

**THE EFFECTS OF CRYSTALLOID RESUSCITATION ON
OXYGEN EXTRACTION IN WHOLE BODY AND GUT
DURING ENDOTOXEMIA**

by

KENNETH WILLIAM GOW

B.Sc., The University of Manitoba, 1991

B.Sc.(Med), The University of Manitoba, 1991

M.D., The University of Manitoba, 1991

FRCS(C), The University of British Columbia, 1997

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Department of SURGERY

The University of British Columbia
Vancouver, Canada

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ABSTRACT

Many investigators advocate aggressive fluid therapy in sepsis, yet changes in the microcirculation may make fluid counterproductive. Sepsis is characterized by a generalized "leak" in capillaries which may promote interstitial edema which in turn, may decrease diffusion of oxygen, increase the distance from capillaries to cells, and alter capillary density. Further, fluid administration may result in capillary hemodilution. Therefore, the author's hypothesis was that crystalloid resuscitation will impair the ability of tissue to extract oxygen.

Four groups (n=8) of anesthetized pigs received either normal saline infusion ($48 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{hr}^{-1}$) or no saline, and E coli endotoxin (50 mg/kg i.v.) or no endotoxin. Whole body and gut oxygen delivery and consumption were measured during progressive hemorrhage. Dual line regression analysis was used to determine the onset of ischemia (DO_{2c}) and oxygen extraction ratio (ER_c). At onset of ischemia, gut was removed to determine degree of interstitial volume and the capillary hematocrit. With use of radiolabelled microspheres as a marker of blood flow, the gut blood flow transit time was determined. Endotoxin significantly decreased ER_c for the whole body (0.82 ± 0.06 to 0.55 ± 0.08 , $p < 0.05$) and gut (0.77 ± 0.07 to 0.52 ± 0.06 , $p < 0.05$). Saline resuscitation also significantly decreased ER_c in the control pigs for the whole body (0.82 ± 0.06 to 0.62 ± 0.08 , $p < 0.05$) and gut (0.77 ± 0.07 to 0.67 ± 0.06 , $p < 0.05$) but did not significantly change the already decreased ER_c in the endotoxin treated pigs. Morphometric techniques revealed that saline resuscitation increased gut interstitial volume ($p < 0.05$), and lead to arterial hemodilution ($p < 0.05$) but not

capillary hemodilution ($p > 0.05$). Using radiolabelled microspheres, saline was shown to increase the relative dispersion of blood flow transit times from 0.33 ± 0.08 to 0.72 ± 0.53 ($p < 0.05$). Thus, saline resuscitation impairs tissue oxygen extraction possibly due to interstitial edema or increased heterogeneity of microvascular blood flow. After endotoxin infusion, where ERc is already decreased, saline resuscitation has a lesser effect. Therefore, the author questions the use of aggressive crystalloid resuscitation for treatment of sepsis in humans.

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Chapter 1

INTRODUCTION

1.1 OVERVIEW

Sepsis is an all too frequent occurrence in critically ill patients . Overall, the cost to health care is staggering (1) with an estimated cost of \$5 to \$10 billion in the US annually. Sepsis will likely be a problem for clinicians not only now but for the foreseeable future (2). The increasing incidence of septic shock continues to be associated with a mortality rates ranging from 40% to 60% despite advances in critical care medicine.

Clinicians have long sought the optimal treatment for patients suffering from sepsis. However, sepsis, by its very nature is a systemic illness whereby many of the toxic effects are the result of the patient's own immune system as it mounts a defense to the infection. The immune system reaction to infection focuses on various aspects of the microorganism but the lipopolysaccharide moiety known as endotoxin has been best described in literature. The immune system through its various leukocytes mounts a defense to the organisms. This defense is in turn mediated by numerous cytokines which have beneficial effects as well as side effects. While the immune system overall is beneficial in its role, if the effects are allowed to go unhindered, the immune system mediators will themselves lead to significant illness. Various toxicity effects occur from these cytokines such that further damage may ensue. Ultimately, the damage done to cells occurs at a microvascular level with ischemia as the final end-point.

While sepsis is a systemic insult, the splanchnic circulation is considered to be particularly affected. There are various reasons why the gut is predisposed to injury. Two explanations are the high metabolic demands which make the gut susceptible to minor deficits and the countercurrent organization of the vessels which leads to possible shunting from arterioles to venules in times of decreased flow. While ischemia in any tissue is potentially harmful, ischemia to the gut is particularly concerning. With the high volume of bacteria that reside in the gut, damage to the mucosal protective layer may result in further bacterial load to the host. Since this translocation of bacteria may ultimately lead to systemic toxicity, the gut has been termed the "Motor of Multiple Organ Failure" (3).

The toxicity that leads to multiple organ dysfunction is ultimately due to tissue ischemia. Ischemia may be defined by the decrease in blood flow. In turn, ischemia results in hypoxia which may be described as the lack of oxygen for aerobic metabolism. This results in anaerobic metabolism which is a less efficient mechanism for energy production and leads to metabolic byproducts. Techniques have been described which allow investigators to determine the onset of tissue ischemia and the tissue's ability to extract oxygen. These studies allow one to determine the potential benefit of various treatments of sepsis.

Treatment of sepsis may be aimed at any step of the pathway from initial insult to the final endpoint of tissue ischemia. While new techniques such as blocking endotoxin or modulating the immune system are being studied (4), time honored therapeutic interventions such as antibiotics and fluid resuscitation are considered the mainstay of therapy (2). While the use of antibiotics is intuitive to

treat the infection, the use of fluid therapy is less than straight forward. Fluid therapy is thought to mediate its benefit by augmentation of filling pressures, replacement of intravascular deficits, decreased viscosity of blood, and a more uniform distribution of blood flow to tissues which is known as capillary blood flow homogeneity. However, since the septic state may be described as a generalized leak in the capillaries, the potential benefit of crystalloid resuscitation is questionable (5). The potential side effects from fluid resuscitation include interstitial edema, hemodilution, and a less uniform distribution of blood flow to tissues which is known as capillary blood flow heterogeneity. Therefore, while some consider aggressive crystalloid resuscitation as essential in the resuscitation of septic patients, physiologic changes that accompany sepsis may not only lead to a limited benefit but could make fluid resuscitation detrimental.

In this thesis, the effect of crystalloid resuscitation is studied on the main outcome variable, oxygen extraction in a porcine model of endotoxemia. The literature pertaining to these topics has been reviewed and is discussed in detail below.

1.2 SEPSIS AND SYSTEMIC INFLAMMATORY RESPONSE SYNDROME (SIRS)

1.2.1 Definitions

The following definitions were established by the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference (ACCP/SCCM) in 1991 (6) (Figure 1).

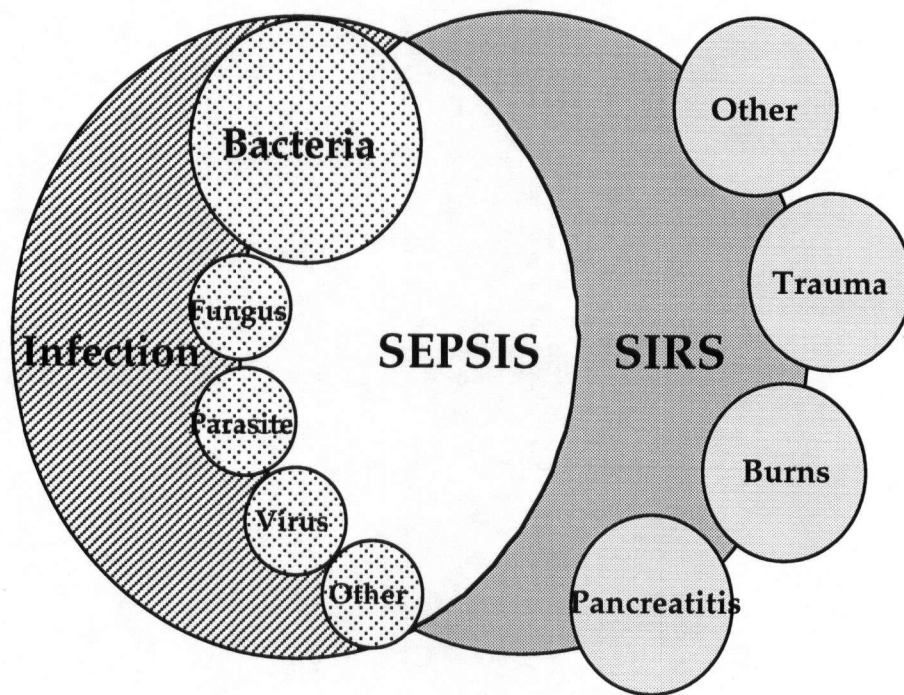


Figure 1. Sepsis and SIRS.

This pictogram illustrates the differences between the terms described by the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference (ACCP/SCCM).

Systemic inflammatory response syndrome (SIRS) - Describes a continuous process, and describes an abnormal host response that is characterized by a generalized activation of the inflammatory reaction in organs remote from the initial insult. When the process is due to infection, the terms sepsis and SIRS are synonymous. SIRS can be seen following a wide variety of insults and includes, but is not limited to, more than one of the following clinical manifestations: (1) a body temperature $> 38^{\circ}\text{C}$ or $< 36^{\circ}\text{C}$; (2) a HR > 90 bpm; (3) tachypnea, with a RR > 20 bpm, or hyperventilation, as indicated by a $\text{PaCO}_2 < 32$ mmHg; and (4) an alteration in the WBC, being either $> 12,000/\text{mm}^3$, or $< 4,000/\text{mm}^3$, or the presence of more than 10% immature neutrophils.

Infection - is a microbial phenomenon characterized by the presence of microorganisms or their invasion of normally sterile host tissue by those organisms.

Bacteremia - presence of viable bacteria in the blood. The presence of viruses, fungi, parasites, and other pathogens in the blood should be described in a similar manner.

Septicemia - has been defined in the past as the presence of microorganisms or their toxins in the blood. However, this term has been used clinically and in the medical literature in a number of ways leading to confusion and difficulty in interpreting data. Therefore, it was recommended to abandon this term.

Sepsis - the systemic inflammatory response to infection. In association with infection, manifestations of sepsis are the same as those previously defined for SIRS.

Severe sepsis - sepsis associated with organ dysfunction, hypoperfusion abnormality, or sepsis-induced hypotension. Hypoperfusion abnormalities include lactic acidosis, oliguria, and acute alteration of mental status. Sepsis-induced hypotension is defined by the presence of a systolic blood pressure of less than 90 mmHg or its reduction by 40 mmHg or more from baseline in the absence of other causes for hypotension.

Septic shock - a subset of severe sepsis and is defined as sepsis-induced hypotension, persisting despite adequate fluid resuscitation, along with the presence of hypoperfusion abnormalities or organ dysfunction.

Multiple organ dysfunction syndrome (MODS) - the previous term multiple organ failure has been replaced with this term. The main change is the use of "dysfunction" used in place of the term "failure" to represent a continuum of physiologic derangements. This can evolve in the absence of an untreated focus of invasive infection and can be reproduced experimentally by the infusion of a diverse spectrum of endogenously derived mediators of inflammation. The term syndrome refers to a pattern of multiple and progressive symptoms and signs that are thought to be related.

1.3 MEDIATORS OF SEPTIC TOXICITY

1.3.1 Mediation of septic toxicity

The body's immune system is paramount in importance in defending against infections. There are various mechanisms at work which comprise a fine balance of stimulatory and inhibitory effects. While for the most part, success results in the

host surviving, the mediators of defense may have toxic effects themselves. The various mechanisms important for immune defense have been shown to affect host tissues as well. The end result of these toxic effects is ischemia at the microvascular level (7) (Figure 2).

1.3.2 Bacterial Endotoxin

While various aspects of bacteria are potentially toxic, the best described has been that of endotoxin. Gram-negative bacteria contain within their cell wall a macromolecular glycolipid termed lipopolysaccharide (LPS) (Figure 3). It comprises two components: the O-specific chain and the core. The O-specific chain is a polymer of oligosaccharides and accounts for the antigenic variability among species and strains of bacteria. The core is composed of an oligosaccharide covalently bound to a molecule lipid called lipid A. Most of the toxicity of LPS resides in the lipid A moiety. The structure of the core region is relatively constant across species and strains of gram-negative bacteria. The terms LPS and endotoxin are not strictly synonymous as LPS refers to the purified glycolipid whereas endotoxins contain small amounts of cell wall proteins, lipids, lipoproteins, and polysaccharides in addition to LPS (8).

There are several proposed mechanisms by which endotoxin effects damage. Endotoxin has been shown to activate complement, the fibrinolytic pathway, neutrophils, and other mediator systems (9) (10). Furthermore, endotoxin has been shown to stimulate macrophages to release cytokines, including tumor necrosis factor (TNF) and interleukin-1 (IL-1), which, in turn, amplify the systemic response

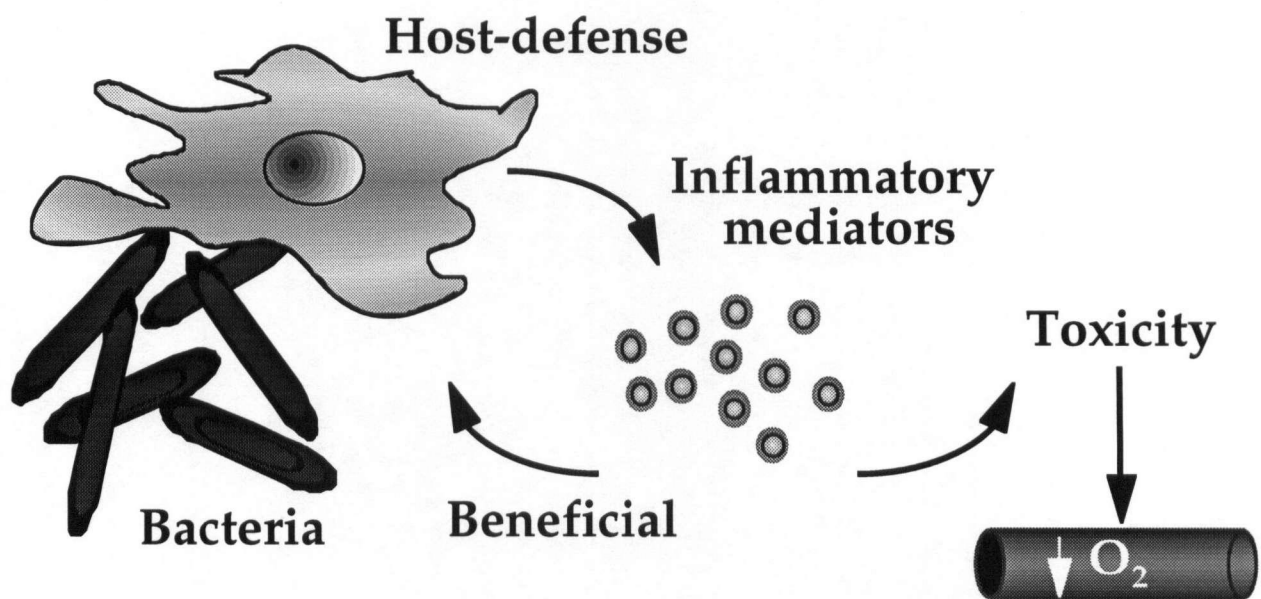


Figure 2. Mediators of Septic Toxicity.

This figure illustrates the overall mediation of the toxicity involved with sepsis. It starts at the interaction between bacteria and the host-defense mechanisms. This interaction is mediated by various mediators leading to various toxicity's ultimately leading to ischemia.

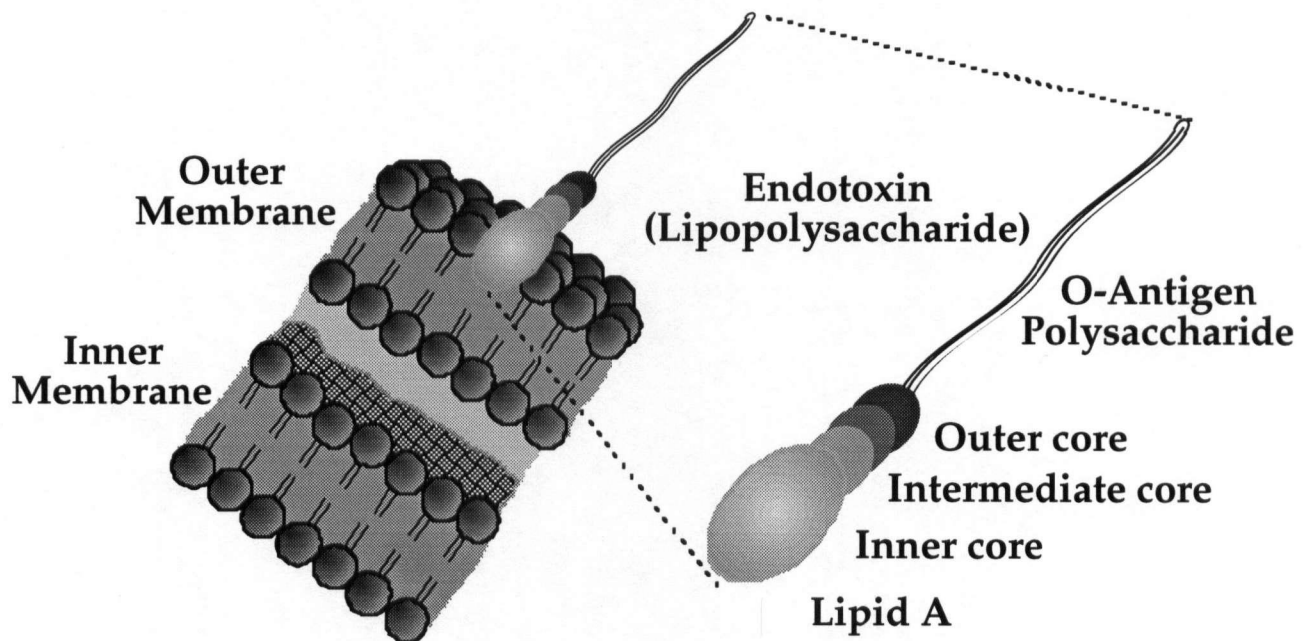


Figure 3. Endotoxin.

This figure illustrates endotoxin (lipopolysaccharide) as part of the cell wall of a gram negative bacteria. Endotoxin is comprised of two components: the O-specific chain and the core. The O-specific chain is a polymer of oligosaccharides and accounts for the antigenic variability among species and strains of bacteria. The core is composed of an oligosaccharide covalently bound to a molecule lipid called lipid A. Most of the toxicity of LPS resides in the lipid A moiety.

to endotoxin by stimulating neutrophils, endothelial cell, platelets, and the release of other cytokines, eicosanoids, platelet activating factor (PAF), endorphins, endothelial relaxing factor, and a variety of other plasma and cellular mediators (11) (10) (12).

Some have speculated that endotoxin leads to mucosal hypoxia as a mechanism of damage. However, VanderMeer et al (13) showed that there is significant mucosal acidosis despite absence of mucosal hypoxia. Other mechanisms by which LPS may cause mucosal acidosis include uncoupling of oxidative phosphorylation (14) (15), inhibiting mitochondrial respiration (16) (17) (18) (17), decreasing availability of substrates (19), increasing metabolism, or decreasing the clearance of H^+ or CO_2 .

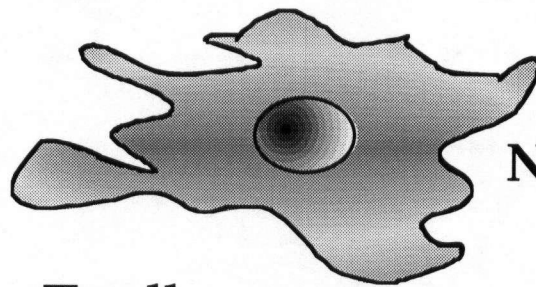
1.3.3 Host Defenses

Baker and Huynh (7) divides host defenses into nonspecific and specific factors. Nonspecific factors include skin, mucus membranes, as well as complement, hemolytic factors, and phagocytosis. Phagocytosis involves interactions with a number of other systems, particularly the complement system. The specific immune system is responsible for recognizing bacteria and creating antibodies to help opsonize them. The specific immune system is a delicately balanced system with cells that have both stimulatory and inhibitory (or regulatory) function. While, for the most part, the immune system is important in preventing infection, the host's response to injury is often worse than the injury itself and can develop into a malignant, uncontrolled SIRS (7). This occurrence represents a state

of disseminated activation of the host's inflammatory systems. While any of the cells of immunity may be involved (Figure 4), it is thought that the macrophage is central to the development of this process (20). Macrophages play a crucial role in regulating the immune response after injury (21). They reside at strategic positions throughout the body, armed to eliminate foreign and infective agents. It is thought that it is the excessive, unregulated, prolonged stimulation of the macrophage in conjunction with other leukocytes and the endothelial system that leads to a vicious cascade of inflammatory mediator release (3). The macrophage is capable of producing directly toxic substances in addition to numerous cytokines and mediators that act synergistically to augment the inflammatory response (7). The various substances including TNF, interleukins, PAF, arachidonic acid metabolites, and nitric oxide are discussed below. Further, a number of investigators have studied the role of neutrophils in the development of SIRS and MODS. Much like the macrophage, it is thought that unregulated activation of neutrophils and the endothelial system are important factors (22) (23). The neutrophils are thought to adhere to the endothelium, creating a microenvironment in which neutrophil-derived proteases and toxic-reactive oxygen species are present at high concentrations. These toxic products can directly injury the endothelial lining, leading to intercellular gap formation, altered vascular permeability, and further neutrophil migration (24). Together, the interaction among macrophages, neutrophils, and the endothelial system augments the inflammatory cascade and results in bystander organ failure (7).

Macrophages

B-cells



Neutrophils

T-cells

Figure 4. Host Defenses.

This figure illustrates the different leukocytes thought to be important in immunity.

1.3.4 Inflammatory Mediators

There are a multitude of toxic mediators thought to have importance in mediating the damages seen in SIRS and sepsis including TNF, IL-1, PAF, prostaglandins, thromboxanes, leukotrienes, nitric oxide, O₂ radicals, interferon, and complement (Figure 5).

1.3.4.1 Peptide cytokines

Some degree of inflammatory response is due to the biological effects and balance of peptide cytokines (22). Both tumor necrosis factor (TNF) and interleukin-1 (IL-1) are important mediators in the pathogenesis of SIRS and MODS. These peptides are synthesized in nearly all organs containing phagocytes and blood mononuclear cells in response to a large number of stimuli including endotoxin, complement, eicosanoids, interferon, other interleukins, and viral antigens. The mechanism of action is thought to be the induction of subsequent effector molecules of SIRS. Infusion of either TNF or IL-1 will lead to lung injury characterized by increased permeability and neutrophil sequestration (22). TNF also causes neutrophil degranulation and superoxide production (22). It appears that TNF and IL-1 act synergistically in inducing shock (25), prostaglandin synthesis, and neutrophil chemotaxis (22). Inhibition of either TNF or IL-1 independently has been shown to prevent lung injury, shock, and death following endotoxin or *E. coli* administration (22).

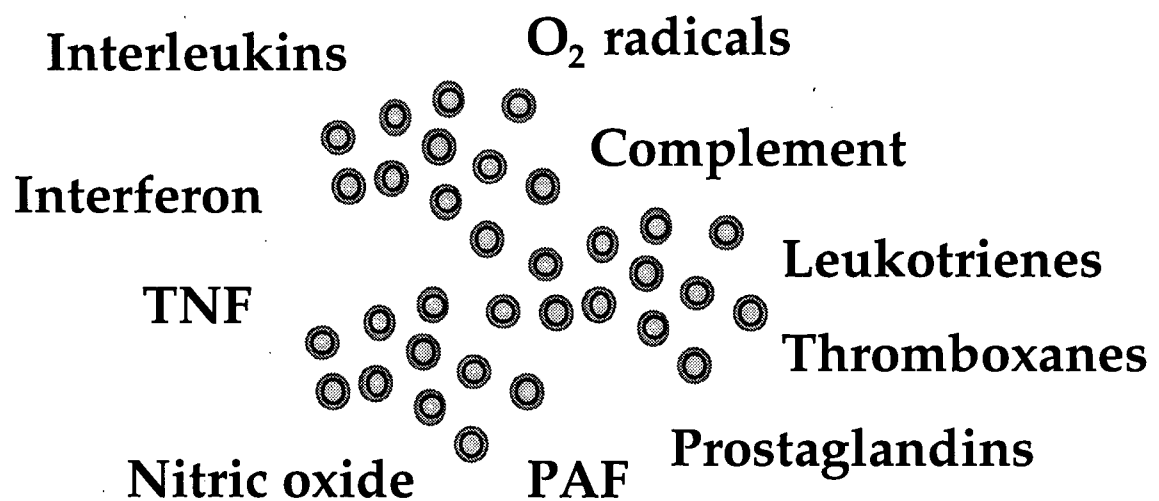


Figure 5. Inflammatory Mediators.

This figure illustrates the various mediators thought to be important in the development of toxicity in sepsis.

1.3.4.2 Platelet activating factor

Platelet activating factor (PAF) is a labile ether lipid first seen to be released by platelets in the presence of antigen and leukocytes. Cipolle et al (22) points to several reasons why PAF is considered an important mediator in SIRS. Infusion of PAF mimics many of the effects of sepsis and endotoxin. Further, PAF is produced during endotoxemia in animals and severe sepsis. Finally, several structurally different antagonists to PAF have been reported to inhibit endotoxin-induced hypotension, lung injury, and mortality.

1.3.4.3 Eicosanoids

Eicosanoids are derivatives of the substrate arachidonic acid (AA), a cell membrane phospholipid. They include prostaglandins (PG), thromboxane (Tx), and leukotrienes (LT). A variety of inflammatory stimuli can directly or indirectly activate phospholipase leading to release of AA from phospholipid pools. Once released, AA is oxidatively metabolized via two major pathways leading to production of the eicosanoids. These derivatives all have varying functions which are beyond the scope of this thesis.

1.3.4.4 Nitric oxide

Nitric oxide (NO) is formed via arginine metabolism and vascular endothelium appears to be the most important source (22). Endotoxin has been shown to stimulate NO formation in vitro (26). The vasodilation and vascular unresponsiveness that occur during sepsis and endotoxemia appear to be mediated

largely via the L-arginine-NO pathway (27). Inhibition of the L-arginine-NO pathway blocks TNF-mediated hypotension in rats (28). While NO may have negative effects, some argue that NO may in fact serve an important role. NO may be protective by inhibiting platelet aggregation and vasodilation, thereby preserving blood flow to important vascular beds to maintain critical oxygen delivery (22). Therefore, inhibiting NO may be detrimental during sepsis in that blood flow to already ischemic areas could be further reduced.

1.3.4.5 Oxygen radicals

Under normal physiologic conditions, 80%-90% of cellular oxygen is consumed at the level of the mitochondrial respiratory chain and is directly reduced to water via the cytochrome oxidase complex (22). In pathologic conditions, fundamental oxygen ($^3\text{O}_2$) can be reduced one electron at a time, giving rise to a cascade of activated oxygen species. The most important species is a free radical, the superoxide anion (O_2^-). Active oxygen metabolites may react to a variety of biologic substrates. They promote DNA scission and base modification, inactivate plasma proteins, cross-link membrane proteins, and most importantly, induce lipid peroxidation in the membrane which may lead to disorganization of its structure and lead to cell death (29). Oxygen free radicals are especially important during ischemia-reperfusion type injuries (22). During the reperfusion phase, severe tissue injury occurs following massive production of oxygen free radicals generated by several mechanisms.

1.3.5 Ischemia and Tissue Injury

The effects described above lead to an alteration in the microvascular flow of blood to tissue the mechanisms of which are described later. Therefore, the inflammatory mediators ultimately results in ischemia as the final endpoint of SIRS and sepsis. Ischemia not only precipitates cellular ATP degradation and impairs energy-requiring cellular function, but it may also activate processes that lead to an increase in oxygen-derived, free-radical production, which can augment or accelerate tissue injury (30).

Ischemia also promotes the formation of xanthine oxidase from xanthine dehydrogenase. Furthermore, there is activation of cellular proteases which converts the xanthine dehydrogenase by proteolysis to xanthine oxidase which uses molecular oxygen as its electron acceptor leading to the generation of superoxide anion and/or hydrogen peroxide (31). Circulating levels of xanthine oxidase have been demonstrated in patients with ARDS (32). It has been hypothesized that liver or gut tissue releases xanthine oxidase, thereby allowing it to serve as a toxic, oxygen-metabolite-generating system in the lung capillary bed (33).

Furthermore, inflammation may be part of the ischemic process or may serve to augment and accelerate the ischemic process. Toxic oxygen metabolites, formed by xanthine oxidase during ischemia, may increase capillary permeability and increase the influx of neutrophils. The arrival of neutrophils and their subsequent activation by factors released from injured cells are likely to augment tissue injury. Local inflammation may also accelerate the ischemic process by causing granulocyte plugging and tissue edema, leading to a further impairment of oxygen delivery (31).

1.4 SEPSIS AND TISSUE ISCHEMIA

The hemodynamic changes that are described in sepsis may be divided into central and microvascular vascular changes (34).

1.4.1 Central hemodynamic changes in sepsis

With regards to the central effects in sepsis, there is an initial stage during which homeostasis is maintained involving various changes including both tachycardia, tachypnea, and fever (12). Ultimately, homeostatic mechanisms fail and signs of circulatory shock ensue and is described as having both a hyperdynamic and a preterminal hypodynamic phase. The hyperdynamic phase of septic shock is typically seen and consists of a high cardiac output, low pulmonary artery occlusion pressure (PAOP), and a low peripheral vascular resistance (12). The hypodynamic stage is manifested by a low cardiac output and an increased systemic vascular resistance.

Despite the increased cardiac output, many investigators have described a global myocardial dysfunction in septic shock (35) (36) (37). The increased cardiac output reflects the increase in heart rate while the stroke volume is usually low (35) (38). Plotted on a Frank-Starling curve against the PAOP, the left ventricular stroke work index was typically reflective of left ventricular failure (12) (39) (40). Furthermore, the magnitude of the myocardial depression correlates with survival as patients capable of maintaining their cardiac output by increasing their heart rate and left ventricular end-diastolic volume are more likely to survive (12). There have been several substances that have been implicated in contributing to this

myocardial depression (41) including TNF, low-molecular-weight water-soluble molecules, and lipid-soluble substances (42) (43) (44). The presence of these depressant factors has been shown to correlate with the severity of cardiac dysfunction and systemic hypoperfusion (45).

1.4.2 Microvascular changes in sepsis

While initial investigations focused on measurable hemodynamic changes, more recent studies have emphasized the role of the microcirculatory changes in sepsis (46) (47). The changes described include: alterations in erythrocyte deformability, viscosity alterations, increased vascular permeability with development of interstitial edema, and changes in microvascular flow with development of capillary heterogeneity. All these changes will go to alter the overall microcirculatory flow of blood and thereby alter the delivery of oxygen from capillaries to cells (Figure 6).

1.4.2.1 Alterations in erythrocyte deformability

The normal state of deformability is the ability of the red cell to alter its biconcave, discoid shape to allow passage through a capillary smaller in diameter than itself (46). Driessen et al (48) emphasized the importance of normal erythrocyte deformability for the maintenance of adequate perfusion of the microcirculation, especially when perfusion pressure is reduced. They further explained that red cell deformability depends on the viscoelastic properties of the cell membrane, the viscosity of the cytoplasm, and the surface area/volume ratio, all of which may

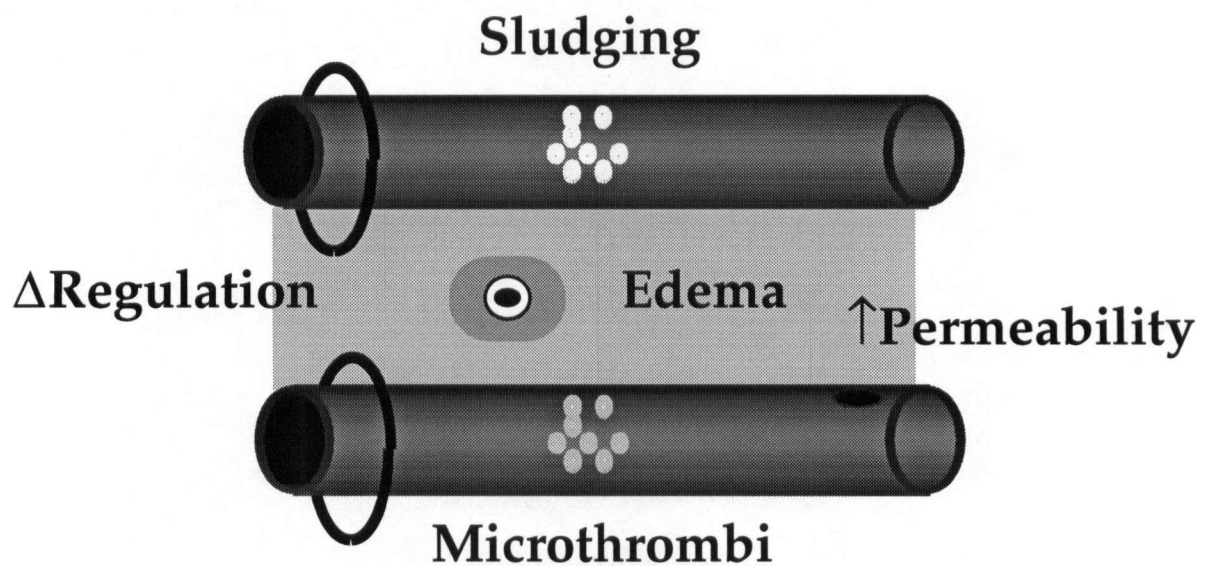


Figure 6. Toxicity.

This figure illustrates the various microcirculatory effects described in sepsis. This includes alteration in autoregulation in the vessels with the ellipses representing the vascular tone, leukocyte sludging and microthrombi formation which leads to vascular obstruction, and increased capillary permeability which leads to interstitial edema as depicted by the shaded area surrounding the cell.

change during shock. When erythrocyte deformability is decreased, the time required for red cell passage through capillaries is prolonged and the cells may themselves impede blood flow and may lead to an impaired ability to extract oxygen as has been demonstrated by Powell et al (49) in septic humans. Furthermore, the increased rigidity of red cells promotes arteriovenous shunting of blood, which further decreases microcirculatory flow during sepsis (50).

1.4.2.2 Viscosity alterations

Blood viscosity is an important determinant of microcirculatory flow. According to Poiseuille's equation, blood flow varies directly with vessel radius to the fourth power and inversely with the length of blood vessels and blood viscosity. Chien (51) studied the rheological factors in the microcirculation during low-flow states and concluded that venular blood viscosity increases due to a decrease in shear stress. The result of increased venular viscosity would be increased post-capillary resistance leading to diminished blood flow and increased transcapillary leakage (51).

1.4.2.3 Increased vascular permeability and interstitial edema

Sepsis has often been characterized as a diffuse increase in microvascular permeability (46) (52) (12). Solomon and Hinshaw (53) showed that endotoxin increases capillary permeability in the skin and muscle tissues independently from changes in hydrostatic and colloid pressures, thereby implying the mechanism to be an alteration in the cell membrane structure. According to Starling's Forces, an

increase vascular permeability will predispose to interstitial edema. Hersch et al (54) studied the histologic and ultrastructural changes in a rat model of hyperdynamic sepsis and showed that despite a preservation of hemodynamic variables, there was a widespread degree of lesions from sepsis including interstitial and intracellular edema. Interstitial edema may in turn lead to an impaired diffusion of oxygen from vessels to cells (55) (34). Heughan et al (56) showed that saline loading will lead to interstitial edema and this was associated with a decrease in tissue oxygenation. Not only can interstitial fluid itself limit the diffusion of oxygen but interstitial edema in sepsis is known to result in an increased distance from cells to capillaries (57). Knisely et al (58) have theorized that such an increased distance will significantly limit the diffusion of oxygen. Other possible changes promoting edema formation include protein leakage (59), separation of tight junctions between endothelial cells (60), dysfunctional rather than destructive changes of vascular endothelium (61), and release of vasoactive agents (62). Finally, tissue edema formation may compress capillaries and thereby limit oxygen delivery (34).

1.4.2.4 Alterations in microvascular flow and capillary heterogeneity

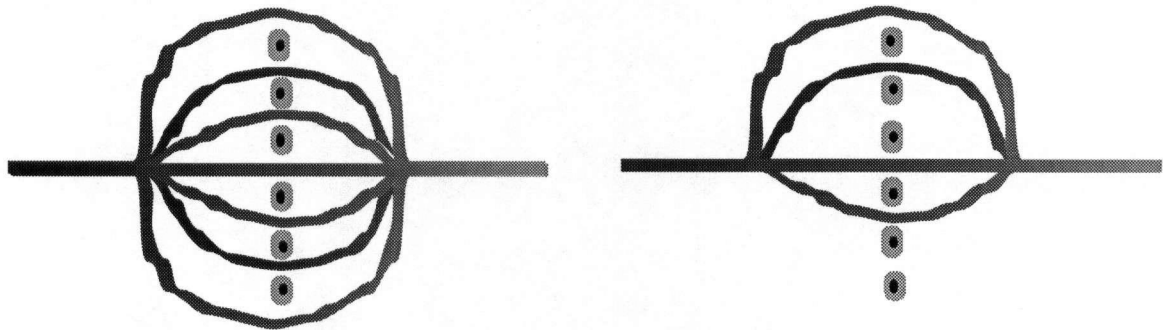
The ability of tissue to alter blood flow to actively metabolizing tissue is of paramount importance in survival. The organism adjusts oxygen extraction in response to changes in oxygen delivery through a balance between vasoconstrictor tone among organ systems and local metabolic vasodilation within tissues (63). However, various components required for normal adaptation to oxygen demands are altered in sepsis including development of vascular obstruction, development of

arteriovenous shunts, and endothelial damage. All these effects lead to an inability to provide normal homogenous blood distribution and thereby leads to a deleterious heterogenous blood flow (Figure 7).

Various components of blood may lead to obstruction of vascular flow. Knisely et al (64) studied patients and animals and found large rigid erythrocyte aggregates in all vessels, and concentrated "sludge" in plugged vessels which lead to obstructed blood flow. Clotting factors may also be activated leading to disseminated intravascular coagulation (DIC) during sepsis (65) whereby fibrin and microthrombosis are deposited in vessels. Finally leukocytes have been shown to cause capillary obstruction during sepsis (65) (41) (66) which not only contribute to vascular obstruction but also has effects on red cell deformability and capillary cross-sectional area (65).

Various studies have shown the occurrence of shunts in various tissue beds in sepsis. Cronenwett and Lindenauer (67) used microspheres to demonstrate arteriovenous shunting of blood in the septic canine. Further, Archie (68) described arteriovenous shunting in a cecal ligation shock model occurring in the splanchnic and renal circulation. Whether these changes represent the effects on the changes of red cell deformability (see above) or represent true anatomical arteriovenous shunts is unclear.

While previously, the endothelial layer was thought of as only a passive tissue, more recent work has described its importance in modulating vascular tone, controlling local blood flow, influencing the rates of leakage of fluids and plasma proteins, modulating the accumulation and extravasation of leukocytes into tissue,



Homogenous distribution

Heterogeneous distribution

Figure 7. Capillary Heterogeneity.

Homogeneous distribution leads to adequate blood flow to the cells. Heterogeneous distribution leads to maldistribution of blood flow to the cells.

and influencing leukocyte activation (69). Therefore, damage to the endothelium and the underlying smooth muscle from hypoxia, inflammation, complement activation, O₂ radical production, and lipid peroxidation, all postulated in the sepsis syndrome, would interfere with the matching of O₂ delivery and metabolic needs by interfering with vascular smooth muscle tone and possible interactions between the endothelium and the smooth muscle (55) (70). Schumacker and Samsel theorize that alteration in endothelial function leads to the loss of autoregulation of the microvasculature which in turn results in the inability of tissue to adjust to reduced oxygen delivery (63) (71). Indeed, sepsis typically is described as having a decreased systemic vascular resistance (72) which reflects the decreased arteriolar and venular tone (36).

Dantzker (55) explains that a loss of autoregulatory ability could explain several of the features seen in sepsis. The effects explained above all point to changes that will affect the ability to adapt to increased oxygen demands. Delivery of oxygen to tissue is ideally done by both an adequate and a homogenous distribution of capillaries. However, Drazenovic et al (73) found endotoxemia in a canine model reduced capillary density in mucosal villi and crypts. Further, Lam et al (57) using intravital microscopy to analyze changes in muscle capillaries in a rat model of sepsis not only found a decreased density of perfused capillaries, but also an increase in capillary heterogeneity, an increase in the inter-capillary distance, an increase in capillary red blood cell velocity, and a decrease in the peak hyperemic response. Thus, while homogenous capillary flow promotes oxygen extraction, all the microcirculatory changes described will result in a heterogenous flow of blood

which may impair oxygen diffusion and extraction by tissue (74) (75) (76) (63) (77) (78) (79).

1.5 SPLANCHNIC ISCHEMIA IN CRITICAL ILLNESS

1.5.1 Protective mucosal barrier

An intact mucosal layer is important to act as a defense against infection. It has been shown that an intact mucosal layer maintains an effective barrier even during states of immunosuppression (80). This implies that the cell-mediated immunity provided by the intestinal wall's lymphocytes, macrophages, and the Peyer's patches, and the mesenteric lymph nodes serves a secondary or supportive role to the epithelium. While the mucosa is an important barrier, the gut is susceptible to ischemia during sepsis or endotoxemia due to the manner that the blood flow is organized.

1.5.2 Splanchnic Metabolic Model of O₂ Control

Metabolic demand theory in the splanchnic circulation proposes that the intestines control local microvascular smooth muscle tone, based on the prevailing tissue PO₂, and independent of nervous or humoral influences. (81). There appears to be two separate microvascular mechanisms by which the intestine can regulate the rate of oxygen delivery (82) (Figure 8). First, local arteriolar tone governs the amount of total blood flow that is available to the individual capillary beds. Control of the precapillary sphincters modulates the number of capillaries that are perfused within a capillary bed, thereby effecting changes in the surface area and the capillary-

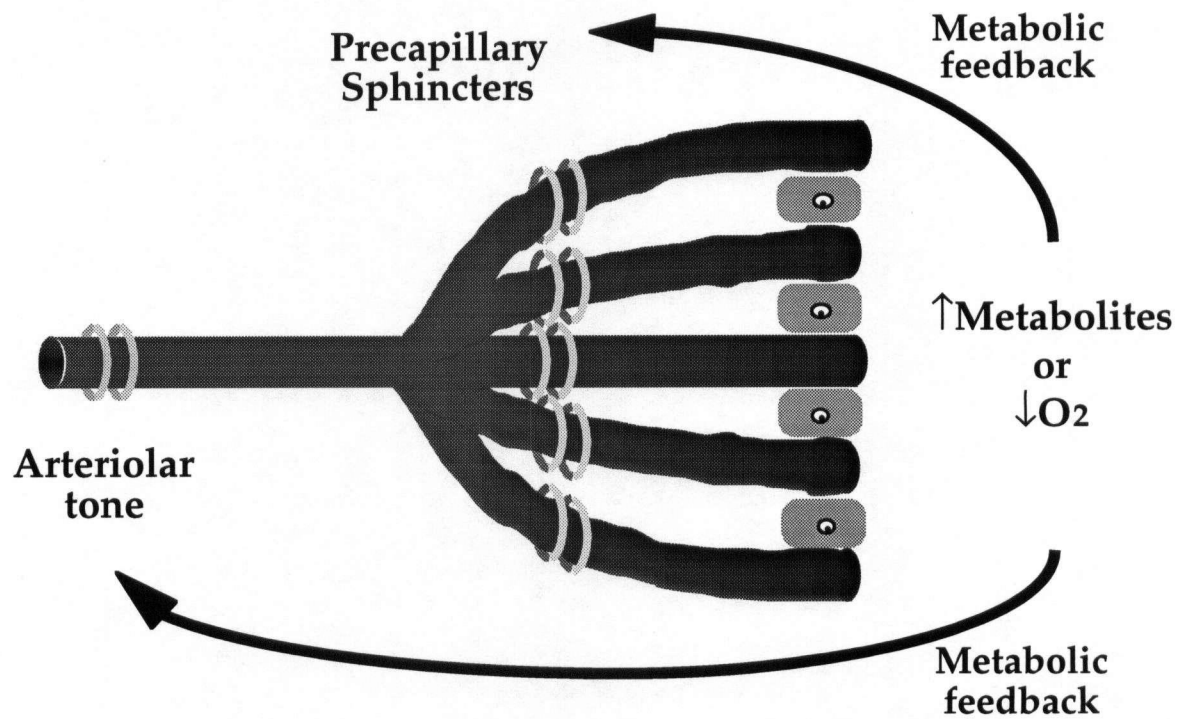


Figure 8. Metabolic model of O₂ control in the gut.

This figure illustrates theoretical model of oxygen control at the microvascular level in the gut. Local arteriolar tone governs total blood flow. Small and moderate decreases in oxygen or build-up of metabolites leads to alteration in the tone of precapillary sphincters.

to-cell diffusion distance (81). Second, with small and moderate decreases in oxygen delivery, the main compensatory mechanism for maintaining a stable oxygen consumption are the precapillary sphincters (83). This mechanism causes an increase in capillary surface area and results in increased oxygen extraction. Only with larger decreases in oxygen delivery does the arteriolar resistance decrease, thereby increasing blood flow to the tissue (84). However, despite this fine control (55), there is a loss of autoregulatory ability in sepsis resulting in a reduced capillary density in mucosal villi and crypts (73). In fact, the reduction in splanchnic blood volume is disproportionately greater than that seen in other tissue beds (85).

1.5.3 Counter-current shunting (CCS)

The microcirculation of the intestinal villus is arranged in a counter-current system of arterioles and venules which is designed to improve absorption. However, the countercurrent shunting has been hypothesized as another reason for the susceptibility of the mucosa to ischemia during sepsis (82) (86) (55) (87) (88). This theory proposes that since the vessels are arranged in a villus with both the arteriole and the venule side by side, O_2 may diffuse from the arteriole straight across to the venule without the villus ever benefiting from the O_2 , thereby effectively shunting oxygen away from the villus (Figure 9). Furthermore, it has been shown that this effect is more exaggerated during times of decreased blood flows (89).

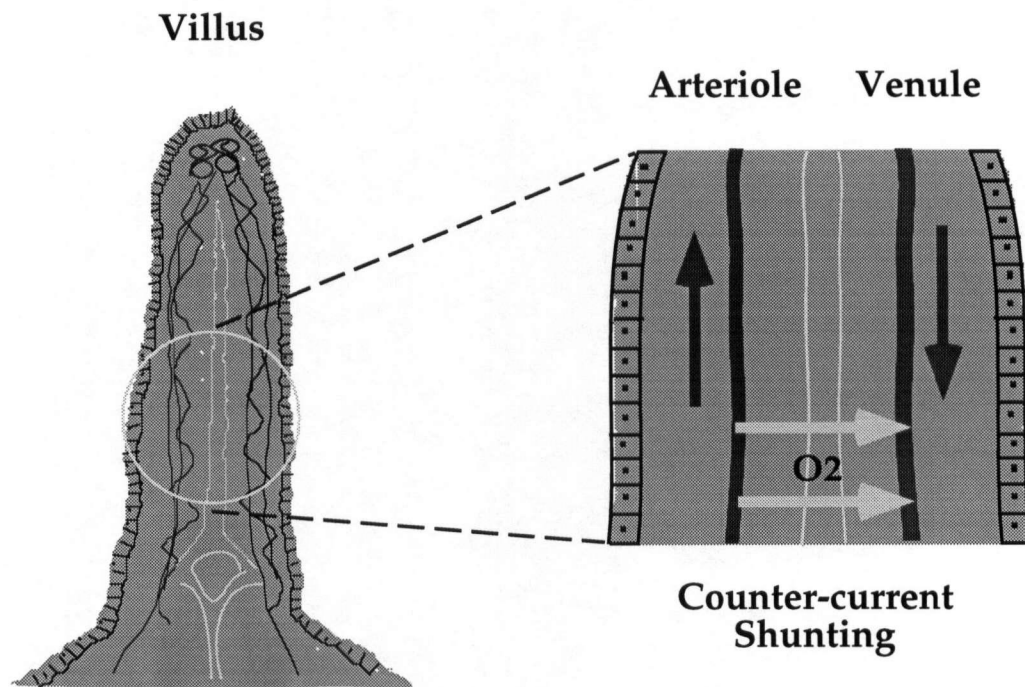


Figure 9. Counter-current shunting (CCS).

This figure illustrates theoretical concept of counter-current shunting in the intestinal villus. It describes how oxygen may shunt across from the arterioles to the venules due to the vessels being arranged side by side.

1.5.4 Gut Ischemia during Septic Shock

While the mucosal barrier serves a particularly important role in preventing infection, its own fine microvascular balance places it at risk in sepsis (90). Fink et al (52) showed that even when the hemodynamic variables were maintained in sepsis, there are significant levels of gut mucosal acidosis. Furthermore, the high metabolic demands of the intestinal surface places the gut at further risk during times of limited oxygen supply (91) (92) (93) (94). Since the gut is susceptible to the effects of sepsis, the protective mucosal barrier is thereby placed in jeopardy (91) (95) (96). Salzman et al (97) have shown that endotoxin infusion leads to increased intestinal permeability to macromolecular hydrophilic solutes. Similarly, O'Dwyer et al (98) injected endotoxin in healthy humans and found increased permeability to nonmetabolizable sugars.

Bacterial translocation is the process by which microorganisms migrate across the mucosal barrier and invade the host. Rush et al in a series of studies (99) have shown that shock in animal models and humans leads to the development of bacteremia and endotoxemia. Further, they have shown that most of the bacteria found in the blood were enteric organisms and by using radiolabelled *E. coli* and demonstrating them escaping from the gastrointestinal tract and into the blood stream, conclude that bacterial translocation is a true phenomenon. Deitch et al (100) had similar conclusions in a mouse model and further determined that the mechanism may be via increased gut permeability via activation of xanthine oxidase leading to development of oxygen free radicals (101). Mainous et al (102) studied the route of bacteremia and determined that bacterial translocation is

primarily via portal blood as opposed to mesenteric lymphatics and occurs in a dose dependent fashion to the degree of insult.

It has been theorized that injury to the gut with the resultant bacteremia and endotoxemia may perpetuate a continuous cycle of injury leading to the development of multiple organ dysfunction syndrome (85). Hence, the term, the gut as the "motor of multiple organ dysfunction" (3) (Figure 10).

1.6. AEROBIC AND ANAEROBIC METABOLISM

1.6.1 Aerobic metabolism

The routine means to derive energy by cells is by aerobic metabolism. Energy is derived from amino acids, glucose, and fatty acids via the Krebs' (Tricarboxylic Acid) Cycle and oxidative phosphorylation. The Krebs' cycle is a series of controlled oxidation-reduction reactions during which the energy, released from the transfer of electrons, is captured. This involves the reduction of NAD^+ to NADH , a shuttling of the electrons from NADH into the mitochondria, transferring the electrons along a series of electron-carrier enzymes, and the capture of energy in the high-energy phosphate bonds of adenosine triphosphate (ATP). Oxygen is the terminal electron acceptor in this scheme and sufficient amounts are required if optimal use of substrate for the generation of ATP is to continue. When oxygen is not present in adequate amounts, the organism relies on the less efficient anaerobic metabolism which generates less energy per substrate when compared to aerobic metabolism (55) (Figure 11).

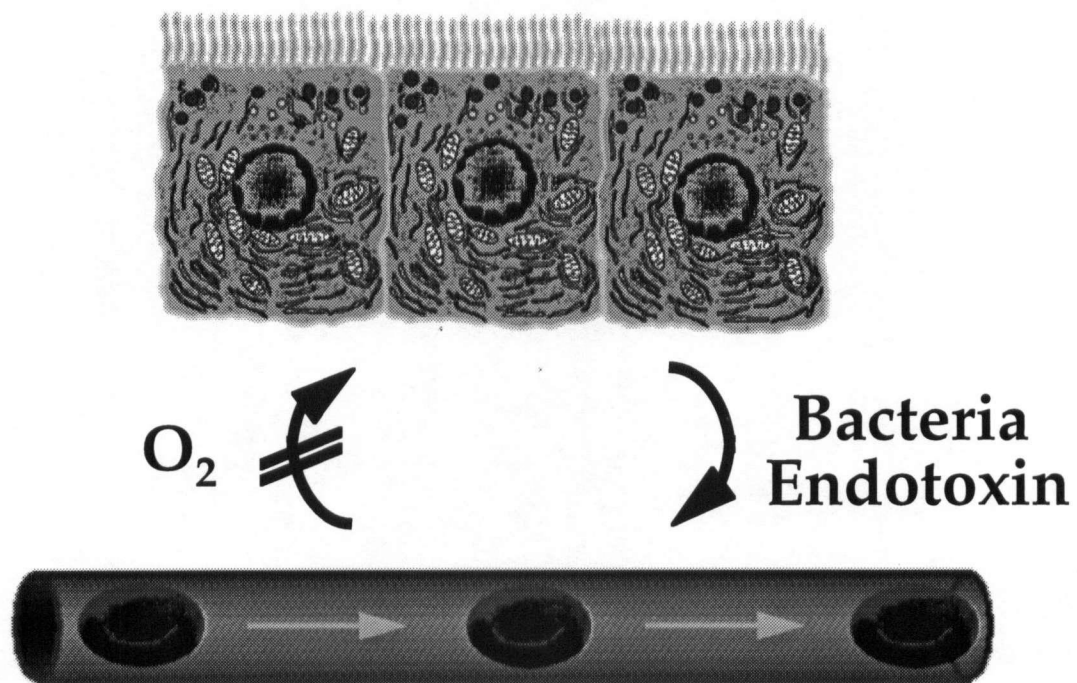


Figure 10. Gut as "Motor of Multiple Organ Dysfunction".

It has been theorized that injury to the gut with the resultant bacteremia and endotoxemia may perpetuate a continuous cycle of injury leading to the development of multiple organ dysfunction syndrome. Hence, the term, the gut as the "motor of multiple organ dysfunction"

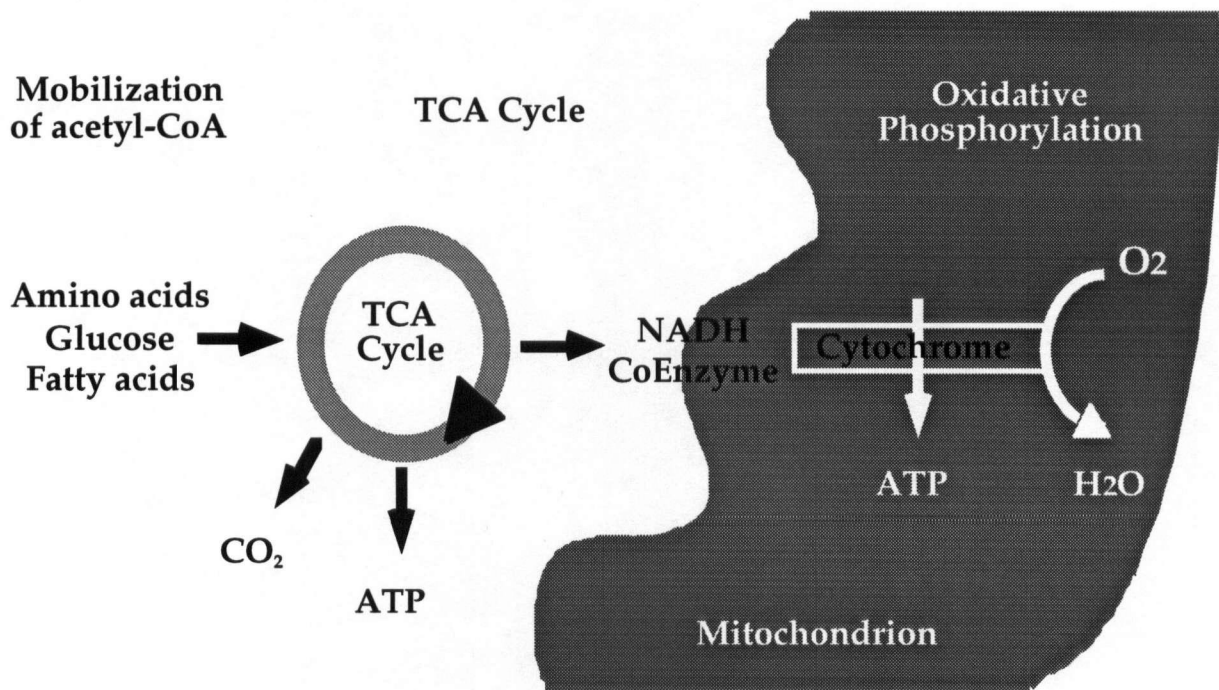


Figure 11. Aerobic Metabolism.

Energy is derived from amino acids, glucose, and fatty acids via the Tricarboxylic Acid Cycle (TCA) and oxidative phosphorylation. The TCA cycle is a series of controlled oxidation-reduction reactions during which the energy, released from the transfer of electrons, is captured. This involves the reduction of NAD^+ to NADH, a shuttling of the electrons from NADH into the mitochondria, transferring the electrons along a series of electron-carrier enzymes, and the capture of energy in the high-energy phosphate bonds of adenosine triphosphate (ATP). Oxygen is the terminal electron acceptor.

1.6.2 Anaerobic metabolism

When oxygen is not available, ATP is generated by glycolysis where pyruvate is the terminal electron acceptor. While the aerobic pathway results in the generation of 36 mmol of ATP per mol of glucose, the anaerobic pathway results in only 2 mmol of ATP per mol of glucose. Not only does this produce much less energy per mol of substrate, but the pyruvate is reduced to lactate which may then contribute to the development of metabolic acidosis (55) (Figure 12).

1.6.3 Oxygen Consumption and Delivery

Investigators have developed techniques whereby the oxygen consumption and delivery variables can be quantified to allow determination of the onset of ischemia and ability to extract oxygen. The technique of decreasing oxygen delivery by progressive hemorrhage and following the effects on oxygen consumption have been described (103) (104) (105). The ability of tissue to utilize O_2 during shock can be determined by plotting the relationship between oxygen consumption (VO_2) and oxygen delivery (DO_2). With progressive decrease in oxygen delivery, the tissue compensates by increasing the amount of oxygen extracted. If the tissue is able to extract enough oxygen then aerobic metabolism is maintained. However, there is a point when O_2 delivery falls below sufficient O_2 requirements after which anaerobic metabolism ensues. This critical point is considered the onset of ischemia (DO_{2c}) (Figure 13).

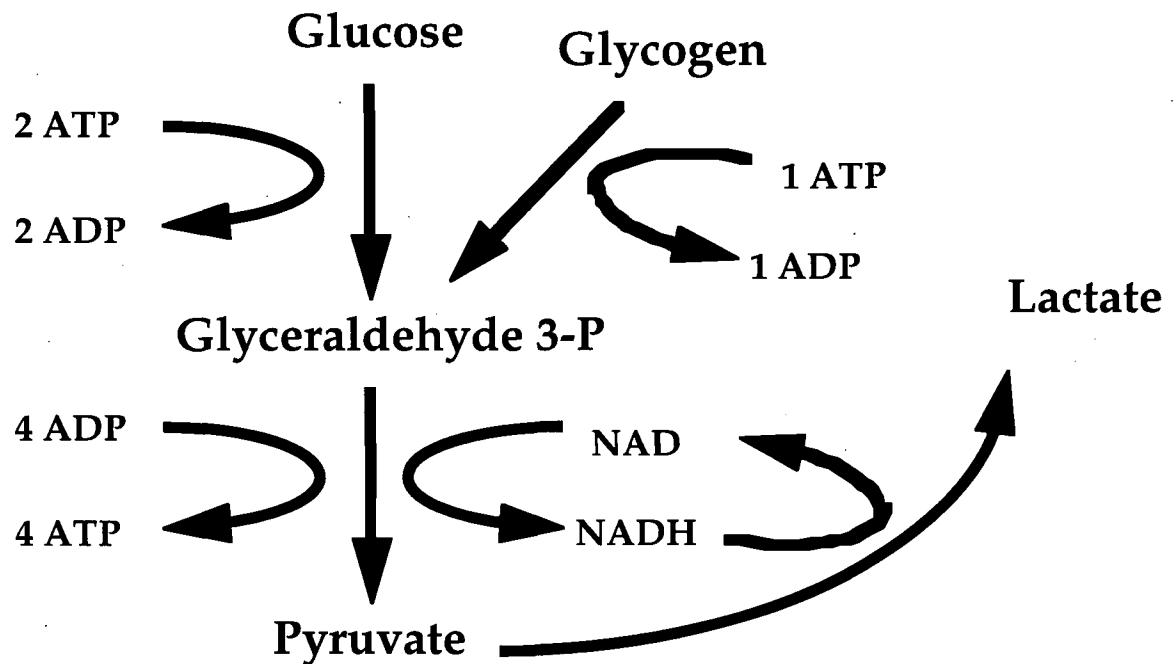


Figure 12. Anaerobic Metabolism.

Anaerobic metabolism of ATP from glycolysis. When oxygen is not available, ATP is generated by glycolysis where pyruvate is the terminal electron acceptor. Not only does this produce much less energy per mol of substrate, but the pyruvate may be reduced to lactate possibly contributing to the development of metabolic acidosis.

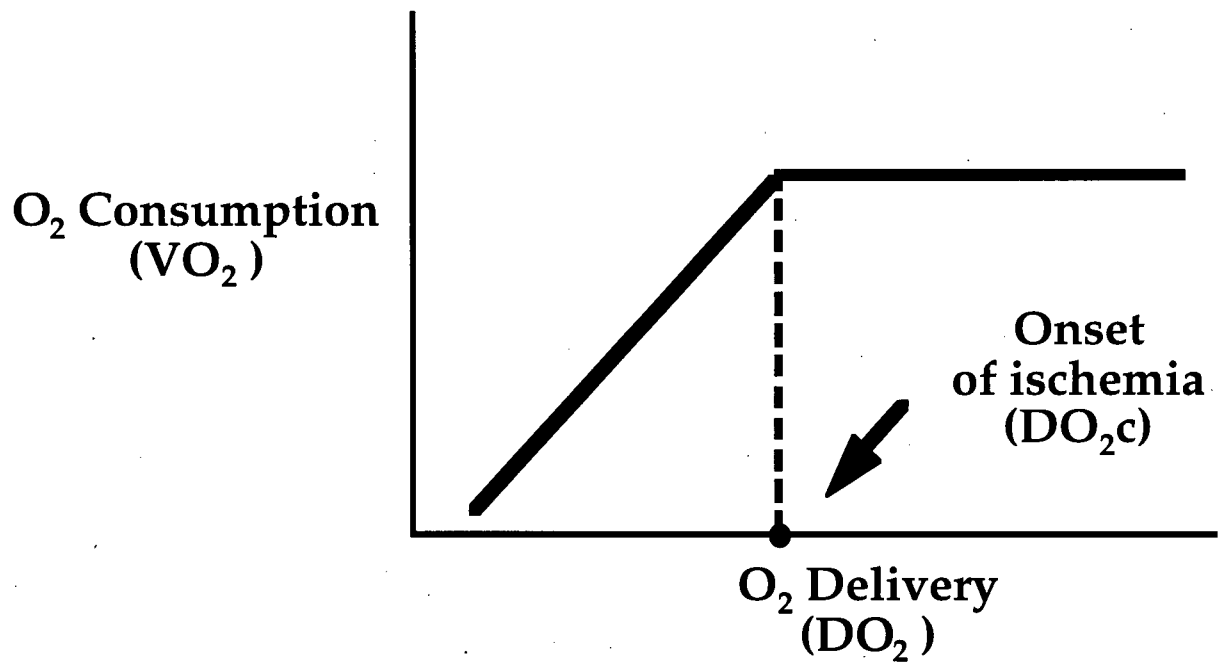


Figure 13. Relationship between Oxygen Delivery and Consumption.

With progressive decrease in oxygen delivery, the tissue compensates by increasing the amount of oxygen extracted. If the tissue is able to extract enough oxygen then aerobic metabolism is maintained. However, there is a point when O_2 delivery falls below sufficient O_2 requirements after which anaerobic metabolism develops. This critical point is the onset of ischemia (DO_{2c}).

1.6.4 Oxygen Extraction Ratio

The ability to extract oxygen is calculated as the oxygen consumption at the onset of tissue ischemia (VO_{2c}) divided by the oxygen delivery at the onset of tissue ischemia (DO_{2c}). This can also be plotted against oxygen delivery to represent the increases in the amount of oxygen extracted as oxygen is delivered (Figure 14). The ability to extract oxygen is based on three major factors: adequate oxygen delivery, unimpeded oxygen diffusion, and the ability to utilize oxygen.

1.6.5 Oxygen Consumption and Delivery in Sepsis

Studies (106) (107) (108) (109) have shown two main changes in the oxygen consumption and delivery curves. First, the oxygen consumption at baseline is at a higher level, representing the increased overall metabolic rate. Secondly, tissues have been noted to have an earlier onset of ischemia (Figure 15). This earlier DO_{2c} may be due to the second major change with sepsis, that of an impaired ability to extract oxygen by the tissue.

1.6.6 Oxygen Extraction in Sepsis

As with studies into the onset of ischemia, the ability to extract oxygen has also been documented to be impaired (106) (107) (108) (109). This can be documented with a lower oxygen extraction ratio curve for the same oxygen delivery (Figure 16). Dantzker suggested three possible reasons for an impaired oxygen extraction and utilization. First, the apparent utilization of anaerobic mechanisms for ATP generation despite high DO_2 may suggest an abnormality of the cells' ability to

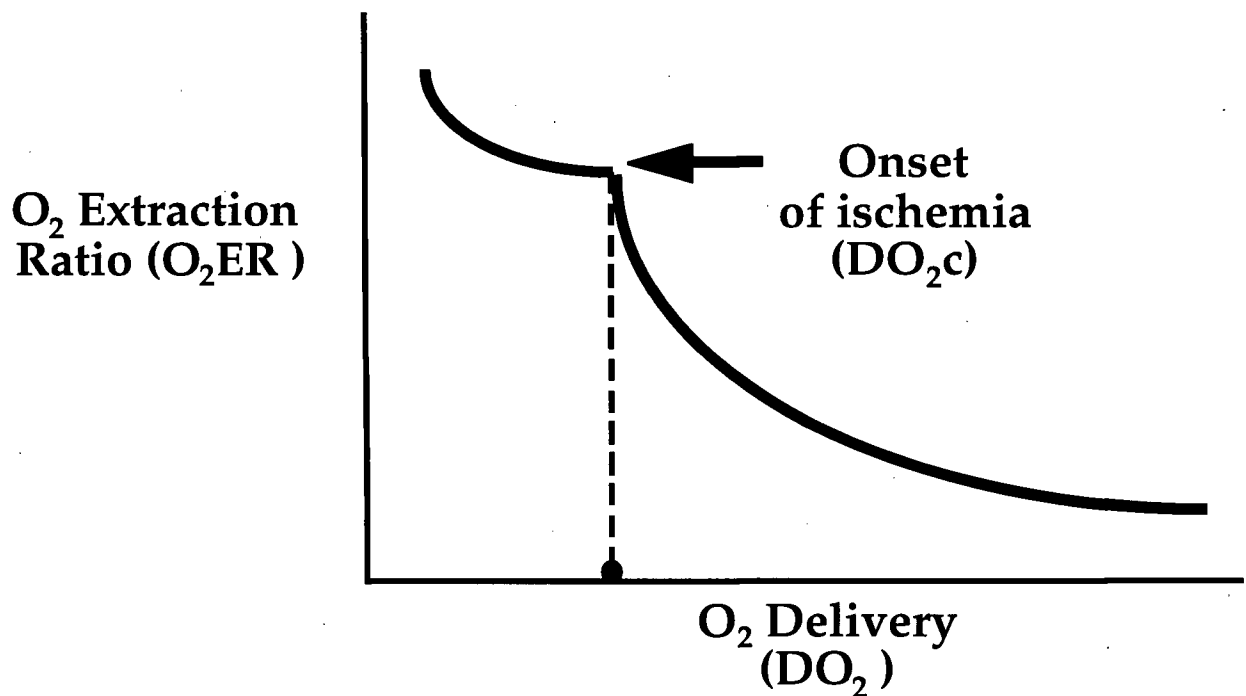


Figure 14. Oxygen Extraction Ratio.

With progressive decrease in oxygen delivery, the tissue compensates by increasing the amount of oxygen extracted. If the tissue is able to extract enough oxygen then aerobic metabolism is maintained. However, there is a point when O_2 delivery falls below sufficient O_2 requirements after which anaerobic metabolism develops. This critical point is the onset of ischemia (DO_{2c}).

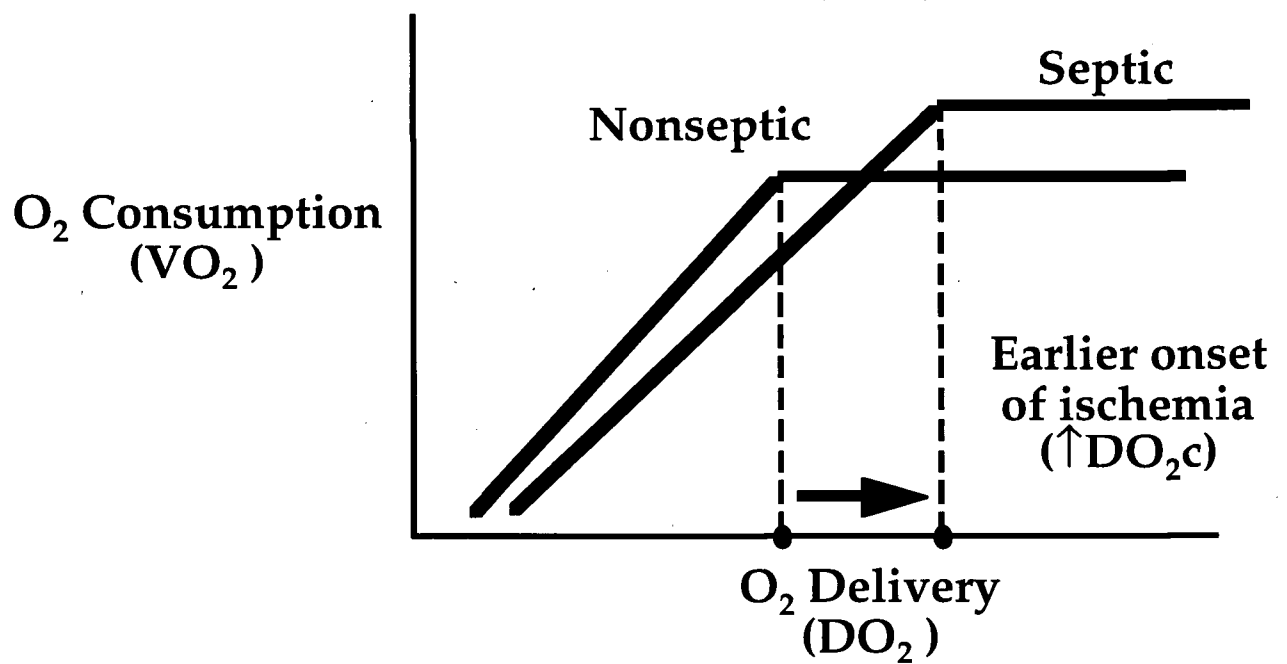


Figure 15. Relationship between Oxygen Delivery and Consumption in Sepsis.

There are two main changes in the oxygen consumption and delivery curves. The oxygen consumption at a baseline is at a higher level and tissues have an earlier onset of ischemia.

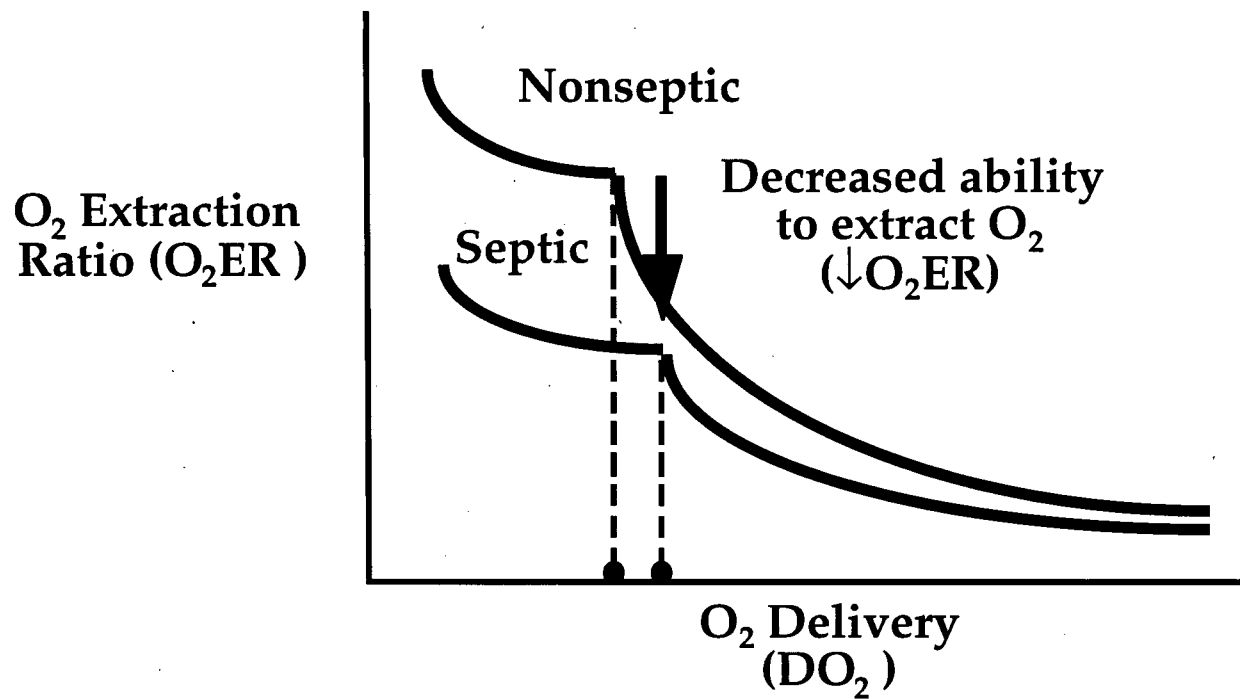


Figure 16. Oxygen Extraction Ratio in Sepsis.

During sepsis, the ability to extract oxygen has been shown to be impaired.

utilize the oxygen that is delivered to it. Indeed, Hersch et al (54) in a study on the histologic and ultrastructural changes in a rat model of hyperdynamic sepsis showed widespread mitochondrial destruction. Second, the finding of a high mixed venous PO₂ in the face of tissue hypoxia may indicate a degree of shunting of blood around metabolizing tissue. Finally, there may be an impaired ability of oxygen to diffuse from the systemic capillaries to the cells requiring oxygen due to interstitial edema.

1.7 TREATMENT OF SEPSIS

1.7.1 Treatment Options

Treatment of sepsis may be focused at any of the levels of septic induced toxicity (Figure 17). Certainly the prevention of infection would be the best strategy. Next would be to treat infection early to limit the effects by either early drainage of infections or treatment with antibiotics (12) (2). New treatments include attempting to block the effects of endotoxin with antibodies and immunomodulation of the host defenses. However these new techniques have meet with conflicting results. Indeed, attempting to modulate the immune system is complicated at best as one needs to balance the advantages and disadvantages of the immune system.

However, all too frequently, patients present far along their course of SIRS. Therefore, the treatments clinicians are left with are an attempt to improve oxygen delivery (47) and maintain adequate arterial perfusion pressure. Two general options are available, including inotropic support and fluid resuscitation.

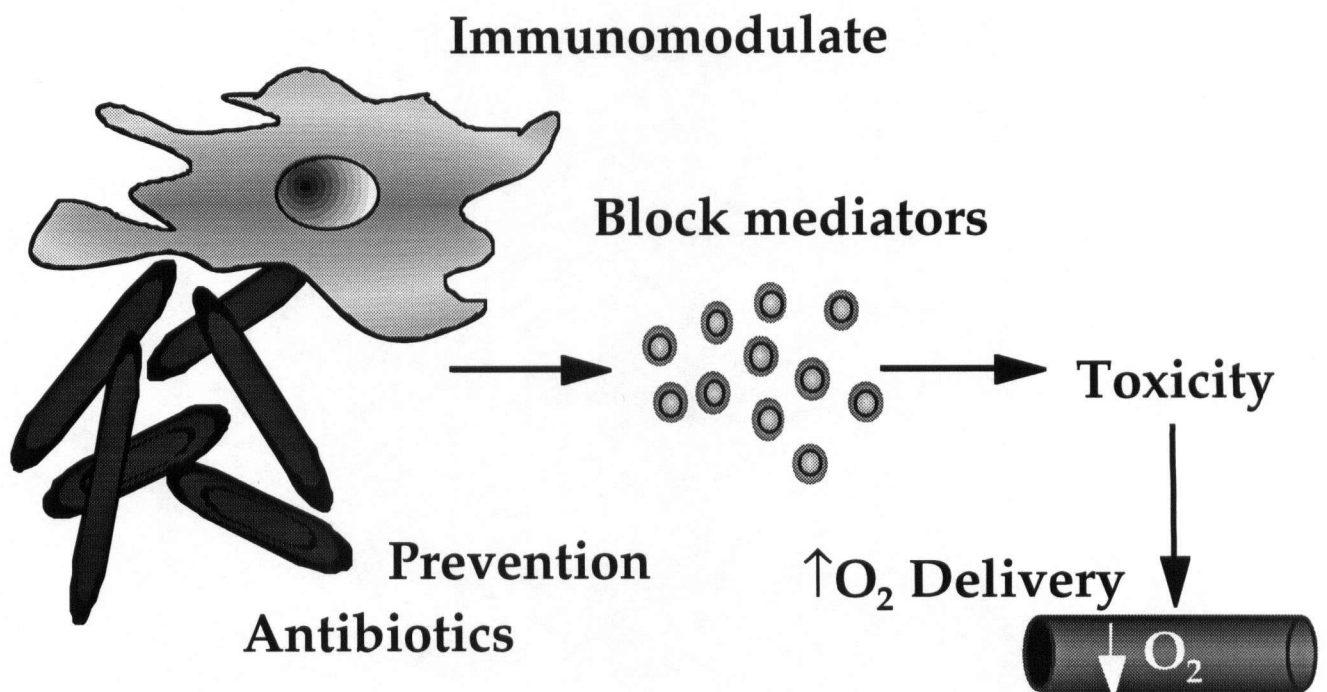


Figure 17. Treatment Options.

This figure illustrates the various treatment options currently being investigated. The currently advocated regimens include prevention, antibiotics, and augmenting oxygen delivery. Areas being investigated include immunomodulation and blocking the mediators of toxicity.

1.7.2 Augmentation of Oxygen Delivery

Oxygen delivery is determined by the following equation:

$$DO_2 = \text{Oxygen content} \times \text{Cardiac output}$$

$$\text{where Oxygen content} = \text{Hgb} \times 1.39 \times O_2 \text{ sat} + 0.003 \times PaO_2$$

From this, one may see that oxygen delivery is dependent on hemoglobin, oxygen saturation, and cardiac output. Increases in any or all of these variable will go to increase the oxygen delivery (47).

To increase hemoglobin, one may transfuse packed red blood cells to increase the hemoglobin concentration (110). This is usually done if the hemoglobin is <100 g/l (111). Many studies showed no benefit in oxygen consumption when transfusing patients above these levels (112) (113).

To increase oxygen saturation, one may increase the oxygen concentration that is given. In many situations, this may require intubation and ventilation to maximize the FiO_2 . A second benefit of intubation and paralysis is a reduction in the work of breathing. Hussain and Roussos (114) showed that early use of mechanical ventilation has been shown beneficial in the management of septic shock.

To increase cardiac output, both vasopressors and fluids are used. Vasopressors are used to try to augment cardiac output by increasing cardiac contractility (47). Of the current inotropes, β -agonists including dopamine, dobutamine, norepinephrine, and epinephrine have been used most often. All but dobutamine also have α -adrenergic action that increases uneven arteriolar

vasoconstriction and may intensify microcirculatory flow maldistributions. Dobutamine has both β_1 and β_2 actions; the latter relaxes previously vasoconstricted arterioles and may improve small vessel blood flow in peripheral tissues (115). Vasodilators play a limited role in the care of septic patients since hypotension is invariably already present (7). Overall, the clinical efficacy and optimal therapy for pressor treatment has not been well documented in prospective randomized trials. Studies have described important side effects that would caution against their use in any situation including increased overall metabolic rate and systemic oxygen demands (116). Further, Ruiz et al report an increased mortality with the use of inotropes in such situations (117). Therefore, since the benefits of vasoactive agents are at present unclear, augmentation of cardiac output is primarily achieved by the administration of fluid resuscitation.

1.7.3 Fluid resuscitation in septic shock

For many clinicians, fluid resuscitation is one of the prime therapies for the treatment of patients in septic shock (118) (2). The rationale for use of fluids has centered on re-establishing normal hemodynamic variables including the augmenting of filling pressures (2) and replacing relative and absolute intravascular volume deficits (2) (119) (120) (121) to augment cardiac function (122). Fluid therapy in septic shock is thought to increase venous return and cardiac output. When fluid therapy is associated with an increase in DO_2 in patients with lactic acidosis, systemic oxygen consumption VO_2 increases and lactic acid levels decrease (123) (124). Other investigators have proposed benefits at a microvascular level.

Hemodilution of blood has been shown to promote survival in critically ill patients (111). The potential benefits include a decreased viscosity of blood (125) leading to an increased velocity of red cells (126) and possibly improved entry of erythrocytes into channels with smaller diameter, thereby decreasing the heterogeneity of blood flow (126) (79).

While both crystalloid and colloidal solutions are frequently used for resuscitation, the choice between these two fluids is controversial. Crystalloids have been generally advocated in view of availability, the lack of potentially infectious complications, and overall costs. However, since only about 1/3 to 1/4 of the saline solution infused remains in the vascular space, large volumes are required in order to achieve therapeutic goals (127). Furthermore, with a significant amount going to the interstitial space, some have raised concerns regarding the development of interstitial edema (128). With these concerns, many have looked to the use of colloids for resuscitation. Colloid fluids may be advantageous because the sustained increases in plasma colloid osmotic pressure (COP) by these fluids will aid in the retention of fluid in the intravascular space (129). Further, evidence shows that sepsis is associated with reprioritization of hepatic protein synthesis with decreased albumin production which contributes to a decreased colloid oncotic pressure (130). Demling et al (131) suggest that it is the hypoproteinemia which may be responsible for the early edema in soft tissues with sepsis. On the other hand, the maintenance of an oncotic gradient by colloid infusion may be difficult in systemic areas in which microvascular permeability is increased. Further, there is concern that egress of larger molecules into the extravascular space may also increase the colloid oncotic

pressure in the interstitium, thereby limiting the effects of the colloid and in fact, may worsen the severity of the interstitial edema and hinder its resorption (127). In view of this complex issue, there is no clear agreement as to the ideal fluid to use in sepsis.

Possible side-effects of crystalloid resuscitation include hemodilution that may lead to compromise of systemic oxygen delivery (132) (118), pulmonary edema (133) (5) (134) (135) (47) or interstitial edema secondary to the increased permeability (136), and hyperchloremic acidosis due to a large volume of saline required for stabilization (137). Indeed, the diffusion of oxygen from red blood cell to the tissue is dependent on surface area for diffusion and the length of the pathway from red cell to mitochondria, both functions of microvascular control, and the diffusion characteristics of oxygen in the tissue (55). Therefore, the effect of tissue edema or inflammation on the diffusing characteristics of the tissue may be significant (55). Further, while some argue that hemodilution leads to a decreased hematocrit which may reduce viscosity and improve blood flow and may thereby be beneficial for some organs like the heart and brain, other organs accommodate poorly to the decrease in oxygen carrying capacity (55).

1. 7.4 Diffusion vs filtration

Diffusion refers to movement of substances in either direction across the capillary. Furthermore, diffusion rate depends on various factors including solubility of the substance in the tissues, the temperature, and the surface area available; it is inversely related to molecular size and the distance over which

diffusion occurs. Filtration refers to the net movement of fluid out of the capillaries and is governed by the Starling law of the capillary.

1.7.5 Starling's Law of the Capillary

Fluid balance between the intravascular and interstitial fluid compartments is determined by the forces operative in the Starling law of the capillary (Figure 18):

$$Q_f = K_f \{P_c - P_i - \delta (\pi_c - \pi_i)\}$$

in which Q_f is the total flow of fluid across the capillary membrane; K_f is the fluid filtration coefficient; P_c is the capillary hydrostatic pressure; P_i is the interstitial hydrostatic pressure; δ is the reflection coefficient; π_c is the capillary colloid osmotic pressure; and π_i is the interstitial colloid osmotic pressure (138) (see below for details).

The interaction of these four Starling forces determines fluid flux between interstitial and intravascular compartments. Capillary hydrostatic pressure is the dominant driving force favoring fluid filtration across the capillaries into the interstitium. Interstitial hydrostatic pressure is usually negative, but can become positive if large amounts of edema fluid accumulate. The plasma colloid osmotic pressure is the only force acting to retain fluid within the intravascular space. In contrast, interstitial colloid osmotic pressure favors fluid retention in the interstitial space and may be diluted by the accumulation of protein-sparse edema fluid. Increases in interstitial hydrostatic pressure and reductions in interstitial colloid osmotic pressure serve to limit edema formation (137).

$$Q_f = K_f (P_c - P_i - \delta [\pi_c - \pi_i])$$

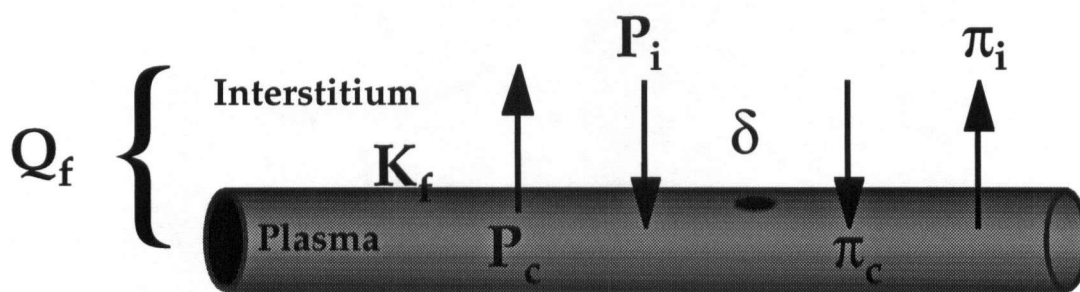


Figure 18. Starling's Forces of the Capillary.

The Starling's Forces determines the fluid flux between interstitial and intravascular compartments. Q_f is the total flow of fluid across the capillary membrane; K_f is the fluid filtration coefficient; P_c is the capillary hydrostatic pressure; P_i is the interstitial hydrostatic pressure; δ is the reflection coefficient; π_c is the capillary colloid osmotic pressure; and π_i is the interstitial colloid osmotic pressure.

The fluid filtration coefficient represents the net amount of fluid crossing the capillary membrane for a given level of Starling's forces. This value varies with changes in the surface area of the functional microcirculation at any one time. Dilation of arterioles, and especially dilation of precapillary sphincters, can increase the number of functional capillaries. Normally, only a fraction of capillaries exhibit blood flow at any one time. The reflection coefficient is a measure of the ability of the capillary membrane to exclude large particles and limit the movement of protein. A membrane completely impermeable to protein would demonstrate a reflection coefficient of 1. The average reflection coefficient for systemic capillaries is approximately 0.9 and for pulmonary capillaries is approximately 0.7 (139) (140). Under conditions of increased capillary permeability, the reflection coefficient may decrease to 0.4 (141), thereby favoring flow of fluid into the interstitium.

The colloid osmotic pressure of plasma is determined by the number of particles in solution that are impermeable to the capillary membrane. Albumin is responsible for approximately 80% of the plasma osmotic pressure (142). The normal plasma colloid osmotic pressure is 21-25 mmHg, and in a critical care population ranges from 18-20 mmHg (143) which will also tend to favor flow of fluid into the interstitium.

1.7.6 Tonometry

As discussed earlier, the splanchnic circulation is particularly vulnerable in sepsis. Since some investigators have shown that the gut becomes ischemic earlier than whole body (86), many have sought a mechanism by which one may detect

changes early enough to prevent mucosal injury. The use of tonometry has been an evolving one. Bergofsky (144) estimated PO_2 and PCO_2 in gallbladders and urinary bladders tonometrically by instilling the organs with saline and after equilibrium, measuring PO_2 and PCO_2 in the intraluminal fluid. Dawson et al (145) carried this idea into use in the intestinal tract with small bowel. Then, rather than placing fluid within the lumen, Kivisaari and Niinikoski (146) measured PO_2 and PCO_2 in tissues by determining the partial pressures of O_2 and CO_2 in saline contained within a Silastic tube which was permeable to these gases. Fiddian-Green et al (147) proposed the idea that tonometry could be used to estimate intramucosal pH (pHi) with the idea that HCO_3^- concentrations in tissue and arterial blood are sufficiently similar to permit substituting the latter value into the Henderson-Hasselbalch equation (Figure 19). Finally, Antonsson et al (148) validated this technique by using microelectrodes to directly measure the ileal pHi and correlating these values to those derived by tonometry during endotoxemia and mesenteric occlusion in pigs.

Clinicians have studied the role of tonometry recently and have showed some encouraging results (149) (150) (151). Gutierrez et al (152) have shown that tailoring therapy to pHi improved overall outcome in a group of critically ill patients. Maynard et al (153) showed that gastric tonometry was the most reliable indicator of adequacy of tissue oxygenation in a group of critically ill patients. Finally, studies have shown such a good correlation to oxygenation of tissue that tonometry may be used to determine the onset of anaerobic metabolism (154) (155).

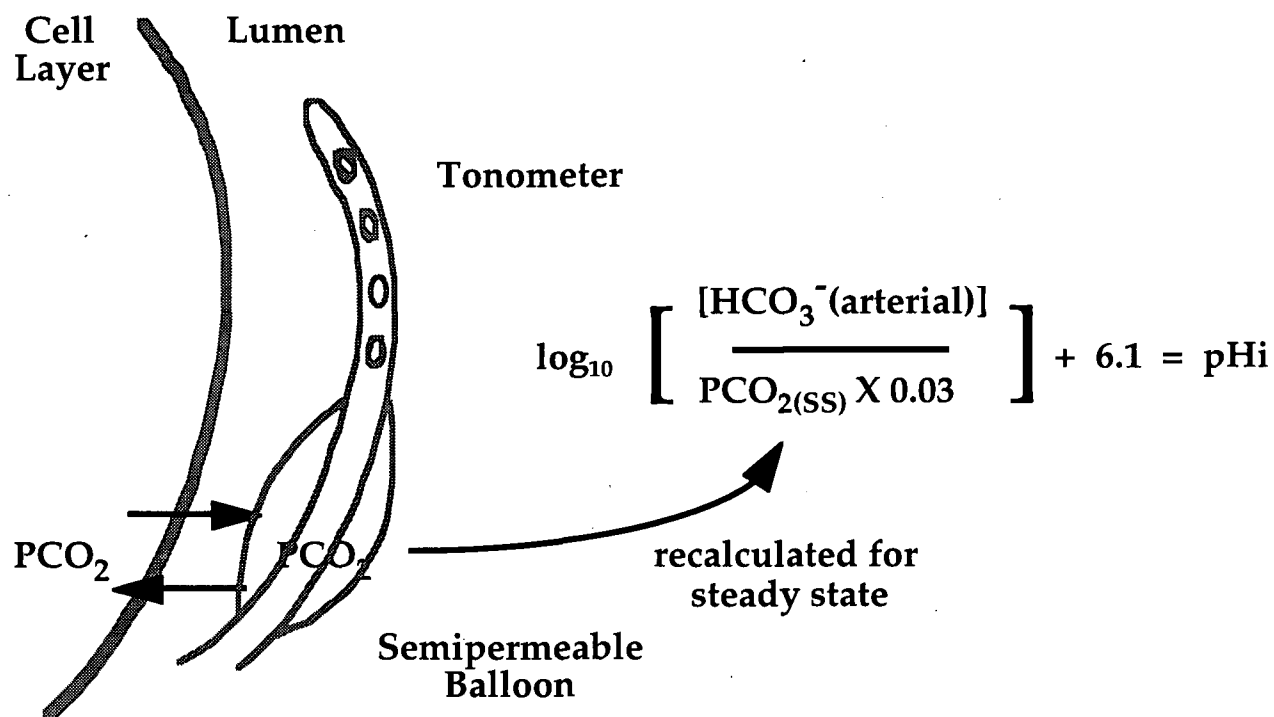


Figure 19. Tonometric Analysis.

This figure illustrates the manner that the tonometer is used to determine the mucosal pH (pHi). The pCO_2 of the mucosal wall equilibrates across the semipermeable membrane with the saline in the balloon. This can then be inserted into the Henderson-Hasselbalch equation using the arterial HCO_3^- to determine the pHi .

1.7.7 Lactate

Several investigators have used lactate as a marker for anaerobic metabolism (74) (106). When there is insufficient oxygen to sustain oxidative phosphorylation, increased glycolysis is called upon to maintain tissue ATP levels. During glycolysis, pyruvate acts as the terminal electron acceptor and leads to the production of lactate (55). However, Danztker (55) points out that there may be some confounding factors which may make the interpretation of serum lactate levels less than straightforward. Serum lactate levels reflect balance in production and the metabolism of lactate. In sepsis there are several reasons for increased production of lactate (156) including inflammatory mediators, cytokines, and other vasoactive substances that impair vasomotor tone, increase microvascular permeability, and facilitate aggregation of leukocytes and platelets. Capillary leakage causes decreased circulating blood volume and cardiac output that is further impaired by the direct effects of sepsis on ventricular function. Ultimately these changes lead to a fall in perfusion and ischemia with the resultant anaerobic metabolism. However, other factors may either raise or lower the level of lactate. Factors raising the levels include a decreased clearance due to decreased perfusion to the liver and kidneys, the main sites of metabolism of lactate (157) (156), and a decrease in the activity of pyruvate dehydrogenase in sepsis (156). Factors leading to an underestimation of anaerobic metabolism include some tissue only producing lactate once all other alternatives to oxidative phosphorylation are exhausted, and as tissue pH falls, glycolytic flux is inhibited thereby providing a feedback on the level of acidosis (55).

Therefore, since lactate levels in the blood reflect the balance of all these complex effects, lactate levels are difficult values to interpret in sepsis (149) (158).

Chapter 2

HYPOTHESIS

Sepsis has been characterized by increased capillary permeability. In view of known changes with respect to the Starling's forces at the capillary level, one would expect crystalloid resuscitation to result in a significant degree of interstitial edema. Interstitial edema may in turn impair oxygen diffusion from capillary to cells and thereby limit the ability of tissue to extract oxygen. Furthermore, interstitial edema along with endothelial edema may worsen an already heterogeneous distribution of capillaries and thereby further impair the ability of tissue to properly match perfusion with oxygen demands. Finally, hemodilution alone may lead to a decrease in the capillary hematocrit such that the delivery of oxygen will be lowered. Therefore, my hypothesis is that crystalloid resuscitation will impair the ability of tissue to extract oxygen in endotoxemia.

Chapter 3

OBJECTIVES OF THE THESIS

The use of fluid resuscitation in sepsis is a generally accepted therapeutic maneuver. However, in studying the physiological implications of fluid therapy, many questions arise as to the potential disadvantages of fluid resuscitation. In this thesis, an attempt is made to address several important questions as to the effects of crystalloid fluids in an endotoxemic porcine model of sepsis.

Specifically, the following questions will be addressed:

1. Does crystalloid resuscitation impair the ability to extract oxygen?

In the clinical scenario, the use of crystalloids has generally been the first line of therapy and often result in improved clinical parameters. However, measuring the oxygen extraction may be a more important parameter as it will assess tissue ability to adapt to sepsis.

To elaborate on the effects of crystalloid resuscitation, several potential mechanisms of action were studied:

2. Does crystalloid resuscitation lead to interstitial edema?

In view of the described generalized leak in sepsis, one would anticipate that aggressive fluid loading will lead to edema and thereby prevent oxygen extraction by tissue. Since it is difficult in the clinical scenario to quantify the degree of interstitial edema, our experiments will examine this possibility.

3. Does crystalloid resuscitation lead to capillary blood flow heterogeneity?

While some argue that fluid resuscitation reduces blood viscosity and allows for improved flow of blood, others argue that there may be endothelial and interstitial edema which may prevent flow of blood and thereby increase an already capillary heterogeneity and thereby impair oxygen extraction.

4. Does crystalloid resuscitation lead to decreased capillary hematocrit?

While crystalloid resuscitation leads to hemodilution at a large vessel level, there have been no papers documenting the capillary red blood cell concentration. This is a possible side effect of fluids which may impair the overall concentration of oxygen delivered to tissues. Using morphometric techniques, one may determine these values.

Chapter 4

RESEARCH PLAN

4.1 THE PORCINE MODEL OF SEPTIC SHOCK

Various considerations for the model of septic shock included the animal to be used and the type of sepsis produced. Pigs are quite similar to humans with respect to renal, cardiovascular, and digestive anatomy and physiology (159) and therefore serve as a useful experimental animal. With regards to the septic model, various types have been described including intravascular infusion of bacteria, peritonitis by bacterial inoculum, cecal ligation and perforation, or infusion of endotoxin. We chose the later for several reasons. Endotoxin, although not a *sine qua non* of sepsis, is considered fundamental in the development of sepsis (8). Further, endotoxin is a stable and relatively pure compound which simplifies some aspects of experimental design. In contrast, bacteria are typically stored frozen, grown in culture and washed several times to remove culture medium and solubilized bacterial products, and later require quantification (8). Finally, Cain and Curtis point out that endotoxin may provide a better experimental model of hyperdynamic sepsis in acute animal experiments than live bacteria as it more closely matches changes in oxygen consumption with a rise by 15% to 20% above the critical DO_2 (160).

Our study also examined the role of fluid resuscitation in the endotoxemic model. While fluid administration in the clinical setting is not standardized, we needed a method of administration which was consistent and rational. A fixed

volume of crystalloid has been described by Fink and Heard (8). A fixed volume as opposed to one titrated to hemodynamic variables has several advantages. First, myocardial performance is impaired by endotoxemia, thus preload may need to be elevated to supranormal levels to maintain cardiac output. Second, sepsis may lead to alterations in ventricular diastolic compliance, thus, left ventricular end-diastolic and pulmonary capillary wedge pressures may be poor estimates for left ventricular end-diastolic volume, the relevant variable for assessing preload. Finally, central venous and pulmonary capillary wedge pressures are typically small numbers, and using conventional catheter-transducer-amplifier systems, there is considerable imprecision (5-15%) (8) in the measurement of these variables; thus, it would lead to imprecise titration of fluid administration.

A porcine model of endotoxemia in which hemodynamic stability is achieved by an aggressive fluid resuscitation protocol has been previously described (52) (161). The model adequately reproduces several features of septic shock in humans including profound systemic arterial hypotension and low SVRI (96). Further, the volumes of fluid used were 25 cc/kg/hr for maintenance and 48 cc/kg/hr for resuscitation. This model has been used in recent studies of sepsis in our laboratory (74). Thus, we used a similar porcine model of endotoxemia with fluid resuscitation being crystalloid administered as a continuous infusion at the same fixed rates.

4.2 MORPHOMETRIC ANALYSIS OF GUT TISSUE

Morphometry is the measurement of structure. Its aim is often to answer questions such as "how many" and "how large" something is. While this is often the goal of morphometry, it is only with stereology that one is able to answer these questions.

Stereology is the three-dimensional interpretation of flat images by the criteria of geometric probability. It can also be defined as extrapolation from two-dimensional to three-dimensional space.

Stereology is practiced by measuring and counting profiles in sections (Figure 20). For most stereological problems, this can be done by superimposing grids of lines or squares and circles over an image. One may adjust sampling within the individual animal to a specific level of acceptable error. Various formulae are available (162) to allow determination of numbers of counts to limit error rates. Counting involves laying the grid on the area of interest and then counting the grid intersections which hit the component of interest. The number of points that hit profiles of the component, divided by the total number of test points equals the volume fraction of that component in the entire specimen. We apply these techniques in the determination of the gut interstitial volume and the capillary hematocrit.

4.3 INFRA-SCAN ANALYSIS OF GUT TISSUE

The infra-scan method of tissue analysis is a recent development in microscopic analysis. It utilizes a microscope attached to a camera which sends

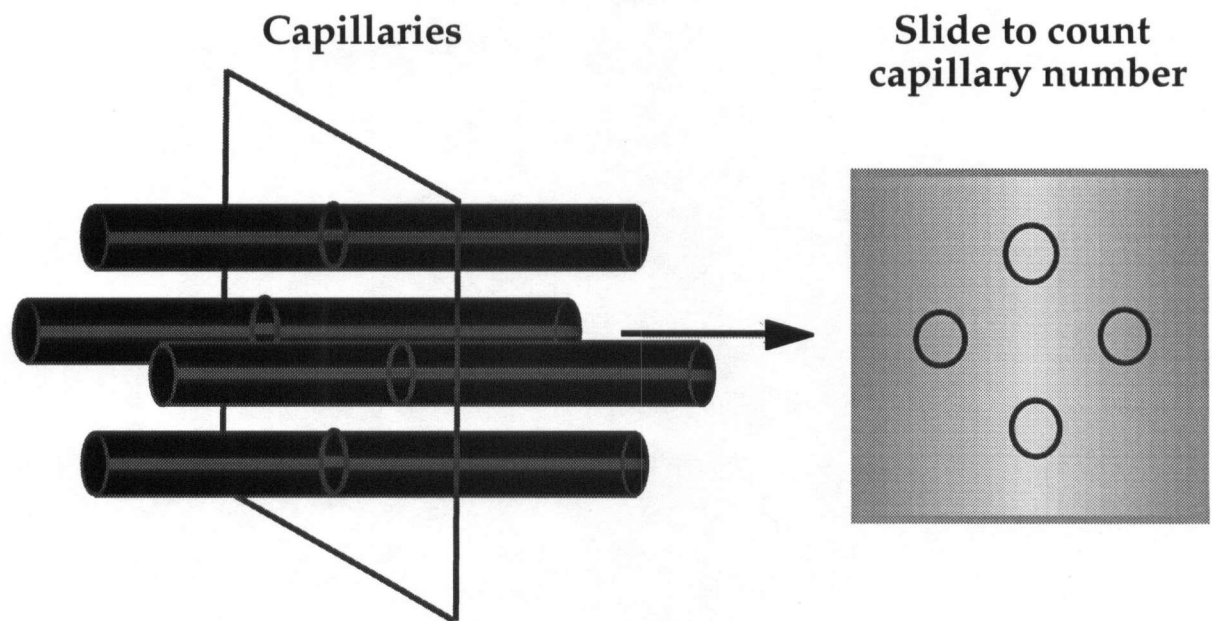


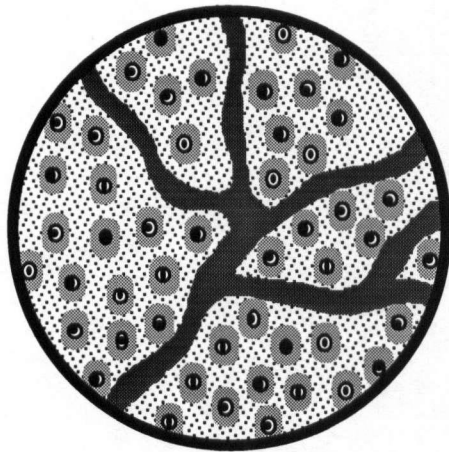
Figure 20. Morphometric Analysis.

Morphometry attempts to determine the number of a certain area interest, in this case capillaries by measuring and counting profiles in sections

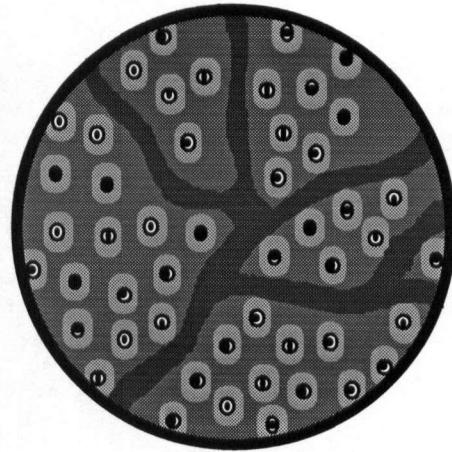
images through to a computer. The investigator is able to choose the image of interest and to select the area in question by use of a cursor. The imaging software (Infrascan, Richmond B.C.) will analyze the entire field for pixels with similar characteristics as the one chosen. The software will then quantify the total images matching the area of interest and determine the fraction area that it represents of the total field (Figure 21). By using visual inspection, the automated analysis may be confirmed. Various areas of interest may be looked at. We were interested in the area fraction of interstitial space as this could be used to estimate the degree of interstitial edema that was present within the gut.

4.4 DETERMINATION OF GUT CAPILLARY HEMATOCRIT

While the effects of fluid loading result in hemodilution at an arterial and venous level, the hematocrit in the microvessels may very well be different (55). Indeed, some investigators believe that the ability of the microvascular hematocrit to change independent of the systemic hematocrit in response to alterations in the microenvironment may account for some of the conflicting observations made during alterations in systemic hematocrit (55). Therefore, since changes in the systemic hematocrit may not reflect the results at a capillary level, we sought to determine this by morphometric technique. Using previously established techniques of morphometric analysis (163) (164), random samples will be cut, fixed, and stained for cutting and processing for photography under a transmission electron microscope. Point counting technique will be used to determine the



**Microscopic
Appearance**



**Infrascan
Analysis**

Figure 21. Infra-scan Analysis.

Infrascan is a technique whereby the slide is placed on a microscope attached to a camera which feeds the image to a computer. The investigator chooses the image of interest and select the area in question. The imaging software analyzes the entire field for pixels with similar characteristics as the one chosen. The software will then quantify the total images matching the area of interest and determine the fraction area that it represents of the total field.

relative volume fraction occupied by erythrocyte and total capillary volume, thereby determining the capillary hematocrit.

4.5 RELATIVE DISPERSION OF RED BLOOD CELL TRANSIT TIME

A major determinant of the transport of oxygen from blood to tissue is the time spent by the red blood cell passing through the vessels, called the red blood cell transit time. The red cell transit time in a given region is dependent on the blood volume and blood flow in that region, both of which may vary. The blood volume and blood flow may each be determined as per studies performed on the lung by Hogg et al (163) and the heart by Allard et al (164). Regional blood volume will be determined using Technetium (^{99m}Tc) labelled red blood cells and regional blood flow will be measured using a reference flow technique and a left atrial injection of 15 μm microspheres labelled with Strontium (^{85}Sr). The different radioactive signals can be used to quantify red cells and microspheres. Since the microspheres are also removed with a reference flow technique, one may correlate the counts of microspheres with the flow of these microspheres. Red blood cell transit time for the entire portion of tissue was determined by the quotient of blood volume and blood flow. By analyzing the red blood cell transit times in various segments of intestine, one may determine the average transit time and the variability in these values. The relative dispersion or coefficient of variation is the quotient of the standard deviation and the mean. As such, it provides one with a measure of the heterogeneity of the microcirculation in question.

4.6 TONOMETRY

Fiddian-Green et al (147) introduced the concept that tonometry could be used to estimate intramucosal pH (pHi) with the idea that HCO_3^- concentrations in tissue and arterial blood are sufficiently similar to permit substituting the latter value into the Henderson-Hasselbalch equation (Figure 19). Antonsson et al (148) have validated this technique by using microelectrodes to directly measure the ileal pHi and correlating these values to those derived by tonometry during endotoxemia and mesenteric occlusion in pigs.

The gastrointestinal tonometer consists of a saline-filled silicone balloon that is placed in the gut lumen. The silicone balloon is highly permeable to oxygen and carbon dioxide. Normal saline is injected into the ileal tonometer balloon and left to equilibrate for 40 minutes. At the end of the equilibration period, the saline will be removed under anaerobic conditions. The first milliliter of saline removed is discarded, as this is not in direct contact with the balloon. The CO_2 content of the subsequent volume of saline removed, which reflects the mucosal CO_2 , will be measured. Simultaneously, the arterial bicarbonate (assumed to be equal to the bicarbonate content of the gut mucosa) will be measured. Using a conversion table, measured PCO_2 will be transposed to a steady state PCO_2 ($\text{PCO}_{2\text{ss}}$), depending on the exact duration of equilibration. Gut mucosal pH will be calculated by substituting the $\text{PCO}_{2\text{ss}}$ and the simultaneously measured arterial bicarbonate in the Henderson-Hasselbalch equation (ie: $\text{pHi} = 6.1 + \text{Log}\{(\text{HCO}_3^-)/\text{PCO}_2\}$).

Chapter 5

EFFECT OF CRYSTALLOID RESUSCITATION ON WHOLE BODY AND GUT IN ENDOTOXEMIA

5.1 INTRODUCTION

In addition to antibiotic therapy, fluid resuscitation is a key component of the management of sepsis and septic shock (2). The goal of fluid resuscitation is to restore intravascular volume to maintain an adequate blood pressure and cardiac output. Restoring a normal cardiac output requires intravascular replacement of third space losses, compensation for venous pooling of blood, and a sufficiently high left ventricular filling pressure to compensate for decreased ventricular contractility during sepsis (72). Importantly, even a normal cardiac output may not necessarily be adequate during sepsis because sepsis is accompanied by impaired tissue oxygen extraction (55) (74) (106) (12). That is, higher oxygen delivery (equals cardiac output times arterial oxygen content) is required to prevent evidence of unmet oxygen demand (76) (12), including decreasing oxygen consumption (74) (106), rising lactate levels (74) (106), low gastric intramucosal pH (152), and organ system dysfunction (55) (76) (12). The causes of impaired tissue oxygen extraction during sepsis have not been fully elucidated. However, impaired oxygen uptake, due to mismatching of oxygen delivery to demand (74) (78) and due to impaired oxygen diffusion from erythrocytes to tissue mitochondria (165), appear to play important roles. Mismatching of oxygen delivery to demand and inadequate oxygen diffusion are due to several microvascular phenomenon; capillary plugging with leukocytes and

other debris (41), capillary endothelial edema and tissue edema (54), and altered microvascular flow (74) (57). While normal response to impaired oxygen delivery is met with a homogeneous capillary recruitment, the microvascular changes associated with sepsis lead to a heterogeneous capillary distribution (74) (57). While some argue that microvascular flow distribution and tissue oxygen extraction may be improved by fluid resuscitation (125), others argue that fluid resuscitation may lead to interstitial and endothelial edema (136) which may further impair microvascular flow and lead to a higher degree of capillary heterogeneity. Considering the multiple effects of sepsis and fluid resuscitation on the intravascular volume, on cardiac function, on the microvascular distribution of oxygen delivery and on tissue oxygen demand, fluid resuscitation in sepsis is a complex intervention. Our goal was to determine whether fluid resuscitation in sepsis impairs tissue oxygen extraction, possibly due to interstitial edema and altered microvascular flow distribution (126) (78).

The splanchnic circulation has been thought to be particularly susceptible to ischemic injury (52) (86) (90). This susceptibility has been attributed to specific dysregulation of organ perfusion (90), to increased overall oxygen requirements by the metabolically active splanchnic organs (86), and to the countercurrent flow in the intestinal villi whereby there is shunting of oxygen away from the tips of the villi (55) (87) (89). Furthermore, it has been proposed that ischemia of the splanchnic circulation may predispose to decreased mucosal integrity and lead to bacterial translocation which may then lead to multiple organ dysfunction

syndrome (166) (52). Thus, we were particularly interested in intestinal oxygen extraction and the splanchnic circulation.

Most describe sepsis as characterized by an increased capillary permeability (136) such that tissues are more prone to develop interstitial edema (136) (54). To further determine whether interstitial edema occurs in tissue in endotoxemia, we determined the volume of interstitial space by morphometric techniques.

Some investigators have suggested that microvascular flow may be improved with hemodilution by reducing hematocrits (111) (125) leading to a decrease in blood viscosity (125) thereby increasing blood velocity and favoring entry into channels with smaller diameters (126). However, hemodilution also may lead to a decrease in the oxygen carrying capacity of blood with a resulting decrease in oxygen delivery at a microvascular level. Further, since hematocrits have only been determined at a large vessel level, this may not reflect the effects of hemodilution at a tissue level (55). Therefore, to further elucidate the possible effects of fluid resuscitation, we sought to determine the capillary hematocrits by morphometric analysis.

Accordingly, we sought to determine whether crystalloid resuscitation in a porcine model of septic shock would impair oxygen extraction capacity of the whole body and specifically of the splanchnic bed. To better understand the mechanism of this effect we measured the dispersion of blood flow transit times in the gut, the volume fraction of interstitium, and the gut capillary hematocrit.

5.2 METHODS

5.2.1 Experimental Design and Protocol

The experimental design was similar to that performed previously in our laboratory (74). Sample size was calculated by analyzing the results of a preliminary study using a sample size of 8 animals per group in endotoxic and control groups. To determine a difference in critical DO_2 of 4 ml $\text{O}_2/\text{kg}\cdot\text{min}$ using a standard deviation of 2 ml $\text{O}_2/\text{kg}\cdot\text{min}$, required four groups of eight animals, for an $\alpha = 0.05$ and $\beta = 0.10$ ("N" Calculator for Macintosh, v 0.9, compuserve #70721,3243). Therefore, a size of 8 animals per group were used as a reasonable estimate of required sample size.

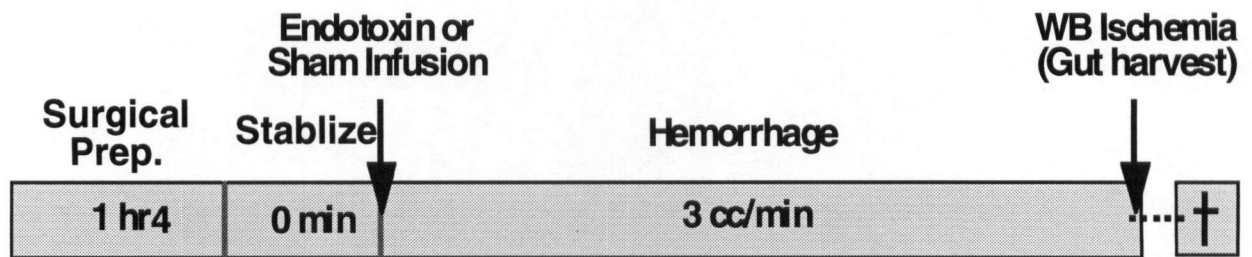
During instrumentation (see below), surgical preparation, and stabilization, all animals received 0.9% Sodium Chloride solution (Baxter, Toronto, ON) infused via the left external jugular catheter at $25 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{hr}^{-1}$. Following this, the animals were randomized to one of four groups: Control/Fluid ($n=8$), Control/No fluid ($n=8$), Endotoxin/Fluid ($n=8$), and Endotoxin/No-fluid ($n=8$) groups. Endotoxin groups received *E. coli* endotoxin $50 \mu\text{g}/\text{kg}$ (0111:B4, Sigma, St. Louis, MO) in 60 ml normal saline over 30 minutes immediately after the baseline data set. Control groups received an infusion of 60 ml normal saline without endotoxin. Fluid groups received an infusion of normal saline at $48 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{hr}^{-1}$ from the baseline measurement set until the end of the experiment. No-fluid groups did not receive any further saline infusion. Following randomization to respective treatment groups, progressive hemorrhage was undertaken at 3 ml per minute using a constant withdrawal pump from the left carotid catheter until the animal died.

Prior to death, when whole body oxygen consumption had fallen by 25%, suggesting that oxygen delivery was inadequate to meet demand, we infused radiolabelled red blood cells, injected radiolabelled microspheres (see below), and rapidly excised the previously prepared segment of jejunum (Figure 22).

5.2.2 Surgical Preparation and Instrumentation

This study was approved by the Animal Care Committee of the University of British Columbia and conforms to National Institute of Health (NIH) standards for animal experimentation. Thirty-two pigs, weighing 25.7 ± 2.8 kg, were fasted overnight and then sedated with 0.5 mg/kg midazolam i.m. (Hofman la Roche, Mississauga, ON). Thirty minutes later the animals were anesthetized using ketamine 500 mg i.m. (MTC Pharm, Cambridge, ON) followed by thiopentol 125 to 250 mg i.v. (Abbott, Montreal, PQ) titrated to effect. Anesthesia was maintained throughout the experiment using ketamine $5 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{hr}^{-1}$ i.v. infusion and 0.5% inspired isoflurane (Anaquest, Mississauga, ON). To avoid changes in whole body oxygen demand, skeletal muscle relaxation was maintained with intravenous pancuronium bromide infusion (Organon, Scarborough, ON) at 6 mg/hr, titrated to effect.

A tracheostomy was performed and an 8.0 mm endotracheal tube (Portex, Wilmington, MA) was inserted and secured. During instrumentation and experimentation the animals were mechanically ventilated (Harvard Apparatus dual phase control respirator pump, model 613, Mills, MA) with 30% oxygen. A low compliance catheter was inserted into the right carotid artery for arterial pressure



TREATMENT GROUPS:

Grp 1:	25 cc/kg.hr	(S)	Non Fluid resuscitated: 0 cc/kg.hr (n=8)
Grp 2:	25 cc/kg.hr	(S)	Fluid resuscitated: 48 cc/kg.hr (n=8)
Grp 3:	25 cc/kg.hr	(E)	Non Fluid resuscitated: 0 cc/kg.hr (n=8)
Grp 4:	25 cc/kg.hr	(E)	Fluid resuscitated: 48 cc/kg.hr (n=8)

Figure 22. Protocol Timeline.

This figure illustrates the protocol for all four groups of animals. S = saline sham infusion, E = endotoxin infusion.

measurement and arterial blood sampling (Figure 23). A catheter was inserted into the left external jugular vein for saline infusion and administration of medications. A pulmonary artery catheter (Criticath model DSP5105H, Ohmeda Medical Devices, Oxnard, CA) was placed via the right external jugular vein for measurement of right atrial pressure, pulmonary artery occlusion pressure, for mixed venous blood sampling, and for cardiac output measurement in triplicate (Thermodilution Cardiac Output monitor Edwards Model 9250, Baxter Health Care, Irvine, CA). A catheter was inserted into the left carotid artery for hemorrhage.

An anterolateral thoracotomy was performed through the fifth or sixth intercostal space on the left. The pericardium was entered and a catheter inserted and secured in the left atrial appendage. This catheter was used for injection of radiolabelled red blood cells and radioactive microspheres for gut blood volume and flow measurements, respectively.

Through a midline laparotomy, the pancreaticoduodenal vein at the second part of the duodenum and the superior rectal vein at the promontory of the sacrum were tied off. Following this, the splenic artery and vein were tied off to prevent autotransfusion and a catheter to sample portal vein blood was inserted via the splenic vein stump. Ligation of these vessels ensured that the gastrointestinal circulation was isolated such that venous drainage passed through the portal vein. Portal venous flow was measured by placement of a 1.5 cm ultrasonic flow probe connected to a Transonic T201 Ultrasonic Blood Flow Meter (Transonic Inc., Ithaca, NY) around the portal vein. An orogastric tube was inserted to allow drainage of the gastric secretions. A tonometric catheter (Tonometrics, Inc., 373 Plantation

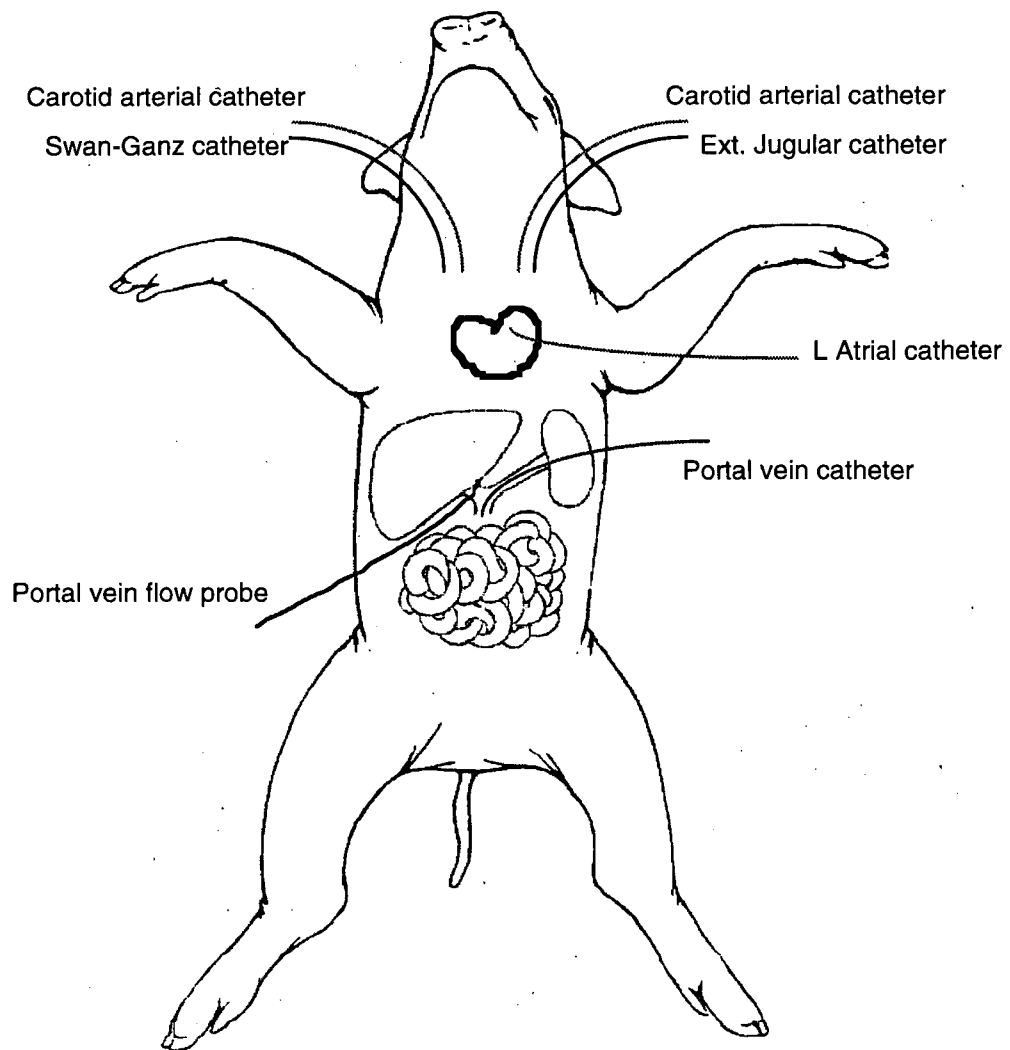


Figure 23. Surgery and Instrumentation.

This figure illustrates the various catheters and probes needed for the experiment.

Street, Worcester, MA) was inserted into the lumen of the ileum 30 cm proximal to the ileocecal valve through a small wound in the antimesenteric border and held in place with a suture. Thirty cm distal to the duodenal-jejunal junction a 60 cm length of jejunum was isolated for later resection for morphometric analysis. Umbilical tapes were brought around the mesentery to allow ligation of the vasculature following injection of radiolabelled microspheres (see below). The mesentery was otherwise not disrupted. The abdomen was loosely closed to facilitate later removal of the jejunal segment.

5.2.3 Hemodynamic Measurements and Calculations

We measured arterial, mixed venous, and portal vein pH, P_{CO_2} , and PO_2 (ABL30, Radiometer, Copenhagen, Helsinki), O_2 content (IL 482 co-oximeter, Instrumentation Laboratories, Lexington, MA) and lactate concentration (YSI 2300STAT lactate analyzer, Yellow Springs Instruments, Yellow Springs, OH) at baseline and every 20 minutes during progressive hemorrhage. Heart rate, mean arterial pressure, pulmonary artery pressure, central venous pressure, pulmonary capillary occlusion pressure, cardiac output, portal vein flows, and whole body oxygen consumption using a metabolic cart (mean of 5 measures at 1 minute intervals, MBM-1000, Deltatrac, Helsinki, Finland) were also recorded at 20 minute intervals. A complete blood count (Coulter counter, Coulter, Miami Lakes, FL) and tonometer measurements was measured every 40 minutes (see below).

Blood oxygen content was calculated as $\text{hemoglobin} \times 1.39 \times \text{blood oxygen saturation} + 0.003 \times PO_2$. Whole body oxygen delivery (WB- DO_2) was calculated as

cardiac output multiplied by arterial oxygen content. Gut oxygen consumption (Gut-VO_2) was calculated as portal vein flow multiplied by the difference between arterial and portal venous oxygen content. Gut oxygen delivery (Gut-DO_2) was calculated as portal vein flow multiplied by arterial oxygen content. The oxygen extraction ratio for both whole body (WB-ER) and gut (Gut-ER) was calculated as oxygen consumption divided by oxygen delivery. From the multiple oxygen delivery - oxygen consumption points obtained during progressive hemorrhage the whole body and gut critical oxygen extraction ratio was determined using Samsel and Schumacker's dual-line regression analysis (167).

5.2.4 Transit times

Gut blood flow and blood volume were determined in the isolated segments of gut after the luminal contents were removed and after the tissue had been fixed for 24 hours in 6% phosphate buffered gluteraldehyde (163). Prior to removal, Technetium ($^{99\text{m}}\text{Tc}$) labelled red blood cells were injected into the left atrial appendage and allowed to distribute in the vascular compartment for 10 minutes. 15 μm microspheres labelled with Strontium (^{85}Sr) were then injected rapidly (10 seconds) into the left atrium. At the time of microsphere injection, blood was withdrawn from the left common carotid artery at 10 ml/minute for 2 minutes into weighed vials for later radiation counting. Next, the arterial and venous vasculature of the gut segment were simultaneously cross clamped and the segment of gut was rapidly excised and immersed in 6% phosphate buffered gluteraldehyde. The 60 cm segments of gut were divided into 30-two cm pieces and each placed into

preweighed vials containing 6% phosphate buffered gluteraldehyde. Each segment was weighed and counted using a Beckman 8000 gamma counter for 3 minutes (164). The blood volume of each 2 cm piece of gut was calculated as counts per minute of each piece divided by counts per minute per ml of the reference blood sample. Blood flow to each 2 cm piece of gut was calculated as microsphere counts per minute from that piece of gut divided by microsphere counts per minute per ml of the reference arterial withdrawal sample. Average transit time for each piece of gut was calculated as blood volume divided by blood flow giving units of time (163). Following calculation of individual transit times, a distribution of transit times for all of the pieces of gut taken together was then determined for each gut segment.

5.2.5 Interstitial Volume

Jejunum from 5 animals in each group was randomly selected (Figure 24). From each of these animals, six of the thirty 2 cm sections of jejunum were randomly selected and the tissue was embedded in glycol methacrylate, sectioned at 2 μ m and stained using methenamine silver. Slides were coded so that the morphometric analysis was blinded. The fraction of bowel wall that was interstitial space was quantitated at 400X using an automated image analysis system (Infrascan, Richmond B.C.) for 5 random fields on each slide. On each slide an area of interstitial space was identified manually. The analysis program then identified the fractional area on the slide that had matching characteristics. In each case the area fraction of interstitial space identified by the analysis package was confirmed by visual inspection.

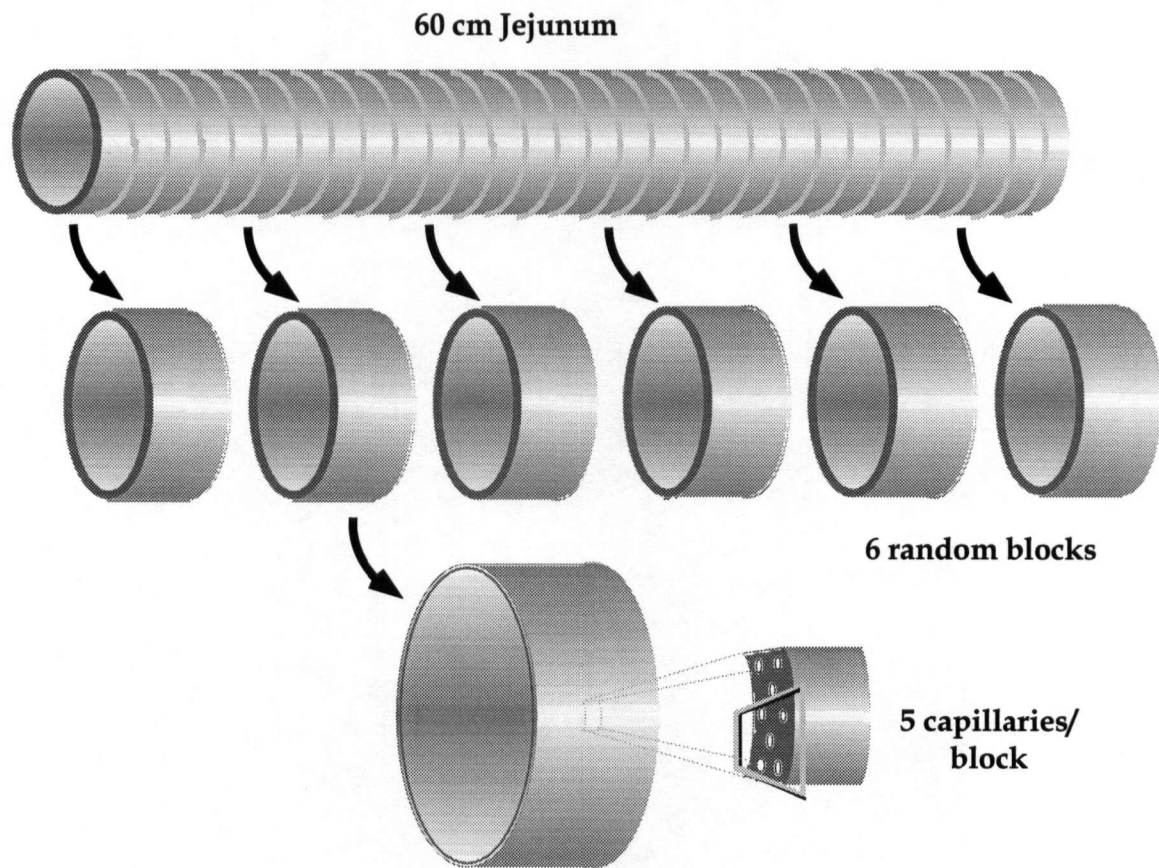


Figure 24. Processing jejunal specimen for morphometric analysis.

Jejunum from 5 animals in each group was randomly selected. From each of these animals, six of the thirty 2 cm sections of jejunum were randomly selected. A portion of each piece selected was embedded in glycol methacrylate. Interstitial volume was determined for various areas while capillary hematocrit was determined by analyzing 5 capillaries/block.

5.2.6 Capillary Hematocrit

Jejunum from 5 animals in each group was randomly selected. From each of these animals, six of the thirty 2 cm sections of jejunum were randomly selected and fixed overnight in 2.5% glutaraldehyde in 0.1M cacodylate buffer. The tissue was postfixed for 2 hours in 1% osmium tetroxide, then stained en bloc for 1 hour in 5% aqueous uranyl acetate and embedded in Eppoxy resin. Thin sections, selected from 1 μ m toluidine blue-stained section, were cut on a Reichert Ultracut ultramicrotome, mounted on 200 mesh copper grids, and stained with lead citrate. Five capillaries (<10 μ m) per section (30 capillaries per jejunal segment) were photographed at 5000X magnification on a Zeiss 10CR transmission electron microscope. Capillary plasma and erythrocyte volume fractions were determined by point-counting using a 400 point grid. The capillary hematocrit was calculated as the quotient of erythrocyte volume fraction and total capillary volume fraction.

5.2.7 Ileal Tonometry

The gastrointestinal tonometer consists of a saline-filled silicone balloon that is placed in the gut lumen. The silicone balloon is highly permeable to oxygen and carbon dioxide. Normal saline (5.0 ml, at room temperature) was injected into the ileal tonometer balloon and was left for 40 min. At the end of the equilibration period, the saline was removed under anaerobic conditions. The first milliliter of saline removed was discarded, as this was not in direct contact with the balloon (since the tonometer tube has a residual volume of one milliliter). The CO₂ content of the subsequent volume of saline removed, which reflects the mucosal CO₂, was

measured using the ABL Blood Gas Analyzer. Simultaneously, the arterial bicarbonate - assumed to be equal to the bicarbonate content of the gut mucosa - was measured. Using a conversion table supplied by the manufacturer (Tonometrics Inc.), measured PCO_2 was transposed to a steady state PCO_2 ($\text{PCO}_{2\text{ss}}$), depending on the exact duration of equilibration. Gut mucosal pH was calculated by substituting the $\text{PCO}_{2\text{ss}}$ and the simultaneously measured arterial bicarbonate in the Henderson-Hasselbalch equation (ie: $\text{pHi} = 6.1 + \text{Log}\{(\text{HCO}_3)/\text{PCO}_2\}$). The use of tonometry to measure changes in ileal intramucosal pH induced by endotoxin infusion in pigs has previously been validated (148).

5.2.8 Statistical Analysis

The relationship between VO_2 and DO_2 for whole body and gut was determined for each animal by finding two best-fit linear regression lines (167) from a plot of VO_2 and DO_2 . The critical oxygen extraction ratio, ERc , was determined at the point of intersection of the two lines (Figure 25). The relationship between lactate and DO_2 for whole body and gut was determined for each animal by finding two best-fit linear regression lines as above from a plot of lactate and DO_2 . To determine whether intravascular volume expansion altered ERc during endotoxemia, we used a two-way ANOVA testing for effect of volume and endotoxin taking $p < 0.05$ as significant. We calculated the mean (μ), second moment (σ^2) and relative dispersion (σ/μ) of the distribution of gut blood flow transit times using standard formulas (74). We also used a 2 way ANOVA to test for

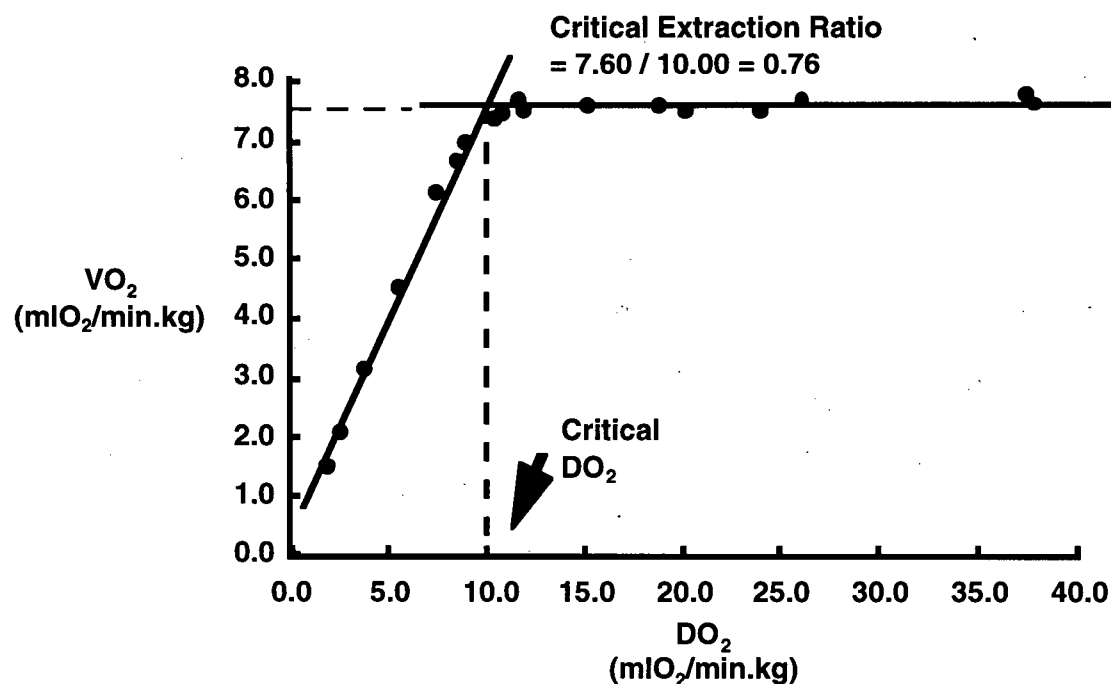


Figure 25. An example of O_2 Consumption and Delivery Curve.

Typical whole body oxygen consumption (VO_2) and delivery (DO_2) data points, measured during progressive hemorrhage, are illustrated. During progressive hemorrhage aerobic metabolism maintains oxygen consumption at approximately a constant value (7.60 $ml O_2/min.kg$) until a critical point is reached (10.00 $ml O_2/min.kg$), after which oxygen consumption falls indicating anaerobic metabolism. Dual regression lines are fit to the data points. The intersection of the two lines identifies the critical oxygen extraction ratio ($=7.60 / 10.00 = 0.76$).

differences due to fluid resuscitation and endotoxin in the relative dispersion of gut blood flow and in the area fraction of interstitial space. A one-way ANOVA was used to test for effect of saline resuscitation on arterial and capillary hematocrit. When a significant difference was found we used a sequentially rejective Bonferroni test procedure to identify individual differences. Results are presented as mean \pm standard deviation throughout the text, tables, and figures.

5.3 RESULTS

5.3.1 Effect of Endotoxin

Endotoxin infusion significantly reduced the critical oxygen extraction ratio in the whole body (Endotoxin 0.55 ± 0.08 versus Control 0.82 ± 0.06 , $p < 0.05$) (Figure 26) (Table 1) and gut (Endotoxin 0.52 ± 0.05 versus Control 0.77 ± 0.07 , $p < 0.05$) (Figure 27) (Table 1). Endotoxin infusion resulted in an earlier onset of ischemia in whole body (Endotoxin 12.2 ± 3.8 versus Control 9.1 ± 1.7 ml O_2 /min.kg, $p < 0.05$) (Figure 28) (Table 1) but not in gut (Endotoxin 24.3 ± 9.0 versus Control 25.3 ± 14.8 ml O_2 /min.kg, $p > 0.05$) (Figure 29) (Table 1). Relative dispersion of RBC flow transit times between segments of jejunum was not affected by endotoxin (Figure 30). This measurement of blood flow distribution is not a measure of the capillary distribution as it includes transit time through all jejunal wall vessels. Endotoxin had no significant effect on the volume fraction of interstitium ($p > 0.05$) (Figure 31). Endotoxin did not affect arterial hematocrit ($p > 0.05$) or capillary hematocrit ($p > 0.05$) (Table 2). Endotoxin significantly decreased the arterial and portal vein leukocyte counts compared to controls at onset of ischemia (Endotoxin arterial $2.2 \pm$

0.5, portal 2.3 ± 0.8 wbc/mm³ versus Control arterial 25.0 ± 11.7 , portal 25.2 ± 12.1 wbc/mm³, $p < 0.05$) (Table 3). Endotoxin reduced the pHi as determined by tonometry at onset of ischemia (Endotoxin 6.91 ± 0.15 versus Control 7.06 ± 0.10 , $p < 0.05$) (Table 4). Endotoxin did not change the arterial lactate values compared to controls ($p > 0.05$) (Table 5). Endotoxin also had no effect on the onset of rise of lactate compared to controls ($p > 0.05$) (Table 6).

Initial oxygen transport (Table 7) and hemodynamic (Table 8) variables did not differ between the Endotoxin and Control groups ($p > 0.05$). At the critical oxygen delivery the Endotoxin groups had lower mean arterial blood pressure (Endotoxin 44 ± 14 versus Control 59 ± 11 mmHg, $p < 0.05$), lower systemic vascular resistance (Endotoxin 516 ± 376 versus Control 1651 ± 408 dynes \cdot sec⁻¹ \cdot cm⁻⁵, $p < 0.05$), and higher pulmonary artery occlusion pressure (Endotoxin 7.7 ± 2.5 versus Control 4.3 ± 1.8 mmHg, $p < 0.05$). In addition the Endotoxin groups survived for a shorter period of hemorrhage (Endotoxin 128 ± 43 versus Control 202 ± 60 minutes, $p < 0.05$) (Table 9).

5.3.2 Effect of Fluid Resuscitation

Fluid resuscitation significantly reduced the critical oxygen extraction ratio in the whole body (Fluid 0.62 ± 0.08 versus No-Fluid 0.82 ± 0.06 , $p < 0.05$) (Figure 26) (Table 1) and gut (Fluid 0.67 ± 0.06 versus No-Fluid 0.77 ± 0.07 , $p < 0.05$) (Figure 27) (Table 1). Fluid resuscitation did not alter the onset of ischemia in whole body (Figure 28) or gut (Figure 29) ($p > 0.05$) (Table 1). Fluid resuscitation significantly increased the relative dispersion of RBC flow transit times in both the control (Fluid

0.81 \pm 0.59 versus No-fluid 0.36 \pm 0.11, $p < 0.05$) and the endotoxin groups (Fluid 0.60 \pm 0.46 versus No-fluid 0.31 \pm 0.04, $p < 0.05$) (Figure 30). Fluid resuscitation significantly increased the area fraction of interstitial space to the same extent in both the Endotoxin (Fluid 63 \pm 20% versus No-Fluid 50 \pm 20%, $p < 0.05$) and Control groups (Fluid 70 \pm 21% versus No-Fluid 45 \pm 19%, $p < 0.05$) (Figure 31). Arterial hematocrit at the onset of ischemia decreased compared to baseline with fluid resuscitation in Control (Ischemic 16 \pm 4% versus Baseline 22 \pm 3%, $p < 0.05$) and Endotoxin (Ischemic 20 \pm 4 versus Baseline 23 \pm 3%, $p < 0.05$) groups (Table 2). However, capillary hematocrit at the onset of ischemia was unchanged from baseline by fluid resuscitation in Control and Endotoxemic animals ($p > 0.05$) (Table 2). Fluid resuscitation decreased the arterial and portal vein leukocyte counts in the control groups at onset of ischemia (Fluid arterial 14.9 \pm 10.5, portal 14.9 \pm 9.9 wbc/mm³ versus Non-fluid arterial 25.0 \pm 11.7, portal 25.2 \pm 12.1 wbc/mm³, $p < 0.05$) (Table 3). Fluid resuscitation did not alter the arterial or portal vein leukocyte counts in the endotoxemic groups at onset of ischemia ($p > 0.05$) (Table 3). Fluid had no effect on the pHi as determined by tonometry at onset of ischemia when compared to non-fluid groups ($p > 0.05$) (Table 4). Fluid did not change the arterial lactate values when compared to non-fluid groups ($p > 0.05$) (Table 5). Fluid also had no effect on the onset of rise of lactate compared to non-fluid groups ($p > 0.05$) (Table 6).

The initial oxygen transport (Table 7) and hemodynamic (Table 8) variables did not differ between the Fluid and No-fluid groups ($p > 0.05$). At the critical oxygen delivery the Fluid groups had a higher mean arterial pressure (Fluid 77 \pm 12

versus No-fluid 59 ± 11 mmHg, $p < 0.05$), higher cardiac output (Fluid 5.7 ± 2.7 versus No-fluid 2.8 ± 0.7 L/min, $p < 0.05$), lower systemic vascular resistance (Fluid 1100 ± 600 versus No-fluid 1650 ± 410 dynes \cdot sec $^{-1}\cdot$ cm $^{-5}$, $p < 0.05$), higher pulmonary artery occlusion pressure (Fluid 6.9 ± 1.1 versus No-fluid 4.3 ± 1.8 mmHg), and a lower hemoglobin (55 ± 11 versus No-Fluid 83.6 ± 13.0 g/l, $p < 0.05$). The fluid resuscitated groups had a greater time of survival after onset of hemorrhage (368 ± 105 minutes) compared to the non-fluid resuscitated group (202 ± 60 minutes, $p < 0.05$) (Table 9).

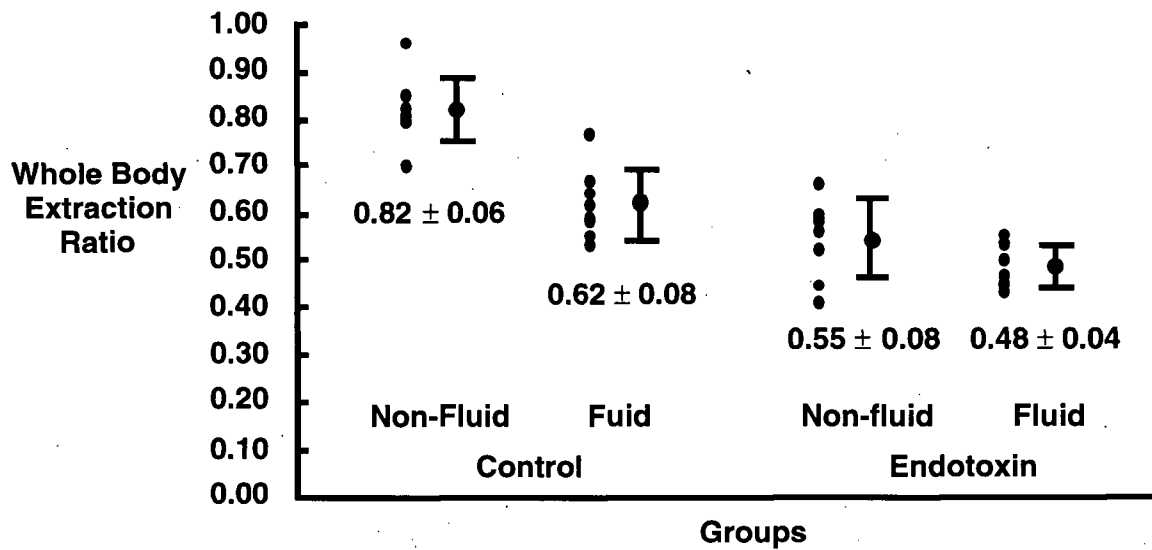


Figure 26. Whole Body Ability to Extract Oxygen.

The whole body critical oxygen extraction ratio for all four groups is illustrated. Fluid-resuscitation reduced the critical oxygen extraction ratio in the Control groups but not significantly in the Endotoxin groups. Endotoxin reduced the critical oxygen extraction ratio (ANOVA, $p < 0.001$). Values are expressed as mean \pm SD.

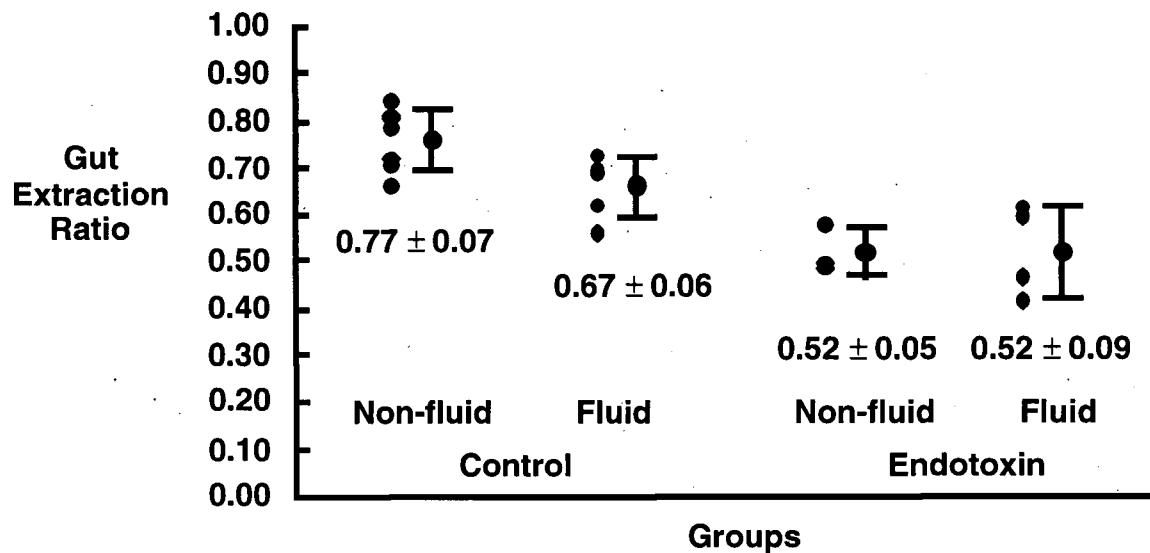


Figure 27. Gut Ability to Extract Oxygen.

The gut critical oxygen extraction ratio for all four groups is illustrated. Like the whole body, fluid-resuscitation reduced the gut critical oxygen extraction ratio in the Control groups but not in the Endotoxin groups. Endotoxin reduced the gut critical oxygen extraction ratio (ANOVA, $p < 0.001$). Values are expressed as mean \pm SD.

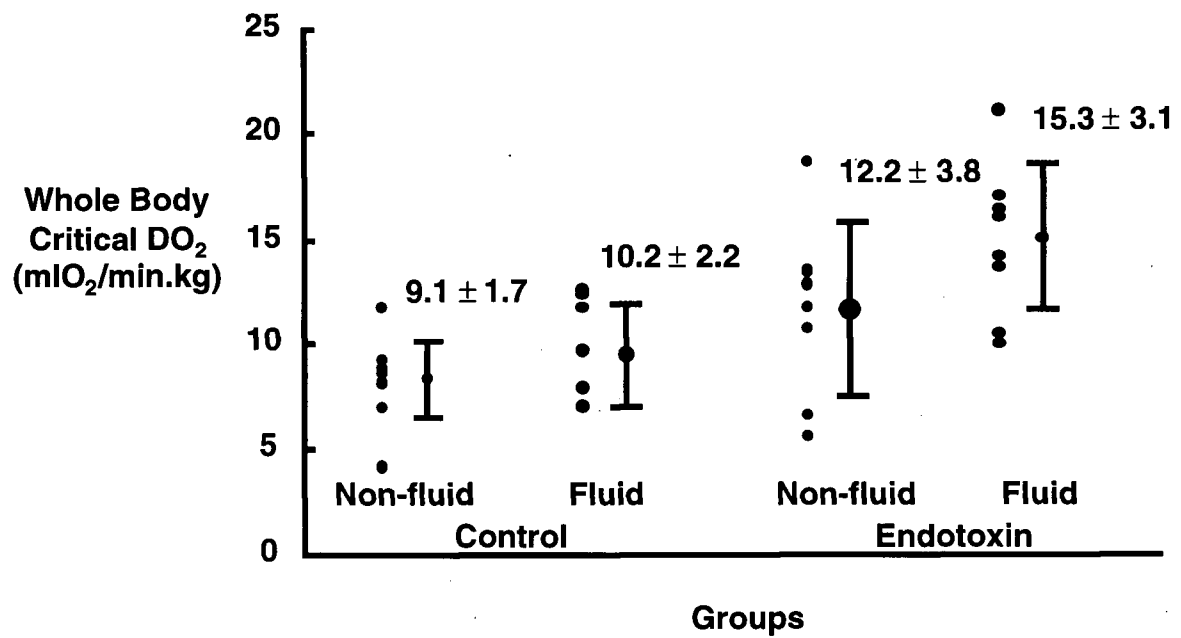


Figure 28. Whole Body Onset of Ischemia.

Critical DO₂ is not altered by fluid resuscitation in the whole body in nonseptic or the septic groups.

However the critical DO₂ is higher in the pooled septic group compared to the pooled nonseptic group.

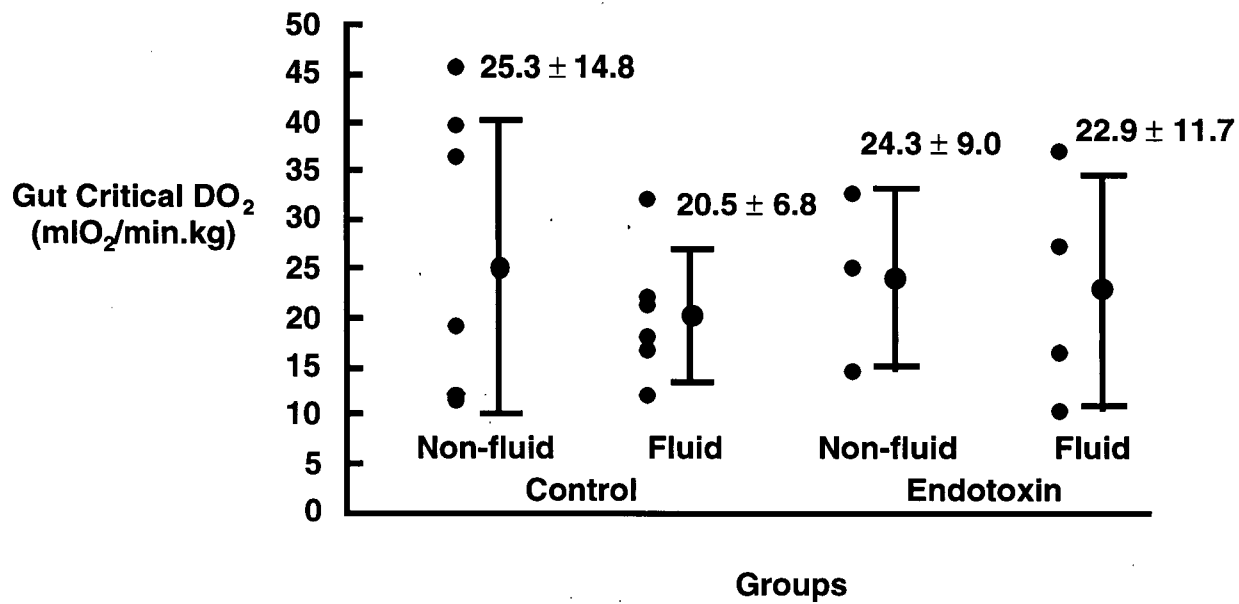


Figure 29. Gut Onset of Ischemia.

Critical DO_2 is not altered by fluid resuscitation in the gut in nonseptic or the septic groups.

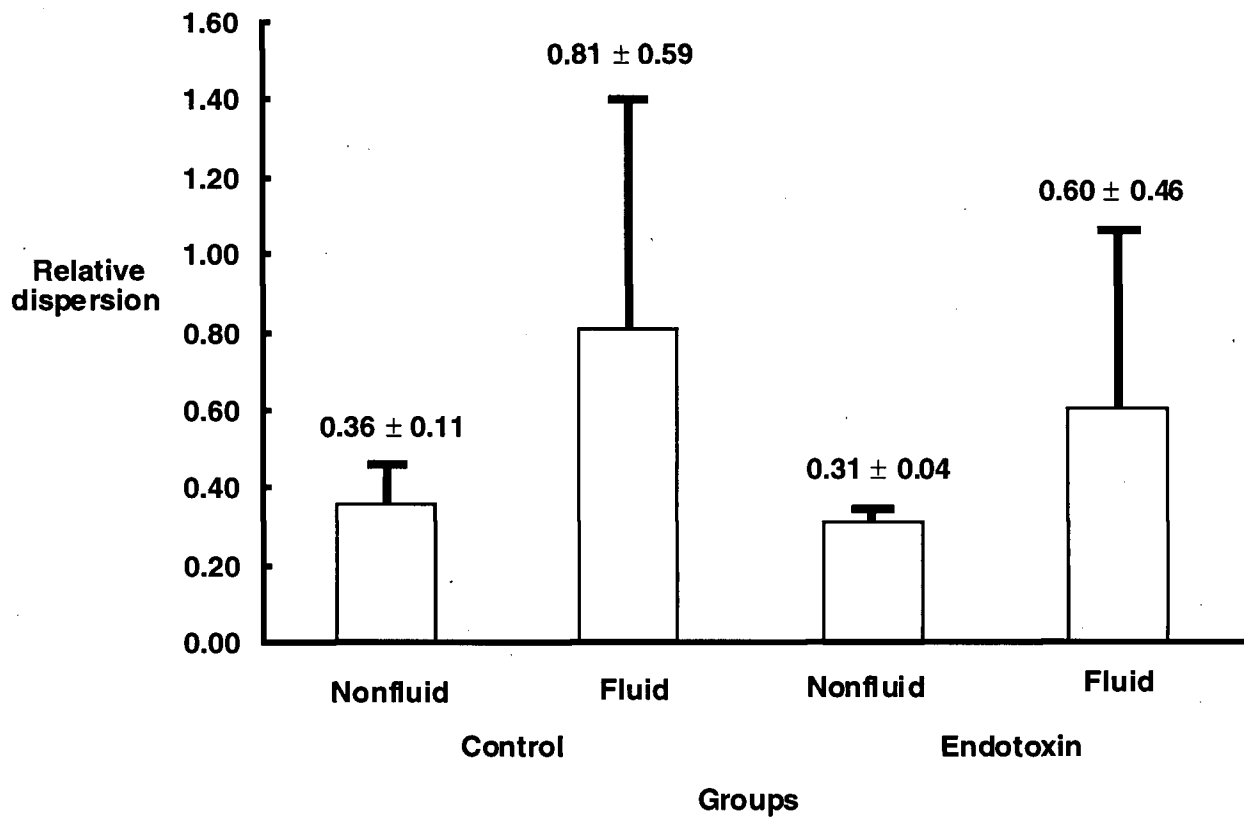


Figure 30. Relative dispersions of gut blood transit times.

The average relative dispersion of total gut blood flow transit times in thirty ~2g segments of jejunum is shown for the four experimental groups. Fluid resuscitation significantly increased relative dispersion (ANOVA, $p < 0.05$). Values are expressed as mean \pm SD.

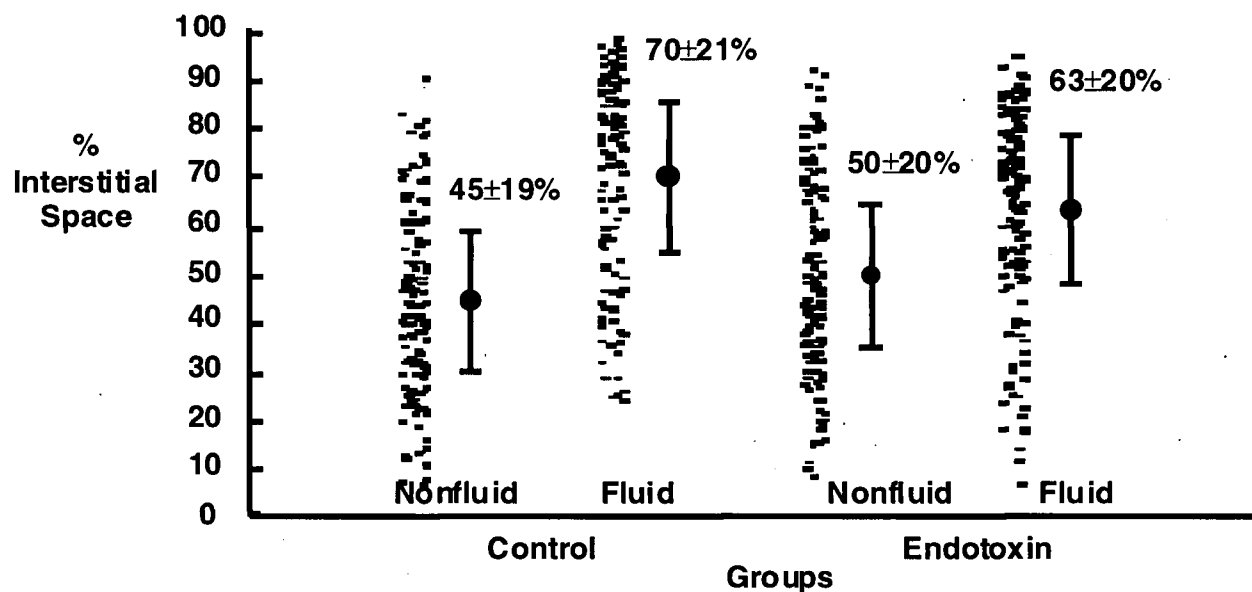


Figure 31. Interstitial Volume in Gut.

Area of interstitial space is illustrated for the four groups. Fluid resuscitation significantly increases the area of interstitial space ($p > 0.05$) however, endotoxin has no significant effect (ANOVA, $p > 0.05$). Values are expressed as mean \pm SD.

Table 1.

Onset of Ischemia and Extraction Ratios

	CONTROL		ENDOTOXIN	
	Non-Resuscitated (n=8)	Fluid-Resuscitated (n=8)	Non-Resuscitated (n=8)	Fluid-Resuscitated (n=8)
WB DO ₂ vs WB VO ₂				
Onset of Ischemia - WB DO ₂ c (mlO ₂ /min.kg of WB wt)	9.1 ± 1.7	10.3 ± 2.2	12.2 ± 3.8†	15.3 ± 3.2†
Critical VO ₂ - WB VO ₂ c (mlO ₂ /min.kg of WB wt)	7.5 ± 1.3	6.3 ± 1.3	6.5 ± 1.5	7.4 ± 1.2
O ₂ Extraction Ratio WB ERc (=WBVO ₂ c/WBDO ₂ c)	0.82 ± 0.06	0.62 ± 0.08*	0.55 ± 0.08†	0.49 ± 0.04†
O ₂ Extraction Ratio WB ERmax (O ₂ ER at final measurement)	0.86 ± 0.03	0.77 ± 0.08*	0.74 ± 0.10	0.81 ± 0.11†
GUT DO ₂ vs GUT VO ₂				
Onset of Ischemia - GutDO ₂ c (mlO ₂ /min.kg of gut wt)	25.3 ± 14.8	20.5 ± 6.8	24.3 ± 9.0	22.9 ± 11.7
Critical VO ₂ - Gut VO ₂ c (mlO ₂ /min.kg of gut wt)	19.6 ± 12.0	14.0 ± 5.6	12.9 ± 6.0	11.8 ± 5.2
O ₂ Extraction Ratio Gut ERc (=GutVO ₂ c/GutDO ₂ c)	0.77 ± 0.07	0.67 ± 0.06*	0.52 ± 0.05†	0.52 ± 0.09†
O ₂ Extraction Ratio Gut ERmax (O ₂ ER at final measurement)	0.87 ± 0.03	0.72 ± 0.08*	0.68 ± 0.07	0.65 ± 0.08†
WB DO ₂ vs GUT VO ₂				
Onset of Ischemia - GutDO ₂ c (mlO ₂ /min.kg of gut wt)	10.6 ± 4.4	13.6 ± 6.1	13.5 ± 7.1	15.3 ± 5.9
Critical VO ₂ - Gut VO ₂ c (mlO ₂ /min.kg of gut wt)	16.7 ± 11.7	14.0 ± 5.1	10.2 ± 5.7	14.0 ± 6.2

* indicates difference between Non-fluid and Fluid-resuscitated groups (p<0.05) in control groups

** indicates difference between Non-fluid and Fluid-resuscitated groups (p<0.05) in endotoxin groups

† indicates difference between the control and endotoxin groups (p<0.05)

Table 2.

Arterial and Capillary Hematocrit

	Arterial Hct(%) @ Baseline	Arterial Hct(%) @ Onset of Ischemia	Capillary Hct(%) Onset of Ischemia
CONTROL			
Non-Resus.	23.0 ± 3.2	25.0 ± 3.5	27.5 ± 8.6
Fluid-Resus.	22.0 ± 3.3	16.1 ± 3.5*†	29.2 ± 14.3
ENDOTOXIN			
Non-Resus.	24.0 ± 3.5	24.4 ± 4.2	25.3 ± 7.7
Fluid-Resus.	22.6 ± 2.6	19.8 ± 3.8**†	25.3 ± 5.3

* indicates difference between Non-fluid and Fluid-resuscitated groups (p<0.05) in control groups

** indicates difference between Non-fluid and Fluid-resuscitated groups (p<0.05) in endotoxin groups

† indicates difference between Arterial Hematocrit at Baseline and at Onset of Ischemia (p<0.05)

Table 3.

Arterial and Portal Vein Leukocyte Counts

	Arterial WBC Baseline	Arterial WBC Critical	Portal WBC Baseline	Portal WBC Critical
CONTROL				
Non-Resus.	13.0 ± 3.8	25.0 ± 11.7*	12.0 ± 4.2	25.2 ± 12.1**
Fluid-Resus.	10.4 ± 7.4	14.9 ± 10.5*¥	8.8 ± 6.6	14.9 ± 9.9**¥
ENDOTOXIN				
Non-Resus.	10.1 ± 3.6	2.2 ± 0.5*†	9.0 ± 3.3	2.3 ± 0.8**†
Fluid-Resus.	10.5 ± 7.9	1.7 ± 0.9*†	9.6 ± 7.3	2.0 ± 1.4**†

WBC are expressed as leukocytes X 10⁹/L

* indicates difference between Baseline and Critical Arterial WBC (p<0.05)

** indicates difference between Baseline and Critical Portal WBC (p<0.05)

† indicates difference between control and endotoxin groups (p<0.05)

¥ indicates difference between Non-Resuscitated and Fluid-Resuscitated (p<0.05)

Table 4.

Intestinal pHi at Baseline and Onset of Ischemia

	pHi at Baseline	pHi at Onset of Ischemia
CONTROL		
Non-Resus.	7.25 ± 0.09	7.06 ± 0.10*
Fluid-Resus.	7.19 ± 0.09	7.07 ± 0.11*
ENDOTOXIN		
Non-Resus.	7.21 ± 0.10	6.91 ± 0.15*†
Fluid-Resus.	7.12 ± 0.26	6.90 ± 0.24*†

* indicates difference between pHi at Baseline and Onset of Ischemia ($p < 0.05$)

† indicates difference between pHi between control and endotoxin ($p < 0.05$)

Table 5.

Arterial Lactate Values

	Art. Lactate
CONTROL	
Non-Resus.	
Initial	2.51 ± 1.06
Critical	3.94 ± 1.24
Fluid-Resus.	
Initial	1.76 ± 0.70
Critical	2.41 ± 0.58*
ENDOTOXIN	
Non-Resus.	
Initial	1.93 ± 0.63
Critical	3.20 ± 0.97
Fluid-Resus.	
Initial	1.53 ± 0.50
Critical	3.07 ± 1.07

Lactate is expressed as mmol/L

* indicates difference between Non-fluid and Fluid-resuscitated groups ($p < 0.05$) in control groups

** indicates difference between Non-fluid and Fluid-resuscitated groups ($p < 0.05$) in endotoxin groups

† indicates difference between the control and endotoxin groups ($p < 0.05$)

Table 6.

Onset of Rise of Arterial Lactate

	CONTROL		ENDOTOXIN	
	Non-Resuscitated (n=8)	Fluid-Resuscitated (n=8)	Non-Resuscitated (n=8)	Fluid-Resuscitated (n=8)
WB DO ₂ vs Arterial Lactate				
Onset of Ischemia - WB DO ₂ c (mlO ₂ /min.kg of WB wt)	13.1 ± 3.3	12.0 ± 4.7	14.9 ± 5.7	12.3 ± 0.8

* indicates difference between Non-fluid and Fluid-resuscitated groups (p<0.05) in control groups

** indicates difference between Non-fluid and Fluid-resuscitated groups (p<0.05) in endotoxin groups

† indicates difference between the control and endotoxin groups (p<0.05)

Table 7.

Oxygen Transport Variables

	PaO ₂ (mmHg)	PaCO ₂ (mmHg)	Arterial pH	Art. Hgb (g/l)	ArtO ₂ content (g O ₂)	Temp (°C)
CONTROL						
Non-Resus.						
Initial	151.0 ± 20.1	29.9 ± 2.9	7.46 ± 0.05	77.3 ± 10.0	103.5 ± 12.3	37.0 ± 0.8
Critical	134.6 ± 19.5	32.1 ± 4.4	7.40 ± 0.06	83.6 ± 13.0	109.7 ± 16.0	38.4 ± 0.5
Fluid-Resus.						
Initial	137.4 ± 31.9	31.2 ± 3.9	7.41 ± 0.05	73.9 ± 9.8	98.3 ± 11.9	36.9 ± 0.7
Critical	109.4 ± 38.6	36.0 ± 11.0	7.28 ± 0.08*	55.0 ± 11.2*	68.6 ± 16.1*	37.9 ± 0.8
ENDOTOXIN						
Non-Resus.						
Initial	159.0 ± 30.3	31.2 ± 6.1	7.45 ± 0.05	78.9 ± 11.1	107.0 ± 14.5	38.0 ± 1.2
Critical	107.2 ± 35.8	35.3 ± 9.9	7.30 ± 0.07	83.4 ± 17.5	105.2 ± 27.5	38.7 ± 0.7
Fluid-Resus.						
Initial	129.7 ± 36.7	33.5 ± 4.3	7.43 ± 0.05	79.1 ± 13.5	102.91 ± 17.2	37.2 ± 0.5
Critical	74.3 ± 19.9**†	43.8 ± 5.8**	7.21 ± 0.08**†	67.9 ± 10.1**†	76.5 ± 15.6**	38.5 ± 0.5

* indicates difference between Non-fluid and Fluid-resuscitated groups (p<0.05) in control groups

** indicates difference between Non-fluid and Fluid-resuscitated groups (p<0.05) in endotoxin groups

† indicates difference between the control and endotoxin groups (p<0.05)

Table 8.

Hemodynamic Variables

	Wedge Pressure (mmHg)	Mean Art.Pres. (mmHg)	Cardiac Output (L/min)	Sys.Vasc.Res. (dynes/sec/cm ⁵)	Portal Flow (L/min)
CONTROL					
Non-Resus.					
Initial	6.17 ± 2.36	77.2 ± 13.8	7.79 ± 1.93	784.30 ± 256.95	0.75 ± 0.17
Critical	4.32 ± 1.78	59.2 ± 10.6	2.76 ± 0.68	1651.76 ± 407.98	0.32 ± 0.21
Fluid-Resus.					
Initial	7.94 ± 1.12	83.3 ± 10.4	8.23 ± 3.60	882.37 ± 407.10	0.86 ± 0.22
Critical	6.89 ± 1.10*	76.7 ± 11.6*	5.74 ± 2.67*	1183.38 ± 604.19*	0.66 ± 0.32*
ENDOTOXIN					
Non-Resus.					
Initial	8.19 ± 2.37	75.7 ± 19.1	6.65 ± 2.45	833.16 ± 483.85	0.70 ± 0.24
Critical	7.71 ± 2.45†	43.5 ± 13.9†	3.64 ± 1.07	515.90 ± 375.47†	0.32 ± 0.14
Fluid-Resus.					
Initial	9.13 ± 1.74	83.5 ± 13.9	6.78 ± 1.93	920.24 ± 412.09	0.73 ± 0.26
Critical	9.49 ± 1.91†	49.3 ± 9.6†	5.90 ± 2.11**	380.14 ± 415.89†	0.53 ± 0.35

* indicates difference between Non-fluid and Fluid-resuscitated groups (p<0.05) in control groups

** indicates difference between Non-fluid and Fluid-resuscitated groups (p<0.05) in endotoxin groups

† indicates difference between the control and endotoxin groups (p<0.05)

Table 9.

Clinical Variables

	Animal wts (kg)	Total fluid (cc/kg)	Total urine (cc/hr)	Time of survival (min)
CONTROL				
Non-Resuscitated	25.4 ± 1.8	69 ± 23	59 ± 30	202 ± 60
Fluid-Resuscitated	27.6 ± 3.9	353 ± 80*	267 ± 160*	368 ± 105*
ENDOTOXIN				
Non-Resuscitated	24.3 ± 1.5	89 ± 18	145 ± 93	128 ± 43†
Fluid-Resuscitated	25.8 ± 2.9	161 ± 61**†	178 ± 115†	171 ± 81†

* indicates difference between Non-fluid and Fluid-resuscitated groups (p<0.05) in control groups

** indicates difference between Non-fluid and Fluid-resuscitated groups (p<0.05) in endotoxin groups

† indicates difference between the control and endotoxin groups (p<0.05)

5.4 DISCUSSION

The major finding from our study is that fluid resuscitation significantly impairs whole body O₂ extraction in control pigs but did not significantly change the already decreased O₂ extraction in the endotoxin treated animals. Impairment of O₂ extraction is associated with increased interstitial edema and increased heterogeneity of transit times but not to altered capillary hematocrit.

Sepsis has substantial effects on the microcirculation that may account for impaired tissue oxygen extraction (74) (126). The host defense response against invading microorganisms can cause the development of the systemic inflammatory response syndrome (SIRS) (6). Endotoxin and other bacterial products trigger macrophages and other inflammatory cells to release TNF- α , IL-1, IL-6 and many other pro-inflammatory mediators (166) (6). These endogenous mediators of the septic inflammatory response have important effects on the microvasculature including leukocyte slowing and microthrombi (168) (52) (41) that may lead to decreased capillary flows, increased capillary permeability - that is, "leaky capillaries" (52) (12) with the resultant interstitial edema (136) and, an alteration in vascular tone (76) (74) (169).

Altered microcirculatory regulation of blood flow may lead to mismatching of oxygen supply to demand resulting in impaired tissue oxygen extraction (78). Lam et al. demonstrate that after cecal ligation and puncture in rats, there was a 36% reduction in perfused capillary density with an increase in heterogeneity of the spatial distribution of perfused capillaries (57). These results are similar to the observations by Humer et al. that the relative dispersion of gut capillary blood flow

increases after endotoxin infusion in pigs and this was associated with impaired oxygen extraction (74). Some time ago, Honig and Odoroff suggested that the dispersion of capillary transit times would have an impact on oxygen exchange by the capillary bed (170). A subsequent theoretical analysis suggested that an increased dispersion of capillary transit times would impair oxygen exchange by increasing mismatch between oxygen supply and demand within small regions of a capillary bed (78). Conversely, Morff has suggested that increased capillary recruitment improves tissue oxygenation in rat cremaster muscle (171).

A number of investigators have suggested that to improve microvascular flow, reduced hematocrits may be useful (111) (125). Hemodilution decreases blood viscosity, may improve capillary red blood cell flux (125), and may improve blood flow distribution within the capillary bed (125) (126). Van der Linden et al found that hemodilution to a hematocrit of 20 or 30% using colloid infusion resulting in an increased critical oxygen extraction ratio during progressive hemorrhage compared to a hematocrit of 40% (125). Tyml has demonstrated that the heterogeneity of microvascular flow in rat skeletal muscle is reduced by hemodilution (126). However, while some argue that microvascular flow distribution and tissue oxygen extraction may be improved by fluid resuscitation, others argue that fluid resuscitation may lead to interstitial and endothelial edema (136) which may further impair microvascular flow and lead to a higher degree of capillary heterogeneity. Therefore, our hypothesis was that not only would endotoxemia increase capillary heterogeneity, but that fluid resuscitation may also increase capillary heterogeneity and thereby impair oxygen extraction.

As per the hypothesis, the author found that fluid resuscitation resulted in impaired O₂ extraction associated increased heterogeneity of transit times. In addition, fluid resuscitation resulted in increased interstitial edema. Therefore, a possible mechanism for impaired O₂ extraction associated with fluid resuscitation is increased heterogeneity of transit times possibly resulting from decreased capillary recruitment secondary to altered morphology of the capillary bed due to interstitial edema.

Since sepsis is characterized by a generalized increase in capillary permeability (52) (12), tissues are predisposed to development of interstitial edema (136). Since crystalloid resuscitation is known to redistribute according to Starling's forces, one would anticipate that interstitial edema would be more prone to occur in sepsis. Further, an increased O₂ diffusion distance associated with interstitial edema could by itself result in decreased interstitial O₂ tension (56), but whether O₂ uptake is affected by interstitial edema is unsupported in studies by others (172).

A key new observation in the current study is that fluid resuscitation significantly increases the relative dispersion of blood flow transit times throughout the gut wall. This may indicate an increased relative dispersion of blood flow transit times in the capillary bed as well as within the larger arterioles and venules within the gut wall. Conceivably, the resultant decrease in capillary diameter due to endothelial edema may impair microvascular blood flow or contribute to leukocyte retention and plugging within the capillary bed (164). In addition, red blood cell rheology may be altered in the septic state (49) and conceivably could be altered by fluid resuscitation resulting in more heterogeneous microvascular flow. As a

result, impaired oxygen extraction may be accounted for by mismatching of oxygen supply to demand. Thus, the potential detrimental effects of fluid resuscitation appear to contribute to overall impaired oxygen extraction in the current set of experiments.

In our previous study (74) impaired O_2 extraction in endotoxin pigs was associated with increased heterogeneity of transit times. In that study endotoxin pigs also received fluid resuscitation whereas controls did not. Similarly, in this study impaired O_2 extraction associated with increased heterogeneity of transit times was observed in endotoxic pigs which received fluid resuscitation. But impaired O_2 extraction in endotoxic pigs was not associated with increased heterogeneity of transit times without fluid resuscitation. Therefore, increased heterogeneity of transit times is not the sole mechanism of impaired O_2 extraction in endotoxemia. Similarly, interstitial edema was increased in association with fluid resuscitation rather than with endotoxemia. Therefore, while interstitial edema with increased heterogeneity of transit times could result in impaired O_2 extraction from fluid resuscitation this is not sufficient to account for impaired O_2 extraction from endotoxemia.

One of the side-effects of massive saline infusion is the development of a metabolic acidosis (137). Acidosis is known to alter the oxygen dissociation curve by shifting the curve to the right. This would lead to a higher P_{50} and therefore a reduced affinity of hemoglobin for oxygen; thus hemoglobin releases oxygen at higher PO_2 , which would tend to raise tissue PO_2 . Therefore, while saline infusion

may have shifted the dissociation curve, it would have done so in favor of improved tissue oxygenation. This would not explain the impaired oxygen extraction that was found.

Capillary transit times are more closely related to O₂ extraction than transit times in the whole gut wall. Capillary transit times was not measured in this study but in a study by Humer et al (74), capillary transit times accounted for about one-half of whole gut wall transit times. In that study altered heterogeneity of capillary transit times associated with endotoxemia and fluid resuscitation was paralleled by altered heterogeneity in whole gut wall transit times. While it is possible that increased heterogeneity of capillary transit times with unchanged heterogeneity of whole gut wall transit times could occur in endotoxic pigs which did not receive fluid resuscitation, one does not have basis for suggesting that this possibility could be a mechanism of impaired O₂ extraction in endotoxic non-fluid resuscitated animals.

Interestingly, the author notes that although arterial hematocrit decreased as anticipated with saline resuscitation, microvascular hematocrit determined using morphometric measurements was not altered by saline resuscitation. Thus, the putative beneficial effect of crystalloid resuscitation on blood viscosity may not extend to the microvasculature. Furthermore, hemodilution does not explain the decreased oxygen extraction seen with fluid resuscitation. However, it is important to point out that the technique of determining capillary hematocrits only included those vessels that had erythrocytes present. As mentioned, there is heterogeneity of blood flow which implies that there will be some vessels which were not perfused at

all and therefore, these would not have been counted as part of the morphometric analysis.

The leukocyte counts in the endotoxemic groups showed a dramatic decrease compared to controls. Since the effect was seen in not only the arterial but also the portal vein samples, one would anticipate not only systemic but splanchnic trapping of leukocytes. Indeed Barroso-Aranda et al (168) showed that endotoxemia is associated with activation of polymorphonuclear leukocytes such that it results in microvascular trapping. They further have shown that obstruction occurs exclusively at the capillary level without involvement of arterioles or venules and suggest that leukocyte capillary plugging may be an important contributor in the fatal outcome after endotoxin administration. Indeed capillary plugging may explain the heterogeneity in capillary blood flow described by some investigators (168) (74) (57) which is thought to lead to the impaired oxygen extraction by tissue (78).

Tonometry has been used by many as an indicator of mucosal ischemia (148) (150) (151) (152). Our results show that tonometry can be used to indicate changes in mucosal acidosis and showed that endotoxemic animals have a larger degree of mucosal acidosis at onset of ischemia. Furthermore, some have used tonometry to indicate onset of ischemia (154) (155). While the author attempted to perform dual line regression between pHi values and oxygen delivery, there were not enough pHi data points to obtain useful numbers to determine onset of ischemia. While more data points would have been ideal, the short period of time of the entire experiment would have required a shortening of the time interval which would perhaps not

allow enough time to adequately equilibrate the gases with the saline in the tonometric balloon.

While many investigators use lactate as a marker of onset of ischemia (74) (106), others consider lactate is a difficult value to interpret (55). While the arterial lactate levels are higher in at onset of ischemia, there appears to be too much variability to make these differences significant. Further, our values show that rise in lactate as determined by a dual line regression with whole body oxygen delivery corresponds to the onset of ischemia as determined with a dual line regression between whole body oxygen delivery and consumption. The only group not to correspond was that of the control non-fluid group. The reasons for this difference are not clear. Possible reasons include an aerobic source of lactate production, early anaerobic production by some tissues, or an inability to metabolize lactate in this group (156).

While current recommendations include early and aggressive use of crystalloids in sepsis, the data suggests that saline resuscitation does not improve the oxygen extraction defect seen in sepsis. Indeed, the saline was shown to lead to increased interstitial edema and a maldistribution of blood flow which may contribute to the oxygen extraction defect. Therefore, the author questions the use of crystalloid resuscitation for treatment of sepsis in humans.

Chapter 6

CONCLUSIONS

A review of the current literature on the microvascular changes associated with sepsis and endotoxemia lead us to our hypothesis that crystalloid resuscitation would impair the ability of tissue to extract oxygen. We found that there is a significant impairment in oxygen extraction capabilities with the addition of crystalloid resuscitation which would therefore support our hypothesis. Potential reasons for this impairment were investigated. First, we studied the potential role of the described increase in capillary permeability leading to interstitial edema. We found an increase in interstitial volume by use of morphometric analysis. This would support the theory that interstitial edema may be a reason for the impaired ability to extract oxygen. Further, alterations in capillary distribution has been theorized as a potential reason for decreased oxygen extraction in shock states. We found there was an increase in blood flow heterogeneity by use of microspheres as markers of blood flow. This effect was seen not only in endotoxemia but with fluid resuscitation. Therefore, an alteration in the capillary flow either by interstitial fluid hindering capillary flow or by fluid impairing endothelial control may be possible. Finally, some reports suggest that fluid resuscitation may lead to hemodilution at a capillary level which may thereby decrease oxygen delivery to tissue beds. We found that while arterial hematocrit is significantly reduced with saline loading, capillary hematocrit was not altered compared to baseline arterial values. Therefore, an alteration in capillary hematocrit would not explain the decreased oxygen

extraction found for the gut and whole body. Thus, while aggressive and early saline resuscitation is considered by many to be a fundamental therapeutic maneuver in sepsis, our study has shown that this therapy leads to significant impairment in oxygen extraction and thereby may prove detrimental.

Chapter 7

SUMMARY: CLINICAL RELEVANCE, FUTURE DIRECTIONS

The ideal therapy for sepsis has still to be defined. There are numerous levels at which therapy may be initiated as was described earlier. We sought to determine the effects of saline resuscitation in our model and found a significant impairment in oxygen extraction. We based our findings on the likely development of interstitial edema as well as an impairment in the microvascular distribution leading to capillary heterogeneity. We would anticipate that if these findings were to be similar to what one sees clinically, that saline resuscitation may worsen an already critical situation. There are several areas of possible further study. Areas would include further studies in the current project, logical follow-up studies, and finally likely future endeavors which may prove beneficial in the therapy of sepsis.

First, there may be other areas of investigations in the current study which may shed further light into the mechanisms of impaired oxygen extraction. While we have shown interstitial edema and heterogeneity of total gut blood flow, there are other aspects which have not yet been studied. While we have looked at the total gut blood flow transit times, we have not yet subdivided these into large, medium, and small vessel as per Humer et al (74). This may give further information as to which vessels are contributing to the heterogeneity. Furthermore, while we have demonstrated heterogeneity in transit times, there are other mechanisms for impaired oxygen extraction which might be investigated. As described earlier, leukocytes have been shown to cause capillary obstruction during

sepsis (65) (41) (66) which may contribute to vascular obstruction. Using morphometric techniques, one may attempt to quantify the number of leukocytes present in capillaries to determine whether this is a contributing factor in the impaired oxygen extraction in endotoxin and saline resuscitated animals. Further, not only has development of capillary heterogeneity been shown to decrease oxygen extraction, but an alteration in the capillary density may also be involved (57). Also with morphometric techniques, the number of capillaries may be quantified. Another technique involves the use of intravital microscopy as has been used to study the microvasculature by others (126) (173). One would expect that increased interstitial edema would lead to decreased number of open capillaries and therefore a decreased capillary density (34). Finally, some investigators have theorized that the inability to utilize oxygen may be an important factor in the oxygen extraction problem seen in sepsis. Mechanisms include uncoupling oxidative phosphorylation (14) (15), inhibition of mitochondrial respiration (16) (17) (18) (17) and mitochondrial destruction (54). One may look at the possibility of mitochondrial destruction as a contributing factor to impaired oxygen extraction. Hersch et al (54) utilized electron microscopy to analyze mitochondrial features and describe significant mitochondrial destruction in a sheep model of sepsis. While all these studies would add further information to our current study, they go no further in suggesting what other therapies may be more successful in improving oxygen extraction in sepsis.

Follow-up studies based on our current investigation includes using the same model with different types of fluid resuscitation. The aim would be to preserve the

beneficial effects of fluid resuscitation but to avoid the side-effects demonstrated in our current study. Possible fluid include other crystalloids and the colloids. As for crystalloids, two other common fluids used include Ringers Lactate and hypertonic saline. With respects to Ringers Lactate (RL), advantages of its use would include less development of hyperchloremic acidosis in view of the decreased concentration of sodium chloride as well as the buffering action of sodium lactate. However, since lactate requires conversion by the liver to pyruvate and then to bicarbonate to act as a buffer, there may be concern during sepsis where the liver has been shown to have decreased function that lactate may accumulate (174). The other potential crystalloid to study would be hypertonic saline. Because sodium is primarily an extracellular ion, infusion of hypertonic saline would be expected to expand the extracellular fluid (ECF) space by a greater amount than the volume infused, because theoretically, water should enter the ECF from the intracellular fluid (128). Further, there may be potential positive inotropic effects as well as systemic and pulmonary vasodilation (128). Horton and Walker (175) have studied hypertonic saline (HS) in a canine model of endotoxemia. They have shown that the addition of HS to RL reduces the total volume of RL required to maintain hemodynamic variables. Further, the net fluid gain was five times less than with RL alone. This would potentially limit the degree of interstitial edema that accompanies most crystalloid fluid resuscitation as occurred in our study. Despite the increased chloride content, it has not been shown to lead to hyperchloremic acidosis as does normal saline (174). However, if HS is rapidly infused, it may precipitate pontine myelinolysis (174) (128). While other crystalloids may be a potential area of investigation,

another area with great potential is the use of colloids. Sepsis has been characterized by a diffuse increase in microvascular permeability (46) (52) (12). Thus, the use of colloids which have been shown to stay in the intravascular space for longer durations (118) would seem obvious. A study involving in vivo microscopy and surface oxygen partial pressure electrodes by Funk et al (173) was performed to compare crystalloids to colloids. They showed that colloids had improved capillary perfusion and tissue oxygenation compared to crystalloid resuscitation. Further, sepsis also is accompanied by a reprioritization of hepatic protein synthesis with decreased albumin production which leads to a decreased colloid oncotic pressure (130). This would, according to Starling's forces, add to the leak of fluid out of capillaries leading to pulmonary edema (176) (177) (178) (179) and interstitial edema (180) (178). Morisaki et al (181) showed that when compared to crystalloid, colloid therapy decreases the progression of extrapulmonary tissue injury in septic sheep and based this on the preservation of microvascular surface area for tissue O₂ exchange. The different types of colloids include albumin and hydroxyethyl starch. Some important properties of albumin include antioxidant activity, binding free fatty acids and endotoxin (182) (128). Because heat treating albumin at 60°C for 10 hours inactivates hepatitis virus and other infectious agents, there is no concern regarding infections with this colloid (118). Adverse reactions to albumin are rare (137). Intravenous administered albumin distributes initially to the intravascular space, but gradually redistributes to the interstitial space. Its intravascular half-life is 16 hours, longer than crystalloids and similar to endogeneous albumin (128). Hydroxyethyl starches (HES) are synthetic colloid derivatives from corn starch. The

agent is prepared by incorporating hydroxyethyl ether into the glucose residues of amylopectin (174). They have been shown to effectively expand plasma volume by 70% at 3 hours and 40% by 12 hours after infusion (174). Zikria et al (183) have suggested that one of the beneficial roles of HES is to alter the microvascular permeability of an ischemic limb, thereby reducing the development of interstitial edema and eventual compression ischemia. Hydroxyethyl starches have been shown to have anaphylactic reactions in 0.8% as well as decreases in factor VIII activity and prolongation of partial thromboplastin time (137) however there have been no increased frequency of bleeding reported. Concerns about the possible immunosuppressive effect of hydroxyethyl starch have also been raised (184). Higher molecular weight particles of hydroxyethyl starch are deposited in the reticuloendothelial system and may affect phagocytic function. However, in a study by Shatney and Chaudry (185) demonstrated that reticuloendothelial clearance rates of labeled lipid emulsions were unchanged after hydroxyethyl starch, and similarly, mortality rates from peritonitis were not altered in animals receiving hydroxyethyl starch. Pentastarch is a modification of the hetastarch formulation as it has a lower average molecular weight, a more homogeneous size of particles, and less hydroxyethyl substitution. These changes allow for a more predictable excretion of pentastarch compared with hetastarch. Its volume-expanding effect lasts 12 hours. Because of its higher colloid oncotic pressure (about 40 mm Hg), it has a greater degree of volume expansion (1.5 times the volume infused) than either 5% albumin or 6% hetastarch (128). Further, it appears to have less effect on coagulation than hetastarch.

Thus, with the potential benefits of colloids over crystalloids, and with the advantages of pentaspan over some other colloids, it places pentaspan as the most rationale fluid to study. One may study the effects in a similar experiment as described with use of pentaspan as the resuscitative fluid and study the oxygen extraction at onset of ischemia. Further, by studying the interstitial volume as an indicator of interstitial volume, one may determine whether colloids improve oxygen delivery by limiting interstitial edema.

While the use of fluid resuscitation to augment oxygen delivery is directed a relatively phase of SIRS, others have studied novel approaches at earlier stages in the inflammatory response. These therapies may be divided into specific and nonspecific. Specific therapies include monoclonal antibody derived against gram-negative bacterial endotoxin has been tested in septic patients in a multicenter, prospective, randomized, double blind fashion (186). Administration of this antibody to ICU patients with suspected gram-negative sepsis early in their septic course resulted in a significant reduction in the mortality in the group who proved to be bacteremic on blood culture. However, when looking at the entire group, there was no significant reduction in mortality. Polymyxin B is another compound which has the property of efficiently binding endotoxin (187). Further, a polymyxin B-impregnated filter is being developed as part of hemofiltration systems to chelate circulating endotoxin (182). Trials are also underway on monoclonal antibodies designed to interfere with neutrophil adhesion (anti-CD18 or CD11), IL-1 receptor, TNF, and PAF (22). Much work needs to be done not only on its potential effectiveness but the timing, potential adverse effects, and the specificity of therapy.

Less specific therapies include eicosanoid inhibitors. Increased activity of phospholipase A2, an important enzyme in the release of arachidonic acid from membrane phospholipids, is correlated with the severity of septic shock (188). Blocking the synthesis or activity of these substances results in increased survival in experimental models (189). Other nonspecific therapies being studied include free radical scavengers, xanthine oxidase inhibitors, and superoxide dismutase to name a few (182).

Whether the crystalloids, colloids, or the novel approaches will have potential in reducing the overwhelming mortality rates remains to be seen. However, it is unlikely that any one therapy will be the "magic bullet" that everyone seeks.

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