Biopharm 1979;17:317-323.

185. Bounous G, Sutherland NG, McArdle AH, et al: A prophylactic use of an "elemental diet" in experimental hemorrhagic shock and intestinal ischemia. Ann Surg 1967;166:312-342.

186. Bounous G, Devroede G, Hugon JS, et al: Effects of an elemental diet on the pancreatic proteases in the intestine of the mouse. Gastroenterol 1973;64:577-582.

187. Guilbert J, Bounous G, Gurd FN. Role of intestinal chyme in the pathogenesis of gastric ulceration following experimental hemorrhagic shock. J Trauma 1969;9:723-742.

188. Neslon AW, Clear JR, Griner LA: Acute surgical colonic infarction in the horse. Am J Vet Res 1968;29:315-327.

189. Allen DW, White NA, Tyler DE: Factors for prognostic use in equine obstructive small intestinal disease. J Am Vet Med Assoc 1986;189:777-780.

190. Allen DW, White NA, Tyler DE: Morphologic effects of experimental distension on equine small intestine. Vet Surg 1988;17:10-14.

191. Snyder JR, Olander HJ, Pascoe JR, Holland M, Kurpershoek CJ: Morphologic alterations observed during experimental ischemia of the equine large colon. Am J Vet Res 1988;49:801-809.

192. Moore JN, White NA, Trim CM, Gana HE: Effect of intraluminal oxygen in intestinal strangulation obstruction in ponies. Am J Vet Res 1980;41:1615-1620.

193. Parker JE, Fubini SL, Car CD, Erb HN: Prevention of intraabdominal adhesions in ponies by low dose heparin therapy. Vet Surg 1987;16:459-462.

194. Singh A: Chemical and biochemical aspects of superoxide radicals and related species in activated oxygen. Can J Physiol Pharmacol 1982;60:1330-1345.

195. McCord JM: The superoxide free radical: its biochemistry and pathophysiology. Surgery 1983;94:412-414.

196. Dickerson RE: Chemical evolution and the origin of life. Sci Am 1978;239:70-86.

197. Del Maestro RF: An approach to free radicals in medicine and biology. Acta Physiol Scand 1980;(Suppl)492:153-168.

198. Bulkley GB: The role of oxygen free radicals in human disease processes. Surgery 1983;94:407-411.

199. Fridovich I: The biology of oxygen radicals. Science 1978;201:875-880.

200. Haber F, Willstatter R: Unpaarigkeit und Radikalketten im Reaktiousmechanismus organischer und enzymatischer. Vorgange Ber Desch Chem Ges 1931;64:2844-2856. cited by Petkau A: Introduction: free radical involvement in physiological and biochemical processes. Can J Physiol Pharmacol 1982;60:1327-1329.

201. Petkau A: Introduction: free radical involvement in physiological and biochemical processes. Can J Physiol Pharmacol 1982;60:1327-1329.

202. Kahn AU, Kasha M, Red: Chemiluminesence of molecular oxygen in acqueous solution. J Chem Phys 1963;39:2105-2106.

203. Foote CS, Wexler S: Olefin oxidations within exited singlet molecular oxygen. J Am Can Soc 1964;86:3879-3880.

204. McCord JM, Fridovich I: The reduction of cytochrome c by milk xanthine oxidase. J Biol Chem 1968;243:5753.

205. McCord JM, Fridovich I: Superoxide dismutase: an enzymatic function for erhythrocuprein (hemocuprein). J Biol Chem 1969;244:6049-6055.

206. Fridovich I: Superoxide radicals, superoxide dismutases and the aerobic life style. Photochem Photobiol 1978;28:733-741.

207. Allen RC, Stjernholm RL, Steele RH: Evidence for the generation of an electronic excitation state in human polymorphonuclear leukocytes and its participation in bacteriocidal activity. Biochem Biophys Res Commun 1972;47:679-684.

208. Babior BM: The enzymatic basis for O_2 production by human neutrophils. Can J Physiol Pharmacol 1982;60:1353-1358.

209. Fantone JC, Ward PA: Role of oxygen-derived free radicals and metabolites in leukocyte-dependant inflammatory reactions. Am J Path 1982;107:397-418.

210. Weiss SJ, LoBuglio AF: Phagocyte-generated oxygen metabolites and cellular injury. Lab Invest 1982;47:5-18.

211. Singh A, Singh H: Timescale in nature of radiation-biological damage: approaches to radiation protection and postirradiation therapy. Prog Biophys Mol Biol 1982;39:69-107.

212. Palor AE, Martin D, Parker JC: The effect of oxygen radicals on pulmonary edema formation. Surgery 1983;94:433-438.

213. Greenwald RA, Moy WW: Inhibition of collagen gelation by action of the superoxide radical. Arthritis Rheum 1979;22:251-259.

214. Morgan AR, Cone RI, Elgert TM: The mechanism of DNA strand breakage by vitamin C and superoxide and the protective roles of catalase and superoxide dismutase. Nucleic Acids Res 1976;3:1139-1149.

215. Petrone WF, English DK, Wong K, McCord JM: Free radicals and inflammation: superoxide-dependant activation of a neutrophil chemotactic factor in plasma. Proc Natl Acad Sci USA 1980;77:1159-1163.

216. Ohmori H, Komoriya K, Azuma A, Kurozumi S, Oto YH: Xanthine oxidaseinduced histamine release from isolated rat peritoneal mast cells; involvement of hydrogen peroxide. Biochem Pharmac 1979;28:233-234.

216. McCord M, Roy RS: The pathophysiology of superoxide; roles in inflammation and ischemia. Can J Physiol Pharmicol 1982;60:1346-1352.

218. Granger DN, Rotili G, McCord JM: Superoxide radicals in feline intestinal ischemia. Gastroenterol 1981;81:22-29.

219. Granger DN, Tayler AE: Permeability of intestinal capillaries to endogenous macromolecules. Am J Physiol 1980;238:H457.

220. Granger DN, Tayler AE: Permselectivity of intestinal capillaries. Physiologist 1980;23:47-52.

221. Granger DN, Sennett M, McElearney P, et al: Effect of local arterial hypertension on cat intestinal capillary permeability. Gastroenterol 1980;78:474.

222. Battelli MG, Della Corte E, Stirpe F: Xanthine oxidase type D (dehydrogenase) in the intestine and other organs of the rat. Biochem J 1972;126:747.

223. Della Corte E, Stirpe F: The regulation of rat liver xanthine oxidase. Biochem J 1972;126:739.

224. Del Maestro R: The influence of oxygen-derived free radicals on in vitro and in vivo model systems. Doctoral thesis Univ. Uppsala (Sweden) 1979. cited by Granger DN, Rotilli G, McCord JM. Superoxide radicals in feline intestinal ischemia. Gastroenterol 1981;81:22-29.

225. Crowell JW, Jerns CE: Effect of allopurinol on hemorrhagic shock. Am J Physiol 1969;216:744.

226. DeWall RA, Vasko KA, Stanley EL, et al: Responses of the ischemic myocardium to allopurinol. Am Heart J 1971;82:362.

227. DeMartino GN, Kuers K: Two calcium-dependent, calmodulin stimulated proteases from rat liver. Fed Proc 1981;40:1738.

228. Chou CC, Dabney JM: Interrelation of ileal wall compliance and vascular resistance. Am J Dig Dis 1967; 12(12):1198-1208.

229. Chou CC: Relationship between intestinal blood flow and motility. Ann Rev Physiol 1982; 44:29-42.

230. Chou CC, Gallavan RH: Blood flow and intestinal motility. Fed Proc 1982; 41:2090-2095.

231. Selkurt EE, Rothe CF, Richardson D: Characteristics of reactive hyperemia in the canine small intestine. Circ Res 1969; 15:532-544.

232. Granger HJ, Norris CP: Role of adenosine in local control of intestinal circulation in the dog. Circ Res 1980; 46:764-770.

233. Shepherd AP, Riedel GL: Differences in reactive hyperemia between intestinal mucosa and muscularis. Am J Physiol 1984; 247:G617-622.

234. Mortillaro NA, Granger HJ: Reactive hyperemia and oxygen extraction in the feline small intestine. Circ Res 1977; 41(6):859-865.

235. Rosenblum W: Dimethyl sulfoxide effects on platelet aggregation and vascular reactivity in pial microcirculation. Ann NY Acad Sci 1983;411:110-119.

236. Granger DM, Barrowman JA: Microcirculation of the alimentary tract II: Pathophysiology of edema. Gastroenterol 1983; 84:1035-1049.

237. Kontos HA. Oxygen radicals in cerebral vascular injury. Circ Res 1985, 57:508-516.

238. Rosenblum WI, El-Sabban F: Dimethyl sulfoxide (DMSO) and glycerol, hydroxyl radical scavengers, impair platelet aggregation within and eliminate the accompanying vasodilation of injured mouse pial arterioles. Stroke 1982; 13(1):35-39.

239. Bulkley GB, Krietys PR, Parks DA, Perry MA, Granger DN: Relationship of blood flow and oxygen consumption to ischemic injury in the canine small intestine. Gastroenterol 1985; 89:852-857.

240. Bounous G: Pancreatic proteases and oxygen-derived free radicals in acute ischemic enteropathy. Surgery 1986; 90(1):92-94.

241. Rijke RPC, Hanson WR, Plaisier HM, Osborne JW: The effect of ischemic villus cell damage on crypt cell proliferation in the small intestine. Gastroenterol 1976, 71:786-792.

242. Davies JJ, Gerring EL: Effect of experimental vascular occlusion on small intestinal motility in ponies. Equine Vet J 1985; 17(3):219-224.

243. Walus KM, Jacobson ED: Relation between small intestinal motility and circulation. Am J Physiol 1981; 241(4):G1-15.

244. Nelson RA, Beargie RJ: Effect of reduced arterial pressure and flow on intestinal motility. Surg Gynecol Obstet 1965; 120:1221-1224.

245. Abe H, Appert H, Casbello J, Howard JM: Nonmucosal seratonin in motility of the small bowel when subjected to acute anoxic trauma. Arch Surg 1973; 106:183-187.

246. Kobold EE, Thal AP: Quantitation and identification of vasoactive substances liberated during various types of experimental and clinical intestinal ischemia. Surg Gynecol Obstet 1963; 117:315-322.

247. Bayliss WM, Starling EH: The influence of blood supply on the intestinal movements. J Physiol (London) 1898; Suppl 23:34-35.

248. Necheles H, Olson WH: Experimental investigation of the effects of trauma and traumatic shock on gastrointestinal motility and secretions. Am J Physiol 1942; 136:32-37.

249. Van Liere EJ, Northrup DW, Stickney JE: The effect of anemic anoxia on peristalsis of the small and large intestine of the dog. Fed Proc 1944; 3:50.

250. Hamilton AS, Collins DA, Oppenheimer MJ: Effects of blood pressure levels on intestinal motility. Fed Proc 1944; 3:17.

251. Vakin KG, Mason JW: The influence of hemorrhage and of depletion of plasma proteins on intestinal activity. Gastroenterol 1945; 4:92-101.

252. Chou CC: Intestinal compliance. Am J Dig Dis 1967; 12(12):1189-1197.

253. Bergan JJ, Gillilaud V, Troop C, Anderson MC: Hyperkalemia following intestinal revascularization. JAMA 1964; 187(1):17-19.

254. Van der Meer C, Valkenberg PW, Snijders PM: Studies on hyperkale mia as a cause of death in intestinal ischemic shock in rats. Circ Shock 1986; 19:329-345.

255. Reimer KA, Jennings RB: Ion and water shifts, cellular in Cowley RA, Trump BE (ed). Pathophysiology of shock, anoxia, and ischemia. Baltimore, Williams and Wilkins, 1982; 132-146.

256. Rose DB: Clinical Physiology of Acid-Base and Electrolyte Disorders. New York, McGraw-Hill, 1984; 254-255.

257. Stick JA, Chou CC, Derksen FJ, Arden WA: Effects of xylazine on equine intestinal vascular resistance, motility, compliance and oxygen consumption. Am J Vet Res 1987; 48(2):198-203.

258. Rijke RPC, Hanson WR, Plaisier HM, Osborne JW: The effect of ischemic villus cell damage on crypt cell proliferation in the small intestine. Gastroenterol 1976; 71:786-792.

259. Haglind E, Haglund H, Lundgren O, Stenberg B: Mucosal lesions of the small intestine after intestinal vascular obstruction in the rat. Acta Chircand 1985; 151:147-150.