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EQUINE COLONIC TORSION -
THE EFFECT OF HEPARIN THERAPY

By

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ABSTRACT

EQUINE COLONIC TORSION - THE EFFECT OF HEPARIN THERAPY

By

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In 20 anesthetized ponies the effects of 1 hour of colonic torsion followed by 1 hour of reperfusion, with and without heparin pretreatment (80 IU/kg of body weight), were determined on systemic arterial pressure (SAP), plasma thromboxane and prostacyclin concentrations, and colonic blood flow (CBF), vascular resistance (CVR) and histologic morphology. Ponies were allotted into 4 equal groups: Control; Control/Heparin; Torsion; Torsion/Heparin. Torsions were created by 720° rotation of the cecum and colon around their long axes. Heparin was administered 30 minutes into the experiment. Data was analyzed ($p \leq 0.05$) using split-plot analysis of variance and Kruskal-Wallis-H tests. Heparin prevented the colonic detorsion-induced hypotension and the increases in CVR and thromboxane, while significantly increasing CBF during reperfusion. It did not alter prostacyclin concentration or histologic appearance of the large colon. This study suggests that heparin is a beneficial treatment during surgery for equine colonic torsion.

This work is dedicated to Dr. John Caron, for without his encouragement and advice it would have never materialized.

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In appreciation for their efforts I would like to thank my committee for their help, especially Dr. John Stick for having the desire to know more about the large colon, and to recognize the efforts of Dr. Jon Patterson for his expertise in large colon pathology and Valerie Crump for her help with the anesthetic equipment.

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INTRODUCTION

In the horse, several types of colic are recognized which differ in respect to the cause, clinical signs, prognosis and treatment. Torsion or volvulus of the large colon, reported in 11 - 17% of horses treated surgically for colic, represents the most severe and fatal form.[1] Survival is dependent upon early recognition, surgical correction and intense supportive care. Even with optimum therapy, survival rates are low, ranging from less than 10 to 42%.[2-4] This review highlights the clinical signs of large colon torsion in the horse, outlines the probable pathophysiology and provides background information on a drug that has been included in its treatment - heparin.

Large Colon Torsion

Horses with strangulating torsions of the large colon present with an acute, severe colic which is unresponsive to analgesics.[1-3] Clinical signs include an unwillingness to stand, diminished gastrointestinal motility, and tympany. Nasogastric reflux can occur as tension increases on the duodenal colic ligament. The abdominocentesis sample, initially normal, with time will have an elevation in both total protein and nucleated cells. Pathognomonic of the condition, though, is the rectal palpation of a distended large colon with a thickened, edematous bowel wall. As the disease process continues, shock is evident; there is a rapid, weak pulse, tachycardia, tachypnea, congested mucus membranes, and hemoconcentration. The horse will often die despite treatment.

The exact cause of colonic torsion remains unknown, but it has been suggested that the large colon is predisposed to displacement because of its limited anchoring attachments.[1] The equine large colon, an extension of the basic mammalian ascending colon, forms a C-shaped loop within the abdomen. It extends cranioventrally from the base of the cecum on the right, to the diaphragm, forming the right ventral colon, reflects caudally to form the left ventral colon until, at the caudal extent of the abdomen, it turns dorsally, forming the pelvic flexure. From this point, the colon backtracks creating first the left, and then, the right dorsal colon. The right dorsal colon progresses caudally, and in front of the root of the mesentery, becomes the transverse colon leading to the small colon. The right ventral colon is attached to the cecum by the cecocolic fold; the dorsal and ventral colons are attached to each other by the mesocolon; and the cecum is attached at its base to the root of the mesentery. The remaining colon is supported only by other abdominal viscera and the body wall.[5] This leaves approximately 80% of the length of the colon free to move within the abdomen.

In theory, any luminal obstruction of the dorsal colon will lead to gas accumulation in the ventral colons as a result of normal fermentation processes. The lighter gas filled colon, unsecured, can then rise in relation to the obstructed colon, initiating a torsion. Other initiating causes of torsion have been hypothesized and include: abnormal motility resulting from parasitic damage to the blood vessels, rolling, change in diet and recent parturition.[6,7]

The blood supply to the large colon is derived from the cranial mesenteric artery as the dorsal colic artery and the ileocecocolic artery; the latter branches to form the ventral colic artery. The dorsal and ventral colic arteries enter the right colon and then traverse the extent of the colon within the colonic mesenteric border, becoming continuous at the pelvic flexure.[5,8] The blood vessels enter into the right dorsal and ventral colons from the cecum. Unfortunately, this is the same site where most torsions occur, and the vascular supply to the entire colon becomes compromised.[1] Torsions of less than 180° may not cause vascular embarassment, but torsions of 270° - 720° have been associated with complete ischemia.[1,4,9]

Pathophysiology

Equine large colon research is limited, but data extrapolated from other species, and from small intestine research, indicate several variables which modify the pathology, including: degree and duration of ischemia, tissue metabolic rates, reperfusion, and bacterial endotoxin. Complete vascular occlusion produces the most rapid progression of injury, from increased mucosal permeability seen after only 10 minutes (and increasing proportionally with the duration of ischemia) to complete loss of intestinal villi after 1 hour, and transmucosal necrosis after 4 hours.[10-13] Similar histopathologic changes have been recorded in experimentally induced small intestinal strangulation obstruction in ponies.[14] In these ponies a grade I lesion (slight separation of villus tip epithelial cells from the lamina propia) was apparent after 30 minutes of ischemia and progressed to a grade IV lesion (complete epithelial separation involving the entire villus, marked lamina proprial and submucosal hemorrhage and edema) after 180 minutes. The severity of the mucosal lesion resulting from ischemia has been shown to be strongly correlated with mortality.[15]

In comparing decreased blood flow with that of total ischemia, the mechanism of injury is more complex.[16] In partial vascular occlusion, autoregulation and increased oxygen extraction act as protective mechanisms against cellular injury.[17,18] The innervated small intestine is able to autoregulate blood flow, keeping it constant, over a wide range of perfusion pressures.[19] In the cat, arterial hypotension (50-55 mm of Hg) reduces small intestine blood flow, but not in proportion to the reduction in perfusion pressure; the intestinal vascular resistance decreases concurrently to maintain blood flow.[17] To further compensate, oxygen extraction is increased during periods of hypoperfusion to maintain oxygen uptake. In both the dog and cat, oxygen uptake becomes flow dependent only below 30 ml/min/100 g.[18,20,21] Above this critical value, even for extended periods of hypoperfusion, oxygen uptake is flow-independent.[18] In limited investigations, the colon, however, does not appear to be efficient at autoregulation, nor at increasing oxygen uptake. In two studies involving the canine colon, colonic vascular resistance increased in response to a reduction in perfusion pressure, while control of colonic oxygenation was only apparent at perfusion pressures greater than 70 mm of Hg.[19,22] It is probable that the equine large colon, like the canine colon, is inefficient at autoregulation and would sustain severe injuries during low flow states, as well as in complete ischemia. Therefore, dependent on the site and its inherent intrinsic control of blood flow and oxygenation, a complexity of tissue injury can result.

The muscularis and the mucosa, the two main morphologic regions of the intestine, have different oxygen requirements.[16] The mucosa with a higher metabolic rate, is more susceptible to the effects of anoxia. Hypoxia at the tips of small intestine villi is a key factor in the development of tissue injury. The normal countercurrent oxygen exchange mechanism between the arterial vessel and the subepithelial capillary network of the villus is altered during ischemia.[16] The transit time of blood through the villus during reduced perfusion pressure is slowed, which increases the time for oxygen exchange to subendothelial capillaries. This markedly reduces the oxygen available to the villus tip and its epithelial cells die due to insufficient oxygenation. This supports the progression of microscopic lesions from the tip of the villus to the base, as seen in the dog, cat, human being and horse.[13,14,23,24]

Unfortunately necrosis is not limited to the ischemic period. It has been shown in the cat that the mucosal injury produced by 3 hours of ischemia and 1 hour of reperfusion is more severe than that produced by 4 hours of ischemia without reperfusion.[25] The injury presumably results from oxygen free radical peroxidation of cell membrane lipids and degradation of basement membrane, extracellular matrix collagen and hylauronic acid. Interruption in membrane integrity results in increased vascular permeability, mucosal structural alterations, and epithelial necrosis.[26-34] It is hypothesized that oxygen free radicals are generated from reactions beginning in the ischemic period. Ischemia-induced cellular catabolism of adenosine triphosphate high energy bonds results in a 10 to 20 fold increase in hypoxanthine, an oxidizable substrate of xanthine oxidase. Xanthine oxidase, thought to exist in normal liver and gastrointestinal cells as an NAD+ reducing dehydrogenase, becomes rapidly and irreversibly converted to the oxidase form during ischemia. Oxygenation during reperfusion then results in rapid, aerobic oxidation of the newly synthesized hypoxanthine by xanthine oxidase, leading to the generation of superoxide (O_2-).[16,32] Evidence to support this hypothesis comes from the observation that superoxide dismutase, a superoxide radical scavenging enzyme[30,33]; allopurinol, a xanthine oxidase inhibitor[26,30,33]; thiopental, a free radical anion scavenger[27]; and, dimethyl sulfoxide, a hydroxyl radical scavenger[26,35] will all reduce the extent of injury following reperfusion in both the cat and the rat. The use of free oxygen radical scavengers is not however always effective in decreasing injury. In complete vascular occlusion, oxygen free radical inhibitors appear to be ineffective.[12,36] It is postulated that reperfusion may only be significant in regional hypotension and not in total ischemia.

Intestinal injury is further augmented by the effects of bacteria and their endotoxins, and bacteria may be crucial to the development of intestinal necrosis. In a model comparing ischemia in germ-free rats with ischemia in conventionally colonized rats, bowel integrity was preserved in germ-free rats, while necrosis occurred in 75% of the conventionally colonized animals.[37] In the healthy horse, gram negative bacteria represent a stable population within the intestine, which during normal growth processes constantly release endotoxin into the intestinal lumen. Detection of endotoxin, therefore, is considered normal within the intestinal lumens. The hepatic reticuloendothelial system and the intact intestinal mucosa prevent bacteria and their endotoxins from entering the systemic circulation.[38,39] However, ischemia-induced mucosal barrier injury facilitates the entry of bacteria and endotoxin into the systemic circulation.[40,41]

Endotoxin transport into the systemic circulation is mediated by both lymphatic transport from the peritoneal cavity to the thoracic duct and by direct absorption into the portal circulation.[40,41] In mice, endotoxin challenge disrupts the zonula occludens between intestinal epithelial cells, providing a route for entry to the systemic circulation from the intestinal lumen.[42] In another mouse model, endotoxin administration, in a dosage dependent manner, promoted bacterial transport from the intestinal lumen into the mesenteric lymph nodes.[43] In ponies, following experimental strangulation/obstruction of the distal jejunum, endotoxin was found in both the peritoneal cavity and in the systemic circulation.[44]

The effects of endotoxins are multiple and have been shown experimentally to increase intestinal capillary permeability, injure cardiac cells and pulmonary endothelium, and decrease endothelial cell proliferation.[33,45-48] It is hypothesized that the delay in endothelial regeneration after focal or diffuse cell loss leads to prolonged exposure of subendothelial collagen fibrils, resulting in continuous activation of cellular and serum factors.[48] In the pony, documented effects of exogenous endotoxin administration include: pyrexia, early leukopenia/late leukocytosis, lactic acidosis, arterial hypoxemia, thrombocytopenia, altered glucose metabolism, and hemodynamic and hemostatic abnormalities.[49-55]

The hemodynamic changes of endotoxin-induced shock consistently cause heart rate elevation and a transient increase in arterial blood pressure and central venous pressure. Arterial blood pressure and central venous pressure return to baseline within 5 minutes, but progressively decline over the next hour. Simultaneously blood flow is redistributed to dilating intestinal mesenteric beds.[49,51] Blood flow in both the small intestine and large colon increases following endotoxin challenge, while intestinal vascular resistance decreases.[51] As a result of the splanchnic vasodilation, peripheral tissue perfusion decreases and cells are forced into anaerobic glycolysis. Within 30 minutes of endotoxin administration systemic lactic acidosis and hypotension have occurred.[50] A major hemostatic change associated with infusion of endotoxin is the initiation of coagulopathies.[45,49] Endotoxin, by activation of complement, factor XII and tissue thromboplastin, can activate the intrinsic and extrinsic coagulation pathways.[56-59] In ponies, experimental infusion of endotoxin results in mild prolongation of coagulation times, marked reduction in platelet numbers, and reduced antithrombin III (AT III) concentrations.[52,53] These findings, although not pathognomonic for disseminated intravascular coagulation, suggest a consumptive coagulopathy. Diffuse intravascular coagulation has, however, been documented in experimentally induced intestinal strangulations of the equine small intestine, as well as in the clinical colic case.[44,60,61] In a study recording the prevalence of hemostatic abnormalities in colic, all horses presenting with either colonic torsion, small intestinal strangulation, or intussusception had an abnormal coagulogram at the time of examination.[62] Altered activated partial thromboplastin times and AT III concentrations were the most frequently observed abnormalities. A decrease in AT III concentration was highly correlated with mortality.[61,63] It is implied that these alterations are the result of endotoxemia.

Of current interest in research is the effect of endotoxin on endoperoxide production, specifically, the production of thromboxane (TxA_2) and prostacyclin (PGI_2). Endotoxin interaction with cellular plasma membranes activates phospholipase C which initiates arachidonic acid metabolism.[64] In a sequence of enzymatic conversions, arachidonate is oxidized to PGG_2 by cyclooxygenase. PGG_2 is then reduced to form PGH_2, a pivitol endoperoxide that is further metabolized to either TxA_2 or PGI_2, depending on the cell and tissue type.[65,66] Within the platelet, PGH_2 is metabolized by thromboxane synthetase to TxA_2 and within vascular endothelial cells, by prostacyclin synthetase, to form PGI_2. The actions of TxA_2, a platelet aggregator and potent vasoconstrictor, are thought to be counterbalanced by the vasodilatory and platelet inhibitory effects of PGI_2. A disturbance in the balance of these two vasoactive amines is hypothesized to be responsible for the pathologic processes, including thrombosis, associated with endotoxemia.[67]

Studies in horses, dogs, calves, rats, and persons have documented increased plasma concentrations of both, following endotoxic challenge. The increase in TxA_2 occurs rapidly, while that of PGI_2 is delayed.[68-76] Similar changes in the rate of production have been documented in _in vitro_ studies with equine peritoneal macrophages.[65] These changes in production are thought to mediate the hemodynamic abnormalities associated with endotoxemia. This theory is supported by multiple studies that have ameliorated the effects of endotoxin by pretreatment with cyclooxygenase inhibitors, including: indomethacin, aspirin, imidazole, phenylbutazone, ketoconazole, flunixin meglumine.[66,69,70,77-81] The latter is the most commonly used drug in the treatment of equine colic and endotoxic shock. Although TxA_2 is generally considered to be destructive, whereas PGI_2, beneficial, selective inhibition of TxA_2 does not appear to benefit the endotoxin challenge horse.[68,82,83] In ponies lactic acidosis, hypotension, and decreased venous return continued despite pretreatment with a thromboxane synthetase inhibitor.[82] Thromboxane synthetase inhibition results in an increased concentration of PGH_2 which is thought to be redirected to PGI_2 production. Both endothelial cells and monocytes are able to metabolize the excess.[84] The balance between these two eicosanoids is delicate and appears essential for normal homeostasis.

In summary, the injury associated with large colon torsion because of the effects of reperfusion and endotoxemia is not simply limited to the ischemic period. Torsion of the colon results in a continuum of local cellular injury and complex, widespread, systemic alterations, of which activation of the coagulation pathway is of major importance. In addition to surgical correction, treatment of the disease includes antimicrobial, nonsteroidal and fluid therapy. Anticoagulant therapy has been widely recommended for the prevention and correction of the associated hypercoagulopathies. Heparin has been the most commonly recommended anticoagulant.[1,2,61]

Heparin

Heparin, the oldest antithrombotic drug still in clinical use, was first identified in the early 1900s by McClean, Howell, and Hott in their search for blood procoagulants in liver extracts.[85-87] Heparin, so called from its identification within the liver, "showed a marked power to inhibit coagulation".[86] Clinical trials began in 1938, and in 1948 heparin was recognized as a clinically useful inhibitor of blood coagulation.[87] In recent years there has been considerable attention directed towards its chemistry and mode of action. The identification of heparin's interaction with AT III has provided a new understanding of how heparin works. Nevertheless, a complete knowledge of its nature and biological actions is far from complete.

Chemically heparin represents a heterogenous group of straight-chain, anionic mucopolysaccharides, more familiarly known as glycosaminoglycuronan sulphate esters, or glycosaminoglycans. The heparins range in molecular weights, from as low as 4000 up to 40,000 daltons (average of 15,000) and are endogenously found in mast cells and in basophilic leukocytes as heparan sulfate, a macromolecule of 750,000 daltons.[87,88] Commercial heparin is prepared from either bovine lung or porcine intestinal mucosa; sites of high mast cell concentrations, and consists of polymers of two repeating disaccharide units: D-glucosamine-L-iduronic acid and D-glucosamine-D-glucuronic acid. Most samples contain 8 - 15 units of each disaccharide, with the percentage of each being variable (Figure 1).[87] The source, as well as the inherent heterogeneity of different chain lengths, have led to numerous discussions about possible differences in potency and in side effects, with some researchers believing that porcine derived heparin is more potent and associated with a lower incidence of thrombocytopenia.[89] In the United States the sodium salt of heparin is available and is standardized to produce a product based on USP units (United States Pharmacopea units), thus eliminating the heterogeneity by expressing dosage in units of anticoagulant activity rather than milligrams of heparin. One USP unit is the quantity of heparin that will prevent 1.0 ml of citrated sheep plasma from clotting for 1 hour after the addition of 0.2 ml $CaCl_2$ solution.[87] No current scientific studies justify the selection of one type of heparin over the other.[90] In addition to the above unfractionated product, fractionated heparin products, comprised of low molecular weights (<10,000 daltons), as well as synthetic heparinoids with specific molecular weights are available and have been advocated as an effective method of prophylaxis, having a greater anti-Xa activity, but a decreased risk of hemorrhage.[85,91,92] To date, however, an increased benefit/risk ratio has not been demostrated in persons.[93,94]

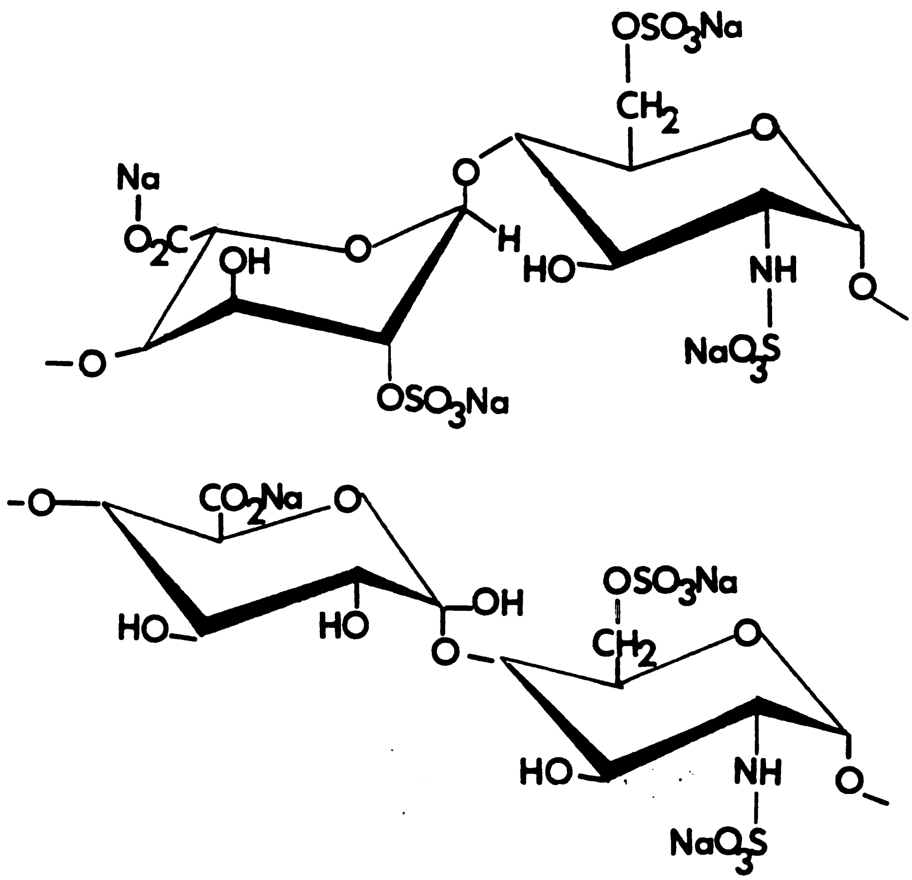

FIGURE 1 - Heparin, a commercial polymer of 2 repeating disaccharide units: D-glucosamine - L-iduronic acid (upper)
D-glucosamine - D-glucuronic acid (lower)

The pharmacokinetics of heparin are nonlinear. Its half-life increases with increasing dosages, while the rate of clearance from blood decreases with increasing dosages. This is modulated by both the uptake by the reticuloendothelial system and by heparin's adherance to the endothelium.[87,88,95] The liver enzyme heparinase depolymerizes and desulfates heparin and the inactive products are eliminated in the urine. With high dosages, heparin itself may appear in the urine. Hepatic disease or renal failure understandably prolongs the half-life of the anticoagulant activity. In general, the half-life in man is given as 90 minutes for typical therapeutic dosages.[88] The route and dosage are dependent on the therapeutic plan desired.

The hemostatic mechanism involves a series of dependent actions consisting of both procoagulant and anticoagulant factors. In the process of vessel wall repair, platelets initiate the first line of defense by accumulating at the site of exposed subendothelial collagen fibrils. Platelet adhesion is dependent upon binding von Willebrand factor, an endothelial secreted large protein multimer, onto the platelet's surface glycoprotein GP Ib. As platelets adhere to the subendothelium they become activated through exposure to collagen and thrombin. Although, thrombin and collagen bind to different platelet surface receptors, each is able to activate the enzyme Phospholipase C, which in turn, hydrolyzes membrane inositol phospholipids. Hydrolysis generates diacyl glycerol and inositol triphosphate which act synergistically to evoke the cellular responses of platelet activation. Major platelet responses include oxidation of arachidonic acid (AA) and release of platelet activating factor (induces cohesion, secretion, and AA metabolism), platelet factor V (essential for thrombin generation on the platelet surface), thrombospondin (important in platelet adhesion and cohesion), platelet derived growth factor (promotes vessel wall repair), platelet factor 4 (capable of neutralizing the anticoagulant activity of heparin), and adenosine diphosphate (an amplifier of platelet activation).[96,97]

Arachidonic acid, following its release from the glycerol backbone of phospholipids, is metabolized within the platelet by two pathways, the lipoxygenase and the cyclooxygenase pathways. The products from the platelet lipoxygenase pathway at this time have an unknown physiologic role. The cyclooxygenase pathway oxidizes AA to PGH_2, a cofactor for collagen induced platelet stimulation, to PGD_2, a minor inhibitor of platelet activation, and eventually to TxA_2. Thromboxane is thought to bind with a platelet surface receptor, similar to collagen and thrombin, and likewise activate Phospholipase C leading to generation of diacyl glycerol and inositol triphosphate. In damaged endothelial cells AA is also metabolized, in this site to PGI_2. Platelet inhibition by PGI_2 is mediated by activation of adenyl cyclase, which increases platelet cAMP. Additionally, PGH_2 released from platelets can be taken up and metabolized by endothelial cells to PGI_2. The balance between the two, PGI_2 and TxA_2, is postulated to play an important role in vascular injury.[96,97]

Thrombin's role in the hemostatic process is to stabilize the platelet hemostatic plug by secondarily generating a fibrin network. Reactions leading to the generation of thrombin are included by what has been commonly referred to as the intrinsic and extrinsic clotting cascade systems and are outlined in Figure 2. The initial trace amounts of factor Xa (in the presence of Ca^{++} and procoagulant phospholipid) act

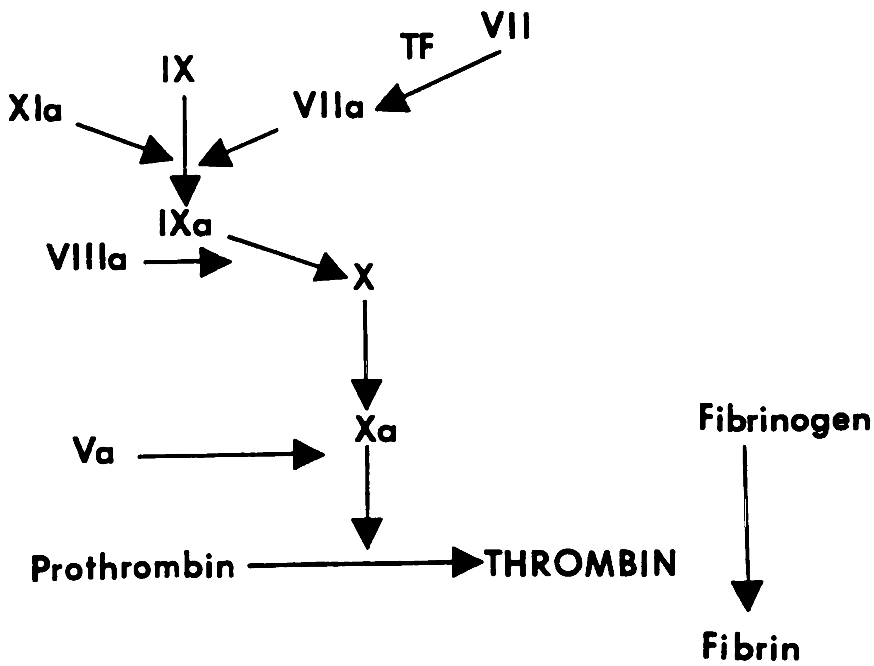

FIGURE 2 - Diagram of the coagulation cascade.

as a positive feedback mechanism on factor VII, rapidly activating it in the presence of tissue thromboplastin (cofactor) to VIIa. While thrombin, in trace amounts, initiates the proteolysis of factors V and VIII to activated factors Va and VIIIa. Factor VIIIa is a required cofactor of IXa, and Va is a required cofactor of Xa. The dual activation of factor IX by both XIa and tissue thromboplastin - VIIa is required for normal hemostasis. Although the reactions generating thrombin occur on cell surfaces, thrombin is able to diffuse into the fluid phase to form fibrin strands by fibrinogen cleavage. In the presence of XIIIa, fibrin is crosslinked to fibronectin, and fibronectin to collagen, stabilizing the forming thrombus. These reactions are essential for normal hemostasis and wound healing.[96,97]

Regulation of coagulation is dependent on numerous interactions. Serine coagulant proteases are inactivated by several protease inhibitors which include AT III, C-1 esterase inhibitor, alpha 1 protease inhibitor, alpha 2 macroglobulin, protein C, plasminogen activator, and heparin cofactor II.[98-101] The AT III reaction appears to be the most important inhibitor of coagulation. It accounts for 75% of the thrombin inhibiting activity in plasma. It can additionally neutralize the proteolytic activity of activated factors IX, X, XI, XII, kallikrein and plasmin.[92,102-105] Antithrombin III inactivates the serine proteases by forming an irreversible 1:1 stoichiometric complex between the protease's active serine site and an arginyl residue near the carboxy terminal end of AT III.[104,106] These reactions proceed at a moderate rate with the proteases preferentially combining with their specific substrates rather than with AT III. However, in the presence of heparin this inactivation is significantly accelerated. Antithrombin

III binds through lysine to heparin's tetrasaccharide chains, undergoes a conformational change and is catalytically converted from a slow inactivator of thrombin to an extremely rapid one without affecting the 1:1 stoichiometry of the thrombin:AT III reaction.[92,107] After formation of the AT III-thrombin-heparin complex, heparin dissociates and initiates the reaction elsewhere.[107]

Although the AT III interaction is thought to be the most significant at this time, heparin can independently affect blood coagulation without AT III. The inhibition of the thrombin-fibrinogen reaction, the inactivation of factor X, Xa, and prothrombin have all been shown in the absence of AT III. These reactions, however, proceed more rapidly in the presence of AT III.[86] Heparin is also known to interact directly with platelets.[108,109] Platelet-bound-heparin inhibits the binding of thrombin, and in this way may prevent platelet aggregation.[86] But this is not heparin's only action, as multiple studies have demostrated that heparin can stimulate platelet aggregation, as well as platelet TxA_2 release.[109-111] A possible explanation for the conflicting reports is the differences in molecular weight fractions, and in the presence or absence of AT III. High molecular weight fractions appear to be more active in induction of platelet aggregation than low molecular weight fractions in the presence of AT III.[108,112,113] The low molecular weight heparin has a higher binding affinity for AT III than it does for platelets. The clinical significance of these facts can only be speculated at this time.

Of recent importance is the identification of heparin-induced release of extrinsic pathway inhibitor (EPI). This inhibitor, thought to be synthesized by endothelial cells, acts as a potent inhibitor of the factor VIIa-tissue thromboplastin complex. Its activity is increased dose dependently following heparin administration. This relationship may in part contribute to heparin's success as an anticoagulant.[114]

In persons heparin is used clinically in three main ways: in the prevention of venous thromboembolism, in the treatment of overt thrombosis, and as an anticoagulant for extracorporeal circulations.[85] Therapeutic regimens are modified according to the therapeutic objective desired. When heparin is used prophylactically in patients at risk for thromboembolism it is given as a low dosage, subcutaneous treatment several hours prior to surgery. The 5000 unit dosage/70 kg of body weight is repeated every 8 -12 hours until the patient is fully ambulatory.[88,115] The rationale for low dose heparin therapy is based on the recognition that factor X's affinity for the heparin/AT III complex is greater than factor X's affinity for prothrombin.[116] Inhibition at this level prevents the positive feedback mechanism of thrombin and therefore, less heparin is necessary than is required for the neutralization of thrombin. Prophylactic therapy has reduced the 25% incidence of surgery-induced deep venous thrombosis to 10%.[115]

Low dosages of heparin are inadequate in the treatment of overt thrombosis, in which high concentrations of circulating thrombin molecules must be neutralized.[85,87,117] Thrombotic treatment incorporates an intravenous loading dosage of 10,000 units followed by dosages of 5000 to 10,000 units intravenously every 4 to 6 hours. Ultimately the dosage and frequency are based on patient response as measured by the activated partial thromboplastin time. The APTT should be maintained near 1.5 times the control prethrombotic value.[87,88,118]

Although heparin is used primarily for its vascular effects, this is only one realm of its activity. It has also been advocated in the treatment of endotoxic shock, the treatment of peritonitis, in the reduction of fibrinous adhesions, and as a protectant against ischemia-reperfusion injury. Its use in the treatment of endotoxic shock is associated with conflicting results. Pretreatment of dogs prior to *E. coli* endotoxin infusion prevented the decrease in both fibrinogen and blood pressure.[119] Pretreatment, as well as post treatment out to 120 minutes after endotoxin administration with heparin has been documented by several investigators to significantly increase survival rates in rats.[120-123] In peritonitis resulting from experimentally induced necrosis of the ileum in dogs, heparin treated dogs were able to resolve the peritonitis, in contrast to those not receiving heparin therapy.[124] Other studies in the dog and in persons have failed to show an increase in patient survival following heparin administration.[123,125] The differences in these outcomes may be due to species differences and to different dosage regimes.

The mechanism by which heparin provides protection against endotoxin is not clear. It has been hypothesized that protection is afforded through the reticuloendothelial system by increasing phagocytosis and preserving the structural integrity of the liver and spleen.[120,126] A second theory suggests that the increased rate of endotoxin clearance is secondary to heparin's prevention of formation of fibrin and intermediate coagulation products, thereby freeing the liver of its role to clear fibrin.[127] Scientific support for either theory is absent.

The treatment of peritonitis and the reduction of adhesion formation have been based on heparin's ability to decrease fibrin formation. Fibrin is formed in any acute inflammatory reaction when thrombin is released from injured tissue and occurs as part of the wound healing process. In experimentally induced peritonitis in both the dog and rat, heparin therapy reduced the duration of peritonitis and increased survival times.[128-131] It is postulated in these studies that heparin renders bacteria more susceptible to host defense mechanisms and antibiotic therapy by preventing the production of fibrin and abscesses that can entrap bacteria. In the absence of sepsis, fibrin can still be detrimental if it forms undesirable adhesions. Heparin has been shown to reduce postoperative abdominal and intra-ocular adhesions.[132,133] The question that arises in concert with reduction of fibrin formation is: How does heparin influence the wound healing process? Reports have been varied, but the most recent investigation concluded that heparin does not negatively influence the healing of abdominal wounds or colonic anastomoses.[134,135] Several studies have investigated heparin's protective effects against ischemia induced injury. In an experimental model of induced cerebral ischemia in Rhesus monkeys, preheparinization reduced the incidence of cerebral infarction.[136] Similar benefits were demonstrated with heparin pretreatment in a rat model of ischemia-induced renal injury.[137] In the dog, heparin significantly reduced the ischemic injury and necrosis of the myocardium following temporary occlusion of coronary arteries; and, heparinization resulted in a significant reduction in the incidence of infarction and edema formation in the canine gracilis muscle following ischemia.[138,139] In rabbits, however, treatment following 4, 4.5, and 5 hours of ischemia was not associated with improvement. In fact, based on histologic examination, treatment resulted in a significantly decreased viability of the ischemic segments.[140] (Although no criteria were given for what determined histologic viability.) In the studies reporting significant protection, the mechanism for protection was not identified. Theories, however, included possible anticoagulant, antiplatelet, and antiinflammatory effects.[138]

The use of heparin in the horse has been based on many of the premises outlined above and on the results of limited primary investigations. Reports of heparin's success in the horse have included the prevention of experimentally induced laminitis, the prevention of endotoxin associated lactic acidosis and clinical distress, and in decreased adhesion formation in ponies following experimentally induced intestinal ischemia.[141-143] Even though scientific data supporting its use has been limited, it continues to be advocated for the prevention and the treatment of disease processes associated with hypercoagulable states including: colic, diarrhea, acute laminitis, septicemia, and endotoxemia.[1,144-146]

As the pharmacokinetics in the horse have not been investigated, a wide range of dosages, empirically based on data from persons, have been recommended and used. In persons a plasma concentration of 0.2 to 0.5 units of heparin/ml, which results in a 1.5 to 2.5 times prolongation in APTT, significantly decreases the incidence of venous thromboembolism.[118,147,148] Prolongation of the equine APTT to 1.5 to 2 times its reference range results in a similar plasma heparin concentration of 0.2 to 0.4 units/ml.[149] Dosages as low as 40 IU/kg of body weight (BW) have been documented to increase the APTT to almost 1.8

times baseline values following injection into clinically normal horses; a six fold increase is seen following 100 IU of heparin/kg of BW.[150] In a recent paper by Gerhards, however, subcutaneous administration of 150 units of calcium heparin/kg of BW, followed with 125 units/kg of BW at twelve hour intervals only resulted in mean plasma heparin values between 0.05 U/ml and 0.17 U/ml, well below the human plasma heparin concentrations thought to be effective.[151] In this study, dosages as high as 150 U/kg of BW of subcutaneously administered heparin had no significant effect on APTT. As Gerhard used calcium heparin, this disparity may be explained as a pharmacokinetic difference between the sodium and calcium salts of heparin in the horse. At this time, current references recommend 40 to 160 IU of heparin/kg of BW subcutaneously two to three times per day.[152]

Associated with the use of heparin are several possible complications including hemorrhage, and in persons, an immune mediated thrombocytopenia.[153] A complication unique to the horse is the reduction of circulating erythrocytes following heparin administration. This reduction, initially thought to result from reticuloendothelial phagocytosis of the erythrocyte is now known to result from erythrocyte agglutination and has been associated with dosages greater than 40 IU/kg of BW.[154-157] A reduction in erythrocyte number is seen approximately 72 hours following the initiation of treatment and return to normal as early as 24 hours after the last heparin dose is given.[154,157] It is speculated that if this agglutination occurs in the microvasculature, that heparin therapy in the horse may be deleterious.[157]

SPECIFIC OBJECTIVES

In many species heparin has been shown to be a beneficial treatment for ischemia and endotoxin induced coagulopathies. These benefits have not been adequately documented in the horse. In fact, heparin may be harmful if erythrocyte agglutination results in decreased peripheral tissue perfusion. To determine if heparin administration is a beneficial or detrimental adjunct treatment for large colon torsion in the horse we designed a study with the following specific objectives:

1. To determine systemic arterial pressure, plasma thromboxane and prostacyclin concentrations, colonic blood flow, colonic vascular resistance and the associated histopathologic changes of the equine large colon following experimental torsion - detorsion.
2. To evaluate the effects of systemically administered sodium heparin (80 IU/kg of BW) on the above parameters following experimental torsion - detorsion.

MATERIALS AND METHODS

Experimental Design

Twenty healthy ponies (4 geldings, 7 mares and 9 stallions) ranging in age from 2 to 30 years and weight from 111.4 to 227.3 kg were randomly and equally allotted into four treatment groups. In each group, a 60 minute surgical recovery period preceded the 120 minute experimental period in which data were recorded. Measured parameters during the experiment included systemic arterial pressure (SAP), colonic blood flow (CBF), colonic vascular resistance (CVR), plasma thromboxane B_2 concentration (TxB_2), plasma 6-keto-PGF_{1a} concentration, colonic tissue pH (pH), and the assessment of colonic histologic morphology. Blood samples for the determination of TxB_2 and 6-keto-PGF_{1a} were collected from the right jugular vein at the start of the 120 minute experimental period (time 0), 60 (immediately following detorsion when applicable), and 120 minutes. Colonic biopsies were harvested at the completion of the 120 minutes immediately prior to euthanasia. The four groups were as follows:

1. Control - All parameters were measured without the effects of heparin or large colon torsion.

2. Control/Heparin - Measurements as in Group 1 with the exception that 80 IU of sodium heparin/kg of BW was administered intravenously 30 minutes into the recording period.

3. Torsion - At the start of the recording period a 720° torsion of the large colon was created within the abdomen by rotating the large colon and cecum about their long axes. Torsion was maintained for 60 minutes, followed by detorsion and 60 minutes of reperfusion.

4. Torsion/Heparin - Similar to Group 3 with the exception that 80 IU of sodium heparin/kg of BW was administered intravenously 30 minutes into the recording period.

Experimental Protocol

All ponies were vaccinated against equine influenza, encephalomyelitis and tetanus, and were treated with an anthelmetic (pyrantel pamoate, 6 mg/kg of BW, PO) at least one month prior to initiation of the study. All ponies were healthy 24 hours before each experiment, as judged from physical examination. Ponies were fed a diet of mixed hay and oats and had access to water up until the time of anesthetic induction.

Anesthesia was induced without preanesthetic using 10% thiamylal (6.6 to 11 mg/kg of BW) administered through a 14 gauge, 12.5 cm catheter placed into the left jugular vein. Halothane and 100% oxygen delivered by a semi-closed anesthetic system by endotracheal tube was used for anesthetic maintenence. Anesthetized ponies were mechanically ventilated. The ponies were positioned in dorsal recumbency, and balanced electrolyte solution was administered through the jugular catheter at the rate of 11 ml/kg of BW/h. The right facial artery was cannulated with PE-240 tubing, which was connected to a pressure transducer and a direct writing oscillograph to continuously record SAP. Through a ventral midline celiotomy, the pelvic flexure of the large colon was identified and exteriorized. While exteriorized, the large colon temperature was monitored by a thermistor probe at the pelvic flexure. Temperature was maintained at 37° C by an overhead heat lamp.

Determination of Colonic Blood Flow and Vascular Resistance

Blood flow to the pelvic flexure of the large colon was recorded continuously by a perivascular electromagnetic flow probe (Appendix) placed around the ventral colic artery, 10 cm proximal to the pelvic flexure. The ventral colic artery was prepared for placement of the probe by careful isolation and ligation of its mural branches over a 2 cm distance. The dorsal colic artery was ligated 10 cm distal to the pelvic flexure.[a] (Figure 3)

Electromagnetic flow probes[b] were calibrated at the completion of each experiment by direct cannulation of the artery with PE-360 tubing distal to the flow probe and measurement of the outflow over time. The weight of the perfused pelvic flexure (extending from the site of the flow probe aborally to the ligated dorsal colic artery) was then determined and blood flow rates standardized per 100 g tissue weight. Selection of the appropriate probe size was made by measuring the artery diameter with a caliper prior to its manipulation. A proper fit to

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[a]The decision to measure blood flow to the pelvic flexure through only the ventral colic artery was made after 3 pilot studies. In these studies blood flow through both the ventral and dorsal colic arteries was examined by the use of electromagnetic flow probes and by direct cannulaiton. The average blood flow through the ventral colic artery represented 81.5% of the total flow to the pelvic flexure, while that through the dorsal colic artery contributed 18.5%. When either artery was occluded, blood flow through the remaining artery increased to compensate. Additionally, surgical isolation of the smaller dorsal colic artery from the adjacent, extensive rete mirabli system was more difficult than to isolate the ventral colic artery and often resulted in thrombus formation. Therefore, only blood flow through the ventral colic artery was measured.

[b]Statham Instruments Division, Oxnard, CA.

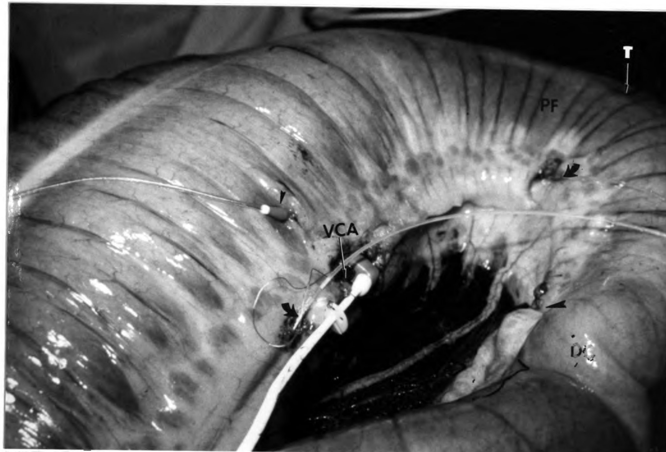

FIGURE 3 - Photograph of the large colon experimental model. The electromagnetic flow probe is shown encircling the ventral colic artery (VCA). The ground lead for the probe (small black arrow) is sutured to the ventral colon. The two arterial pressure lines are indicated by the curved black arrows. Temperature thermistor (T) is inserted at the pelvic flexure (PF). The large black arrow indicates the site of ligation of the dorsal colic artery. (DC = dorsal colon).

record mean blood flow was achieved with a probe 10-20% smaller than the vessel diameter. All but 2 ponies were instrumented with a 4.0 mm diameter probe. The remaining two small ponies (111.4 and 113.6 kgs in body weight) were instrumented with a 3.0 mm diameter flow probe. To further ensure that the probe did not restrict blood flow, a 20 gauge needle, which was connected to PE-90 tubing, a pressure transducer and direct writing oscillograph was inserted into the ventral colic artery 5 cm proximal to the flow probe. The pressure gradient between this arterial pressure line, and to a similar one inserted into the artery at the pelvic flexure, was used to determine flow probe size.[c] The arterial perfusion pressures recorded at the pelvic flexure were used in the determination of colonic vascular resistance. Colonic vascular resistance (R) was calculated by dividing the colonic arterial pressure (P) by colonic blood flow (Q): R (mm Hg/ml/min/100g) = P/[(Q'/k)* 100], where k = tissue weight in grams of the perfused segment.

Determination of Plasma Thromboxane and Prostacyclin Concentrations

Venous blood samples were collected from a 14 gauge, 12.5 cm catheter inserted into the right jugular vein. Samples were collected into plastic test tubes containing cold 100 mM EDTA (pH = 7.4) and 10 mM of the prostaglandin synthesis inhibitor sodium meclofenamate (1:20 with blood). Samples were stored on ice, centrifuged for 10 minutes and the plasma harvested and stored at -70°C until assayed by radioimmunoassay.

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[c]Pilot studies performed on two ponies identified a normal pressure gradient of 5 mm of Hg between these two sites in the absence of the flow probe. If the pressure gradient exceeded 5 mm of Hg, a larger probe size was selected.

The plasma was analyzed for TxB_2 and 6-keto PGF_{1a}, the stable breakdown products of TxA_2 and PGI_2, respectively. Standards were prepared by diluting authentic TxB_2 or 6-keto-PGF_{1a} to various concentrations in prostanoid-free horse plasma. Standards were checked for parallelism. TxB_2 and 6-keto-PGF_{1a} antisera were reconstituted in phosphate-buffered saline containing 0.5% gelatin (PBSG) at a dilution which would bind approximately 40% of the radiolabeled prostanoid in the absence of unlabeled prostanoid. The labeled compound was diluted with PBSG to a radioactive level of 5000-6000 cpm/assay tube. One-hundred microliters each of the radiolabeled prostanoid, antibody, and standard or sample were mixed together and incubated for 16-24 hours at 4°C to allow binding. The binding reaction was terminated by the addition of 1.0 ml dextran-coated charcoal suspension to precipitate the unbound prostanoid. After centrifugation at 300 g for 12 minutes (0°C), supernatant fluid was collected into vials containing 15 ml of scintillation coctail[d] and the radioactivity determined in a Beckman Model LS-3150P scintillation counter. The cross reactivity of the TxB_2 antibody with 6-keto-PGF_{1a}, PGF_{1a}, PGF_{2a}, PGE_1, PGE_2, PGD_2, PGA_1, PGA_2, PGB_1, PGB_2, dihydroxy-keto-E_1, dihydroxy-keto-F_{1a}, 5-HETE, 12-HETE, and 15-HETE was < 0.1%. The cross reactivity of the 6-keto-PGF_{1a} antibody with PGF_{1a} = 7.8%, 6-keto-PGE_1 = 6.8%, PGF_{2a} = 2.2%, PGE_1 = 0.7%, PGE_2 = 0.6%, and with TxB_2, PGD_2, PGA_1, PGA_2, PGB_1, PGB_2, 15-keto-PGF_{2a}, 15-keto-PGE_2, dihydroxy-keto-E_2, dihydroxy-keto-F_{2a} was < 0.1%.

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[d]Safety Solve, Research Products International, Mt. Prospect, Il.

Determination of Colonic Tissue pH

Colonic tissue pH was monitored by a glass pH electrode[e] inserted into the tunica muscularis at the pelvic flexure. Measurements were obtained in 7 ponies: one Control, and two from each of the remaining groups.

Histopathologic Examination

A 2.5 x 2.5 cm, full thickness tissue biopsy was harvested by sharp dissection from the area of the pelvic flexure. The segment was pinned to a cardboard base under tension to prevent fragmentation and disruption of the tissue layers, and was fixed in 10% phosphate-buffered formalin. Following fixation, the specimens were trimmed, embedded in paraffin, sectioned at 4 µm, and processed routinely.

Hematoxylin-and-eosin-stained sections were examined by light microscopy and evaluated in a blind manner for morphologic evidence of 1)vascular responses and 2)tissue degeneration and necrosis. Each parameter was scored from 0 to 3 according to the following scheme:

1) Vascular responses: 0 = histologically normal; 1 = marked hyperemia within the mucosa and submucosa; 2 = marked hyperemia and edema; edema present diffusely in the submucosa and occasionally within the lamina propria; 3 = marked hyperemia and edema, and moderate to marked hemorrhage; hemorrhage multifocal or diffuse within the submucosa and/or in the lamina propria.

2) Tissue degeneration and necrosis: 0 = histologically normal; 1 = moderate to marked multifocal necrosis of the subepithelial lamina

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[e]Khuri Regional Tissue pH Monitor, Vascular Technology, Inc., Chelmsford, MA.

propria at the tips of mucosal folds; 2 = changes seen in grade 1 above, and in addition, mild to marked multifocal degeneration and/or necrosis of mucosal epithelium of mucosal fold tips; 3 = changes seen in grade 2 above, but with degeneration and/or necrosis of mucosal epithelium involving at least the distal one-half of mucosal-folds; in some areas, necrosis extended into the recesses of mucosal folds.

Each histopathologic sample received a combined score ranging from 0 to 6 based on the extent of their vascular and tissue degeneration/necrosis responses.

Statistical Analyses

Statistical analysis of all parametric data (except pH), was performed using a between-within/split-plot analysis of variance. Post ANOVA comparisons between and within treatment group means were made with a modified Bonferroni t-test.[158] Small sample size precluded statistical analysis of the pH data. The non-parametric histopathologic scores were compared using a Kruskal Wallis-H test. Significance level was set at $p \leq 0.05$ in each analysis.

RESULTS

I. Hemodynamics

A. Systemic Arterial Pressure (SAP) (Figure 4)

In the Control and the Control/Heparin groups, SAP remained stable (means between 90 and 105 mm of Hg) throughout the recording period. In the Torsion group, SAP was not significantly different from baseline (86.9 $\pm$ 7.3 mm of Hg) during the 60 minutes of colonic torsion, but immediately following detorsion SAP decreased and was significantly lower than Control group pressures for 20 minutes. In the Torsion/Heparin group, SAP remained stable and was not significantly different from baseline (94.7 $\pm$ 11.3 mm of Hg) or the Control group at any time during or following colonic torsion.

B. Colonic Blood Flow (CBF) (Figure 5)

Mean colonic blood flow ranged from 41.2 $\pm$ 14.7 to 54.8 $\pm$ 11.7 ml/min/100 g of tissue weight in the Control and Control/Heparin groups during the experiment. In both the Torsion and Torsion/Heparin groups, CBF was decreased to 0 ml/min/100 g by torsion of the large colon. In the Torsion group, following correction of the torsion, CBF failed to recover and remained significantly low for 50 minutes. In the Torsion/Heparin group following colonic detorsion, CBF returned to flows similar to the nontorsion groups and was significantly higher than the non-heparin treated Torsion group for 40 minutes.

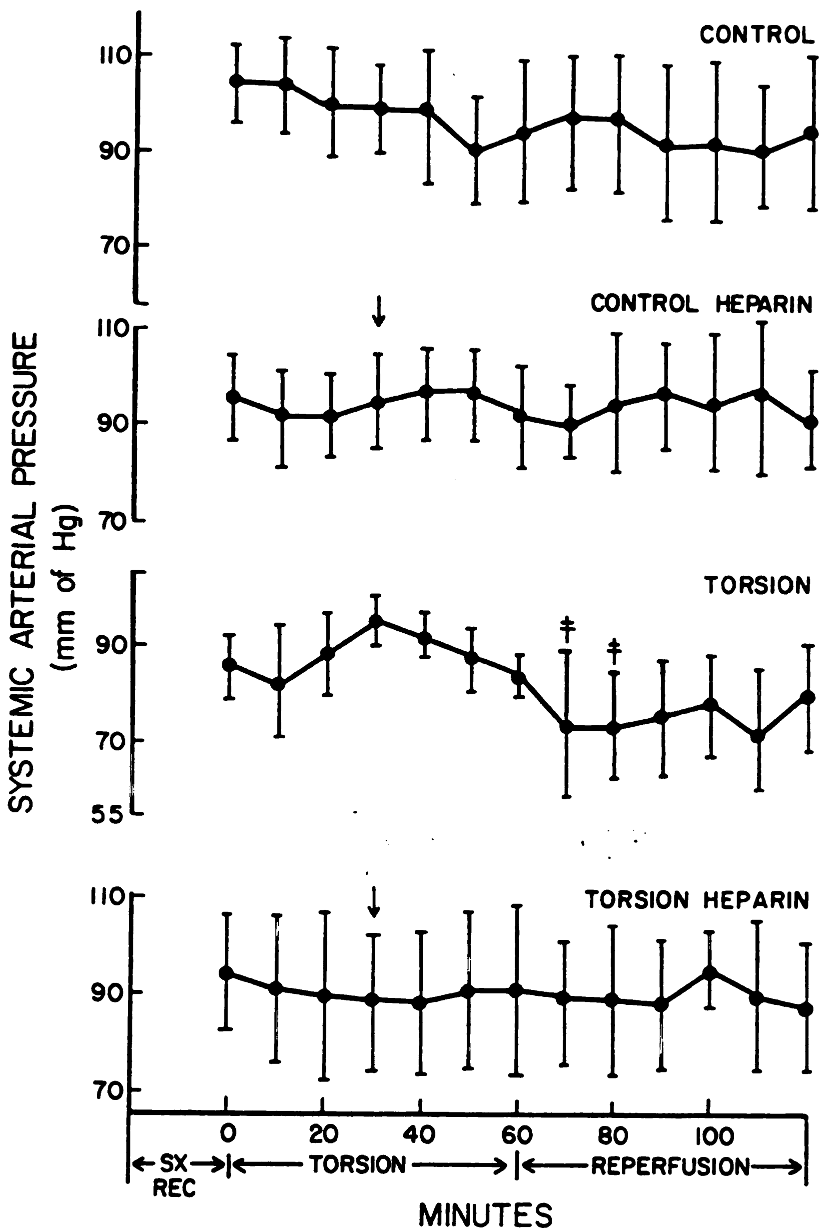

FIGURE 4 - The mean (± SEM) systemic arterial pressure of Control, Control/Heparin, Torsion, and Torsion/Heparin groups during the 120 minute experiment. The arrow indicates the time of heparin administration. Significant differences ($p \leq 0.05$) identified:
x - different than baseline; + - different than Control group;
* - different than Torsion group.

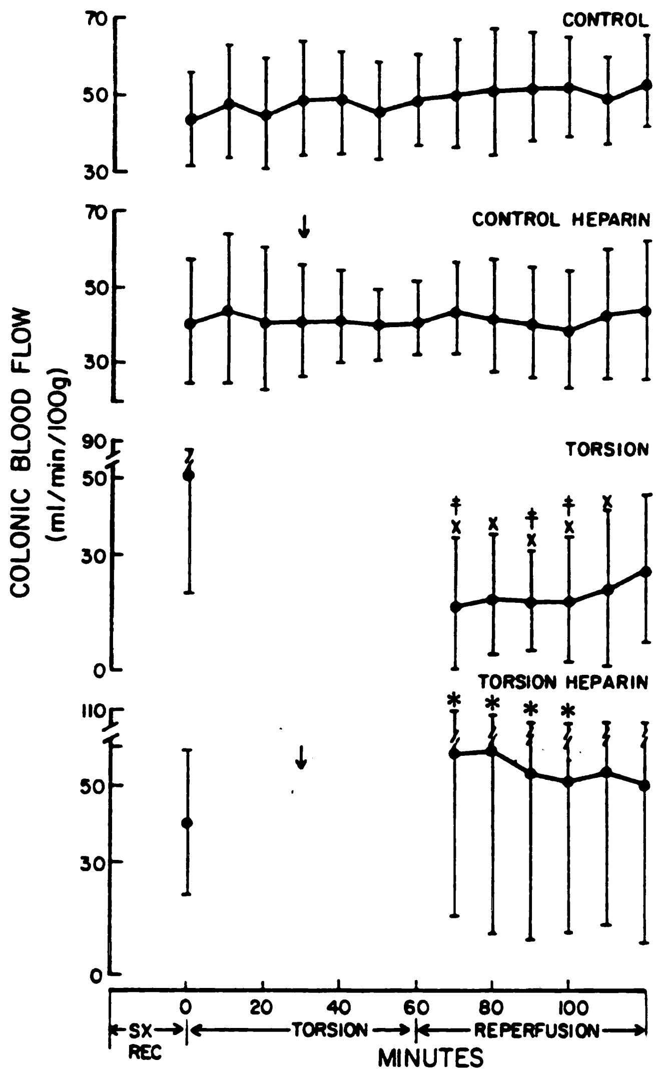

FIGURE 5 - The mean (± SEM) colonic blood flow of Control, Control/Heparin, Torsion, and Torsion/Heparin groups during the 120 minute experiment. The arrow indicates the time of heparin administration. Significant differences ($p \leq 0.05$) identified: x - different than baseline; + - different than Control group; * - different than Torsion group.

C. Colonic Vascular Resistance (CVR) (Figure 6)

Mean CVR was less than 2.5 mm of Hg/ml/min/100 g in all groups at the onset of the recording period and remained so in the Control and Control/Heparin groups throughout the experiment. In the Torsion group, following colonic detorsion CVR was significantly elevated for 50 minutes, in comparison to baseline (1.76 $\pm$ 0.80 mm of Hg/ml/min/100 g) and Control group resistances. In the Torsion/Heparin group, this post-detorsion elevation in CVR did not occur and CVR was significantly lower than that of the Torsion group at 70, 80, 100 and 110 minutes.

II. Colonic Tissue pH (Figure 7)

Colonic tissue pH in both the Control and Control/Heparin groups remained greater than 7.0 during the experiment. In contrast pH progressively declined during the 60 minutes of colonic torsion in both the Torsion and Torsion/Heparin groups to 6.65 and 6.52, respectively. Following correction of the torsion, pH returned towards normal in both groups, with the Torsion group remaining slightly lower.

III. Plasma Prostanoid Concentrations

A. Thromboxane B_2 (Figure 8)

In the Control and Control/Heparin groups, plasma TxB_2 concentrations were not significantly different within or between treatment groups at any assay time. In the torsion group, plasma TxB_2 concentration was significantly elevated immediately following colonic detorsion compared to baseline concentration (58.21 $\pm$ 56.05 pg/ml versus 23.28 $\pm$ 22.11 pg/ml, respectively). This post-detorsion elevation in TxB_2 did not occur in the Torsion/Heparin group.

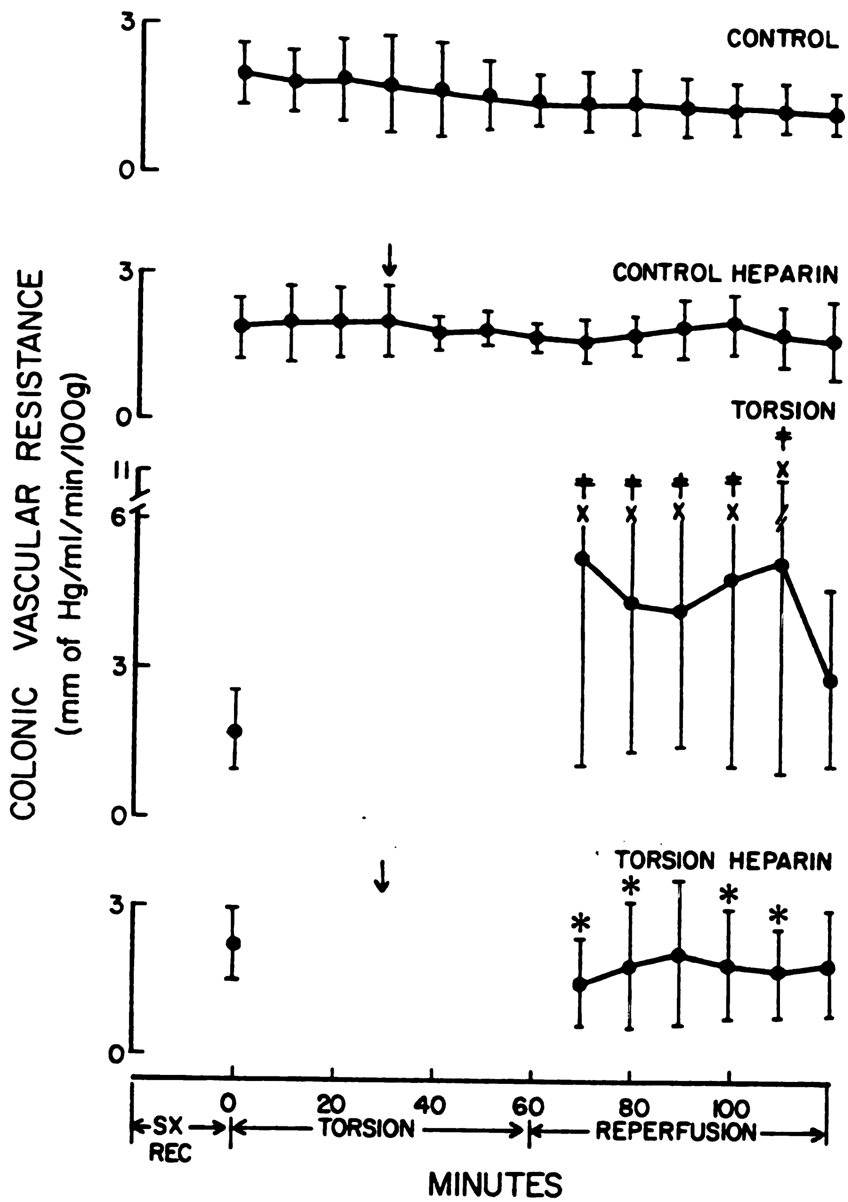

FIGURE 6 - The mean (± SEM) colonic vascular resistance of Control, Control/Heparin, Torsion, and Torsion/Heparin groups during the 120 minute experiment. The arrow indicates the time of heparin administration. Significant differences ($p \leq 0.05$) identified:
x - different than baseline; + - different than Control group;
* - different than Torsion group.

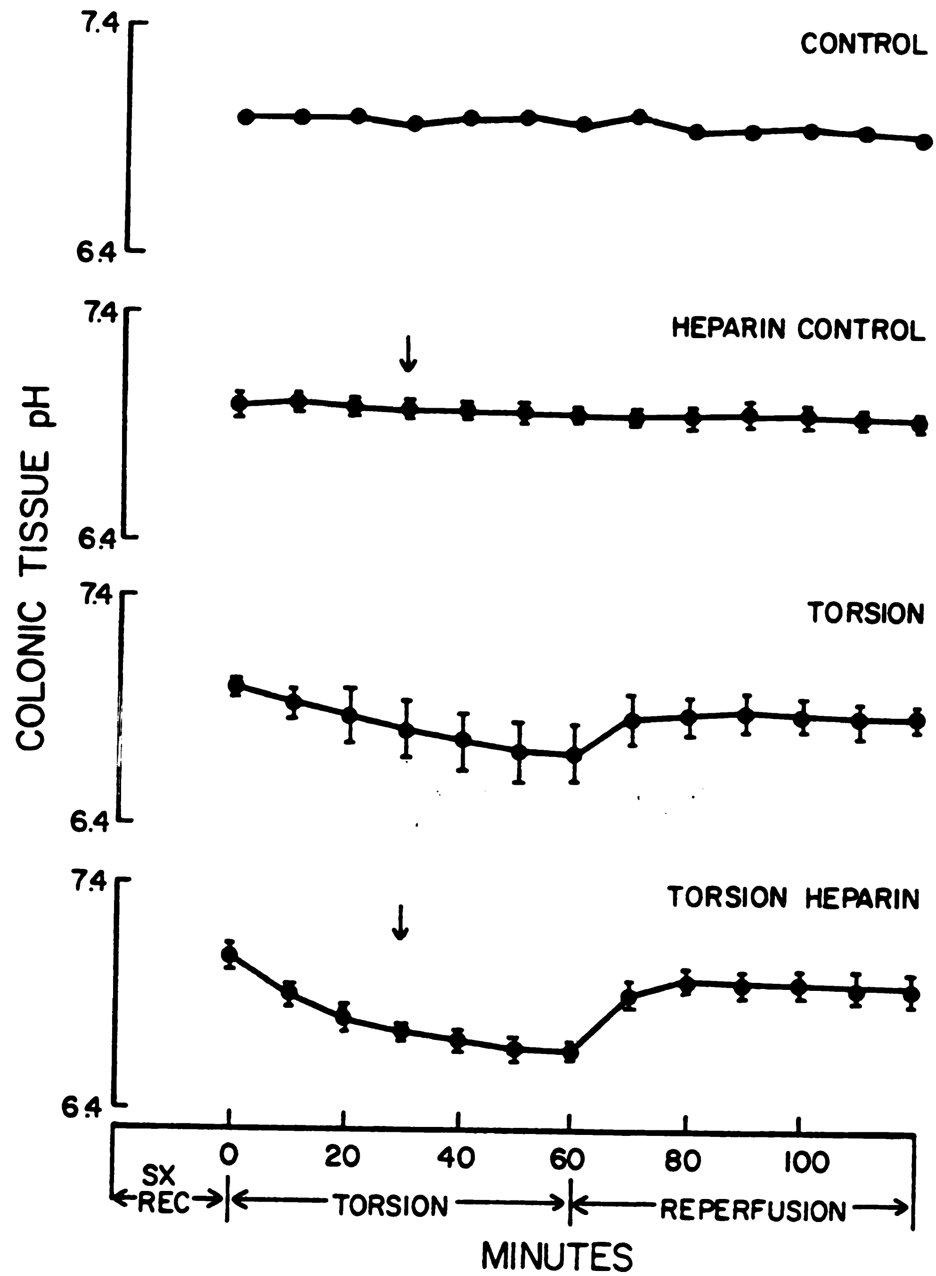

FIGURE 7 - Pelvic flexure colonic tissue pH in 1 Control pony; and 2 each Control/Heparin, Torsion, Torsion/Heparin ponies. The arrow indicates the time of heparin administration.

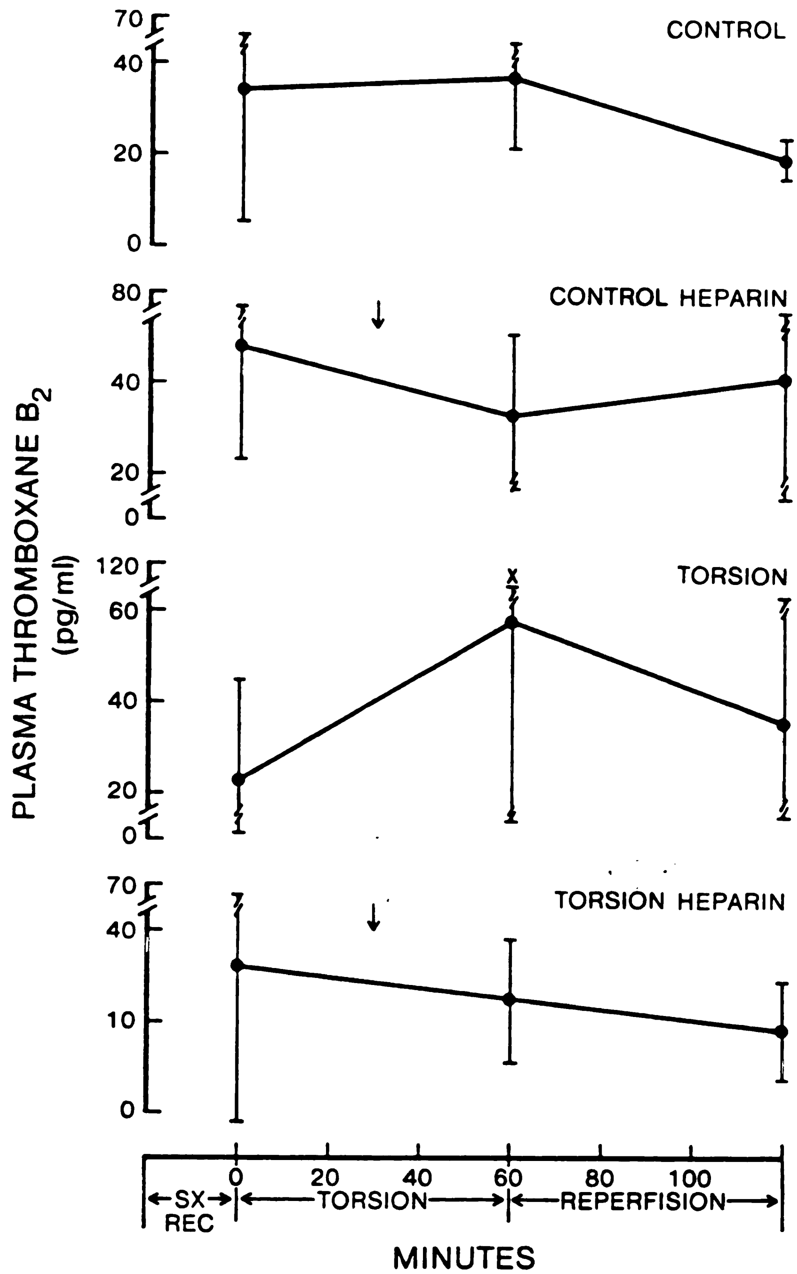

FIGURE 8 - The mean (± SEM) plasma Thromboxane B_2 of Control, Control/Heparin, Torsion, and Torsion/Heparin groups during the 120 minute experiment. The arrow indicates the time of heparin administration. Significant differences ($p \leq 0.05$) identified: x - different than baseline; + - different than Control group; * - different than Torsion group.

B. 6 -keto -PGF_{1a} (Figure 9)

In all four treatment groups, plasma 6-keto-PGF_{1a} concentration increased with time. Concentrations were not statistically different in either the Control or Control/Heparin group. In both the Torsion and Torsion/Heparin groups, plasma 6-keto-PGF_{1a} was significantly elevated at the completion of the experiment in comparison to baseline values (2.77 $\pm$ 2.69 pg/ml versus 12.66 $\pm$ 11.53 pg/ml and 3.89 $\pm$ 3.30 pg/ml versus 14.05 $\pm$ 14.14 pg/ml, respectively).

IV. Histopathology (Table 1)

Four Control group and 5 Control/Heparin colonic biopsy samples were reviewed. In all but one tissue biopsy, a combined score of 2 or less was assigned. (Figure 10) Changes were consistent with normal to mild, diffuse hyperemia of the submucosa, lamina propria and mucosal fold tips. The hyperemia/congestion was characterized by marked distension of blood vessels, primarily veins and venules, by erythrocytes. Edema, characterized by separation of bands of collagen fibers in the submucosa by clear spaces or homogeneous, pale eosinophilic, proteinaceous material, was present infrequently, but when present, was mild and multifocal in nature. In some instances the submucosal lymphatic vessels were markedly dilated. The submucosal edema resulted in thickened tissue sections. The necrotic/degenerative changes involved moderate, multifocal necrosis of the subepithelial lamina propria. This was characterized by the presence of necrotic debris and fragmented cell nuclei immediately beneath the epithelium at

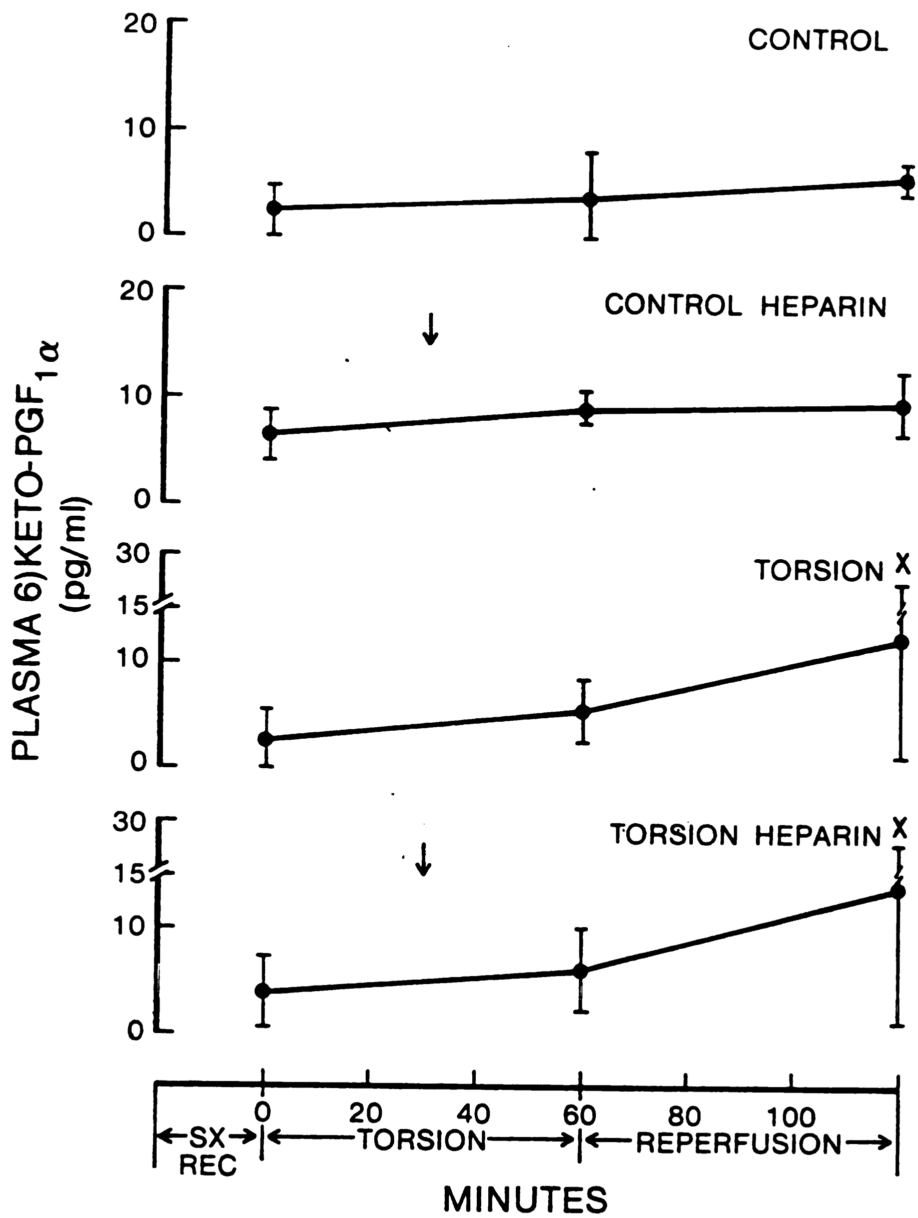

FIGURE 9 - The mean (± SEM) plasma 6 -keto -PGF_{1a} of Control, Control/Heparin, Torsion, and Torsion/Heparin groups during the 120 minute experiment. The arrow indicates the time of heparin administration. Significant differences ($p \leq 0.05$) identified: x - different than baseline; + - different than Control group; * - different than Torsion group.

TABLE 1 - HISTOPATHOLOGY SCORES

GROUP	BIOPSY	VR*	DN**	COMBINED SCORE
CONTROL	1	1	1	2
	2	0	1	1
	3	2	3	5
	4	0	1	1
CONTROL HEPARIN	1	0	0	0
	2	0	1	1
	3	1	1	2
	4	0	1	1
	5	2	0	2
TORSION	1	0	1	1
	2	3	3	6
	3	3	2	5
	4	3	2	5
	5	3	3	6
TORSION HEPARIN	1	3	2	5
	2	3	2	5
	3	2	1	3
	4	3	2	5
	5	3	2	5

* VR = vascular response
** DN = degeneration and/or necrosis

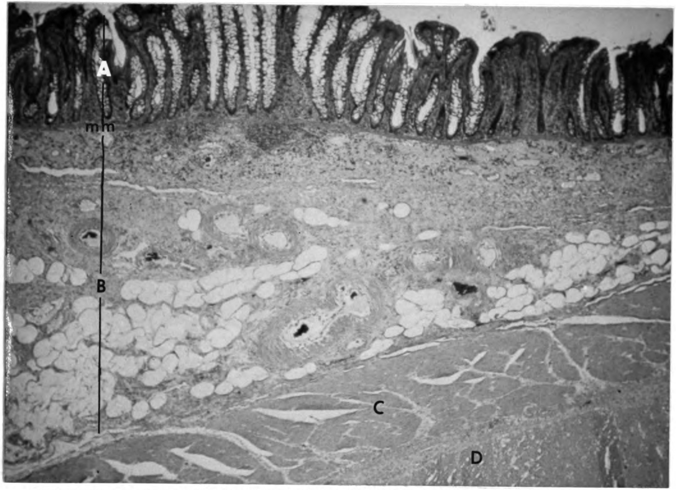

FIGURE 10 - Normal colonic architecture is shown in this photomicrograph of a biopsy from Control pony #2: Tunica mucosa (A); muscularis mucosae (mm); tunica submucosa with adipose tissue (B); inner circular (C) and outer longitudinal (D) layers of the tunica muscularis. H & E x2.5

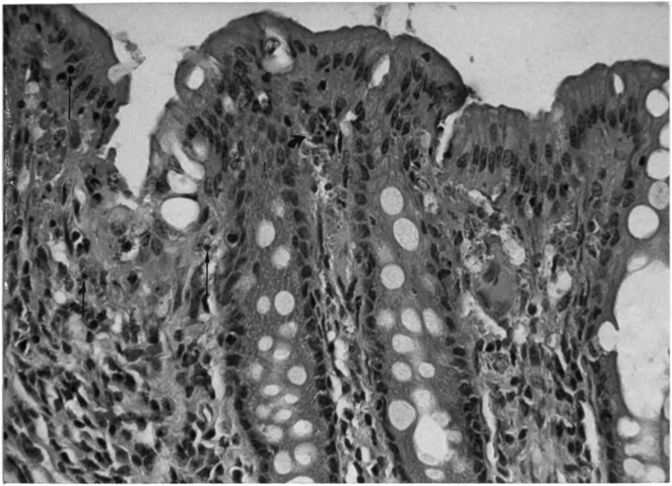

FIGURE 11 - Superficial lamina propria necrosis characterized by pyknotic (long arrows) and fragmented (curved arrows) nuclei within the lamina propria (A) of the mucosal layer is present in this biopsy from Control pony #1. H & E x25

the tips of at least one out of ten mucosal folds. (Figure 11) In the remaining tissue section, a Control group biopsy, a combined score of 5 was given. In addition to the above changes, this section had moderate, multifocal necrosis of the surface epithelium on fold tips, and mild, multifocal necrosis of the mucosal fold epithelial lining. The extensive necrotic changes identified within this tissue section were not similar to the other 8 biopsies examined. Regardless, no statistical difference was identified between the rank sum scores of either group.

In both the Torsion and Torsion/Heparin groups, histologic changes were considered severe in all but one Torsion sample. In this latter section changes included moderate, multifocal necrosis of the lamina propria. The section was assigned a score of "1". The remainder of the biopsy sections received combined scores between 3 and 6 (Figure 12). These sections characteristically had moderate to severe, diffuse edema within the submucosa, moderate to severe, diffuse congestion within the lamina propria and submucosa, and severe, multifocal to diffuse hemorrhage characterized by extravascation of erythrocytes into the stroma of the lamina propria and submucosa. Necrotic changes ranged from moderate, multifocal necrosis of the lamina propria and superficial epithelium to severe, diffuse necrosis and degeneration of surface epithelium on mucosal fold tips. Degenerating epithelial cells were shrunken or irregularly shaped, with hypereosinophilic cytoplasm. Necrotic epithelial cells were distinquished by hypereosinophilic cytoplasm and pyknotic or fragmented nuclei. In severe necrosis the eroded mucosal epithelium was replaced by a layer of granular, eosinophilic, necrotic debri comprised of fragmented nuclei,

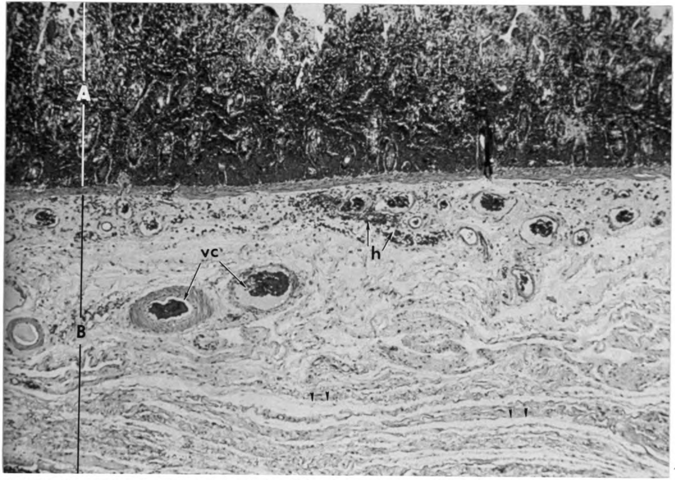

FIGURE 12 - The changes present in this photomicrograph of Torsion pony #2 [severe, diffuse hemorrhage within the mucosa (A) and vascular congestion (VC), edema separated collagen fibers (black arrows), and hemorrhage (h) within the submucosa] are typical changes resulting from colonic torsion. H & E x4

erythrocytes, and bacteria.(Figure 13) Torsion of the large colon resulted in histopathologic changes significantly different than the combined nontorsion group (Control & Control/Heparin). There was no statistical difference associated with heparin therapy between the Torsion and Torsion/Heparin groups.

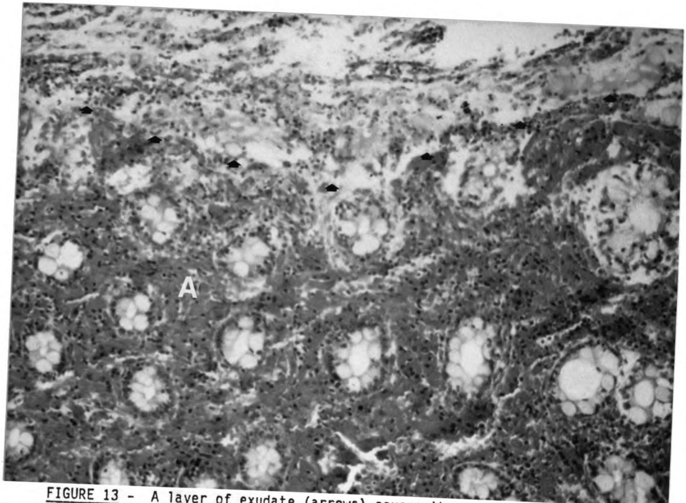

FIGURE 13 - A layer of exudate (arrows) covers the mucosal surface of this colonic fold from Torsion pony #5. Severe, diffuse hemmorhage is present within the lamina propria (A). H & E x10

DISCUSSION

Baseline values of SAP, CBF, CVR, TxB_2, and 6-keto-PGF_{1a} in all ponies and all values of the Control group were considered within physiologic limits of normal when compared to values obtained in other species. Systemic arterial pressure and CVR were consistent with reported equine data.[159,160] Colonic blood flow has been reported to range from 18 + 8 ml/min/100 g in persons to 123 $\pm$ 57 ml/min/100 g in dogs using several different methods.[161,162] (Table 2) Mean CBF in this study (49.8 $\pm$ 13.3 ml/min/100 g) was greater than previously reported in ponies.[51] This disparity in blood flow rates is explainable by technique differences.[168] The electromagnetic flow probe, a direct measurement of blood flow, provides a more accurate measurement of blood flow than the indirect microsphere measurement technique.

A wide range of TxB_2 and 6-keto-PGF_{1a} concentrations in the horse have been published. (Table 3) The measurement of eicosanoids is technically difficult and concentrations are increased by sampling that causes platelet damage or delay in the addition of cyclooxygenase inhibitor. Additionally the specificity of the assay may vary. Therefore, although serum values, representing complete platelet activation are expected to be 1000 - 10,000 times greater than plasma values, variation even in plasma samples is expected.[169,170] These factors combine to make between laboratory comparisons, with the exception of trends, difficult. However, because normal basal levels of

TABLE 2 - COLONIC BLOOD FLOW

SPECIES	CBF ml/min/100g*	TECHNIQUE	REF
Man	18 $\pm$ 8	Inert gas	160
Cat	22 $\pm$ 7	Inert gas	160
Dog	82 $\pm$ 10	^{86}Rb clearance	161
	123 $\pm$ 57	^{86}Rb clearance	162
	41 $\pm$ 6	Microspheres	163
	39 $\pm$ 1	^{133}Xe clearance	164
Rhesus Monkey	91 $\pm$ 47	Microspheres	165
Rat	42	^{86}Rb clearance	166
Pony	28 $\pm$ 7.5	Microspheres	51
Pony	49.8 $\pm$ 13.3	Electromagnetic Flow Probe	Control Grp Mean

* Mean $\pm$ SEM

TABLE 3 - EQUINE PROSTANOID CONCENTRATIONS

	SOURCE	CONCENTRATION**	REF
	Serum	14.3 ng/ml	78
	Serum	43.8 ± 7 ng/ml	79
	Serum	28.1 ± 0.01 ng/ml	80
TxB_2	Serum	11.3 ± 0.8 ng/ml	81
	Serum	29.1 ± 4.2 ng/ml	83
	Plasma	125 pg/ml*	79
	Plasma	1300 pg/ml*	69
	Plasma	34.2 ± 29.1 pg/ml	Control Grp Baseline
	Serum	204 pg/ml	78
	Serum	400 ± 30 pg/ml	83
	Plasma	221 pg/ml	78
6-keto-PGF_{1a}	Plasma	300 pg/ml*	79
	Plasma	250 pg/ml*	69
	Plasma	2.1 ± 2.9 pg/ml	Control Grp Baseline

* Estimated from graph
** Mean ± SEM

the parent compounds, TxA_2 and PGI_2, in persons do not exceed 2 pg/ml, the published equine values appear high.[171,172] In contrast, our baseline values of TxB_2 and 6-keto-PGF_{1a} concentrations are similar to the data from persons.

Colonic torsion and its correction (detorsion) causes a decrease in tissue pH during large colon torsion, systemic hypotension, significantly low reperfusion CBF and significant elevations in CVR, TxB_2, and 6-keto-PGF_{1a}. The mechanisms inducing these changes cannot be definitely determined from this experiment, but there are several possible explanations. The decrease in tissue pH is explained by the lack of blood-borne oxygen during torsion causing cellular metabolism to convert from oxidative-phosphorylation to anaerobic glycolysis. The end product, lactic acid, caused the decrease in tissue pH. The remaining physiologic changes may have occurred because of 1) the effect of local vascular injury, and/or 2) the effect of endotoxin release.

Ischemia induced degeneration of the vascular endothelium results in the exposure of subendothelial collagen, endothelial release of thromboplastin and prostacyclin, platelet adherence, and ultimately, in the initiation of the coagulation cascade.[173-180] The initiation of blood coagulation by damaged vessel walls can occur through either the extrinsic or the intrinsic pathway. The intrinsic pathway is triggered when factor XII (Hageman factor) contacts collagen; tissue thromboplastin, released from injured endothelial and smooth muscle cells, activates the extrinsic pathway.[181-183] Thrombin, the end product of the common pathway, further amplifies the cascade. As platelets become activated thromboxane is released, which like thrombin amplifies coagulation, but in addition causes contraction of vascular tissue.[184,185] These reactions, initiated by ischemia, could account for the changes seen in TxB_2, 6-keto-PGF_{1a}, CVR, SAP, and CBF.

The post-detorsion elevation in plasma TxB_2 maybe the systemic reflection of the ongoing coagulopathy occurring within the large colon. When released into the systemic circulation, TxA_2, potentially mediates an increase in pulmonary vascular resistance, decreasing cardiac return, and thereby the decrease in SAP. This relationship between TxB_2 and SAP is shown in Figure 14, and has been seen in other experimental studies.[69,70,74,75] Thromboxane locally causes vasoconstriction and the increase in CVR. Plasma concentration of 6-keto-PGF_{1a} increases progressively as PGI_2 is released from ongoing vascular injury and thrombin stimulation of endothelial cell AA metabolism.[186,187] The final result of these local and systemic changes, including thrombus formation, increased vascular resistance, and systemic hypotension, is the significant decrease in colonic blood flow.

Endotoxin, alone, can also initiate these same changes. Endotoxin can activate the intrinsic coagulation system through factor XII (Hageman factor) and the extrinsic system by increasing thromboplastin production and through Hageman factor activation of factor VII.[56,188,189] Hageman factor can additionally convert prekallikrein to kallikrein, which in turn releases bradykinin from HMW kininogen.[190] Bradykinin, which can increase tissue permeability and cause peripheral vasodilation, has been implicated as the cause of the endotoxin associated systemic arterial hypotension.[190] Two recent studies suggest that bradykinin is responsible for the second of the characteristic biphasic hypotensive periods that follow endotoxin challenge.[191,192] Administration of a competitive bradykinin antagonist will attenuate this decrease.[191]

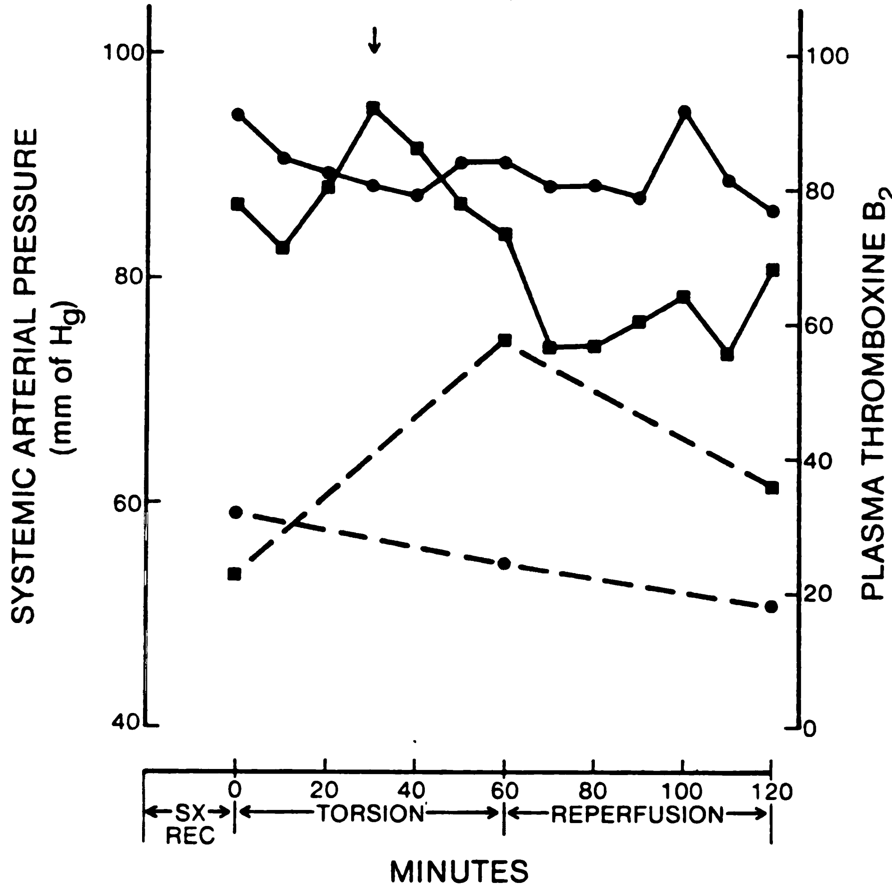

FIGURE 14 - The interrelationship of systemic arterial pressure (solid lines) and plasma TxB_2 concentration (broken lines) is shown in 1 h of colonic torsion followed by 1 h of reperfusion. In untreated ponies (Torsion group, depicted by []) systemic hypotension coincided with the post-colonic detorsion elevation in plasma TxB_2 concentration. In heparin treated ponies (Torsion/Heparin group, depicted by O), there was no increase in plasma TxB_2 concentration following detorsion and systemic arterial pressure remained stable. Arrow indicates the time of heparin administration.

Endotoxin released from the ischemic colon could therefore, through the activation of the coagulation system, mediate platelet release of TxA_2, endothelial release of PGI_2 and the generation of bradykinin, resulting in systemic hypotension, increased CVR and the decrease in CBF. Similar changes have been documented in equine endotoxin shock models.[55,69,70,75] In these studies the immediate increase in pulmonary vascular resistance and decrease in systemic arterial blood pressure is temporally associated with the elevation in TxB_2 seen following endotoxin administration. The prolonged hypotension associated with endotoxin administration is thought to result from the vasodilatory effects of increased PGI_2.[69,70] Cyclooxygenase inhibition prevents these changes.[55,69,70,78,79] Based on these studies, and on the evaluation of the colonic biopsies harvested following large colon torsion, in which the superficial mucosal barrier was extensively disrupted, it is easy to postulate that the alterations seen following colonic detorsion could be initiated by endotoxin. It is more likely, however, that the hemodynamic changes seen result from both the vascular and endotoxic insult.

In evaluating the second objective of this experiment, the effect of heparin therapy, no untoward effects on the hemodynamics, plasma prostanoid concentrations, colonic tissue pH, or in the histologic appearance of the large colon were identified in the Control/Heparin ponies. The potential negative effect of a heparin-induced decrease in peripheral perfusion possibly caused by erythrocyte agglutination was not seen. Colonic blood flow was not different from that of the Control ponies, nor was the histologic appearance. Heparin pretreatment prior to colonic detorsion maintained SAP, prevented the elevation in plasma TxB_2, and permitted the return of CBF and CVR to normal.

The mechanism of heparin's protective action is speculative, but there are several potential routes. As an anticoagulant, heparin catalyzes the AT III reaction with thrombin, and with coagulation pathway factors IX, X, XI, XII, and kallikrein.[92,102-105] After catalyzing the reactions heparin can dissociate and initiate reactions elsewhere. Independent of AT III heparin can directly bind with and inactivate factors X, Xa, and prothrombin and stimulate the release of endothelial EPI which inhibits the factor VIIIa - thromboplastin complex.[114] Additionally, platelet bound heparin prevents thrombin from binding and causing platelet stimulation.[108,109] The heparin and heparin/AT III reactions are important. They decrease the amount of thrombin being generated, as well as inactivate that already present. Overall there is a decrease in platelet activation and TxB_2 release. In addition, by inactivating factor XII (Hageman factor) and kallikrein, the release of bradykinin is prevented. Effectively diminishing the production of bradykinin and the release of TxA_2 will, as seen in this experiment, prevent the SAP and CVR alterations. (Figure 14) With higher perfusion pressures, decreased CVR, and decreased thrombi formation, CBF following detorsion returned to baseline rates.

Heparin did not prevent the endothelial release of PGI_2, as is evident by the increase in 6-keto-PGF_{1a}. The release of PGI_2 causes vasodilation and prevents platelet aggregation.[96,97] Overproduction of PGI_2 is assumed to be checked by the decrease in thrombin induced secretion. These actions are necessary in the prevention of thrombus formation and occur in response to any endothelial injury. Therefore, the lack of interference with PGI_2 concentration by heparin is probably beneficial.

Heparin did not prevent the tissue injury caused by the torsion-induced ischemia. Failure of histologic protection by heparin was expected. Based on other investigations, mucosal damage is an immediate and ongoing result of ischemia.[10-13] In the experiment, heparin is not administered until 30 minutes after the large colon torsion is created and is prevented from entering the colon after administration because of the torsion. The result is that the histologic appearance of the colon is not different than the torsion group not receiving heparin therapy. However, the increase in colonic blood flow associated with heparin therapy could have potentially mediated the release of oxygen free radicals, causing a more severe injury (the classic reperfusion injury). In this experiment this was not apparent and the effect of increased blood flow was considered beneficial.

Two further potential mechanisms by which heparin may have exerted its action include the potentiation of the reticuloendothelial system and the prevention of platelet activating factor (PAF) actions. It has been hypothesized that heparin increases phagocytosis of bacterial endotoxins in the liver, yet there is little evidence for this.[120,126,127] Whether this occurs, and is in effect in this experiment, cannot be answered. The actions of PAF, in contrast, have been better defined. Recent evidence supports the role of endotoxin stimulation of membrane phospholipase A_2 resulting in PAF synthesis.[193,194] Intravenous administration of PAF produces many of the symptoms of endotoxic shock including the systemic hypotension.[194,195] The strongest evidence for a role of PAF in endotoxic shock is the observation that specific PAF antagonists will not only prevent the effects of PAF, but when administered to endotoxin challenged animals will prevent the associated abnormalities.[194-196] Heparin administration blocks PAF inhibition of adenylate cyclase.[197] This results in an increased platelet concentration of cAMP, which inhibits platelet aggregation. Whether PAF plays an important role as a mediator of endotoxic shock in the horse is not known. At this time it is only known that PAF will cause equine platelets to aggregate in vitro.[198] In conclusion, heparin has many possible routes of action in which to initiate beneficial reactions.

SUMMARY

In summary, torsion of the large colon created many disturbances in the recorded in the Control group. Colonic tissue pH progressively declined throughout the 60 minute torsion period, and following colonic detorsion, systemic arterial pressure and colonic blood flow were significantly decreased, while colonic vascular resistance and plasma TxB_2 and 6-keto-PGF_{1a} were significantly increased. Pretreatment with heparin 30 minutes prior to correction of the torsion prevented the systemic hypotension, the increases in colonic vascular resistance and plasma TxB_2 concentration, and significantly increased colonic blood flow following detorsion. It did not, however, alter the 6-keto-PGF_{1a} concentration, nor the histologic appearance of the large colon. On the basis of these findings, sodium heparin, at a dosage of 80 IU/kg of body weight, is recommended as a beneficial treatment during surgery for torsion of the equine large colon.

APPENDIX

APPENDIX

ELECTROMAGNETIC FLOW PROBES

Perivascular electromagnetic flow probes work on the basis of Faraday's law of electromagnetic induction, $E = (BLV) * 10^{-8}$, where E is the EMF in volts, B is the magnetic field, in gauss, L is the lumen diameter in cm, and V is the velocity of liquid in cm/sec.[199] An electromagnet housed within the probe is excited by a pulsed current, producing a magnetic field at right angles to the direction of flow. As blood flows through the magnetic lines of force, a pulsatile voltage (EMF) is developed. The EMF, which is directly proportional to the velocity of the blood flow through it, is sensed by the electrodes located at diametrically opposite points on the vessel lumen. The flow signal voltage is amplified, averaged, and displayed by a meter, and recorded by a direct writing oscillograph.[200]

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