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# Systemic inflammatory response to traumatic injury and the progression to multiple organ dysfunction syndrome

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**TITLE:**

**Systemic Inflammatory  
Response to Traumatic  
Injury and the Progression  
to Multiple Organ  
Dysfunction Syndrome**

**DATE: October 9, 1994**

**Systemic Inflammatory Response to Traumatic Injury  
and the Progression to  
Multiple Organ Dysfunction Syndrome**

by  
JoAn L. Monaco

A Thesis  
Presented to the Graduate Committee  
of Lehigh University  
in Candidacy for the Degree of  
Master of Science in Behavioral Neuroscience

Bethlehem, PA  
September 25, 1994

Approved and recommended for acceptance as a thesis in partial fulfillment of the requirements for the degree of Master of Science.

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## ABSTRACT

Multiple organ dysfunction syndrome (MODS) is now recognized as a pathological process that depends, in part, on the systemic inflammatory response following injury. Using an isolated, perfused hindlimb model in male Sprague-Dawley rats, the early inflammatory response to injury was studied.

Hindlimb circulation was isolated and perfused with a modified Krebs's buffer (Ph 7.4) containing albumin, washed human blood void of white blood cells (WBCs), amino acids, and glucose, aerated with O<sub>2</sub>/CO<sub>2</sub> (95:5). Prior to cannulation, hindlimbs in the injured group sustained bilateral femur fractures and a one minute bilateral quadriceps muscle crush injury. Oxygen consumption, lactate, thromboxane (TxB<sub>2</sub>), prostaglandin F<sub>1α</sub> (PGF<sub>1α</sub>), tumor necrosis factor (TNF), and interleukin-6 (IL-6) were measured in the perfusate prior to cannulation and at 5, 20, and 80 minutes of perfusion.

Over the 80 minute time course, the following results were noted: 1) no change in lactate or oxygen consumption indicating the hindlimbs were well-perfused; 2) no change in TNF for either group; 3) TxB<sub>2</sub> release increased in both groups but was only significant in the injured group; 4) the 80 minute IL-6 levels were augmented, however, injury did not alter this response in hindlimbs perfused without WBCs. Interleukin-6, therefore, is an important early mediator released directly from the endothelium yet its release is most-likely dependent on the presence of WBCs.

Using the isolated, perfused hindlimb preparation, these experiments are among the first to outline the early inflammatory response in massive tissue injury.



## INTRODUCTION

### **Multiple Organ Dysfunction Syndrome**

Multiple organ dysfunction syndrome (MODS) is defined as the presence of altered organ function in an acutely ill patient such that homeostasis cannot be maintained without intervention (Bone, Balk, Cerra, Dellinger, 1992). This syndrome is the leading cause of mortality in the critically ill surgical patient (Bone et al., 1992) accounting for 50% to 80% of surgical intensive care unit deaths with costs exceeding \$150,000 per patient (Baue, 1990; Deitch, 1990; 1992; Demling, LaLonde, Saldinger, Knox, 1992).

Multiple organ dysfunction syndrome was first recognized in the late 1960's as the sequential failure of lung, liver, and kidney after trauma which usually resulted in death (Baue, 1975). It has since been seen in other injurious events such as hypovolemia, septic shock, severe inflammation, prolonged inadequate resuscitation (Cerra, 1992), and soft tissue injury, all of which have perfusion deficit as a common link. This results in a relative oxygen deficit that occurs because the body is either unable to accommodate the increased need for oxygen, that occurs with the hypermetabolic state, or the tissues cannot extract delivered oxygen. In the hypermetabolic state, the body tends to maintain central circulation (to the heart, brain, etc.) at the expense of other structures such as the skin, muscle, gut, and kidneys. The ischemia in such "secondary" structures leads to an increase in toxic metabolites, inflammatory cytokines, a loss of gut-barrier function, and soft-tissue injury, all of

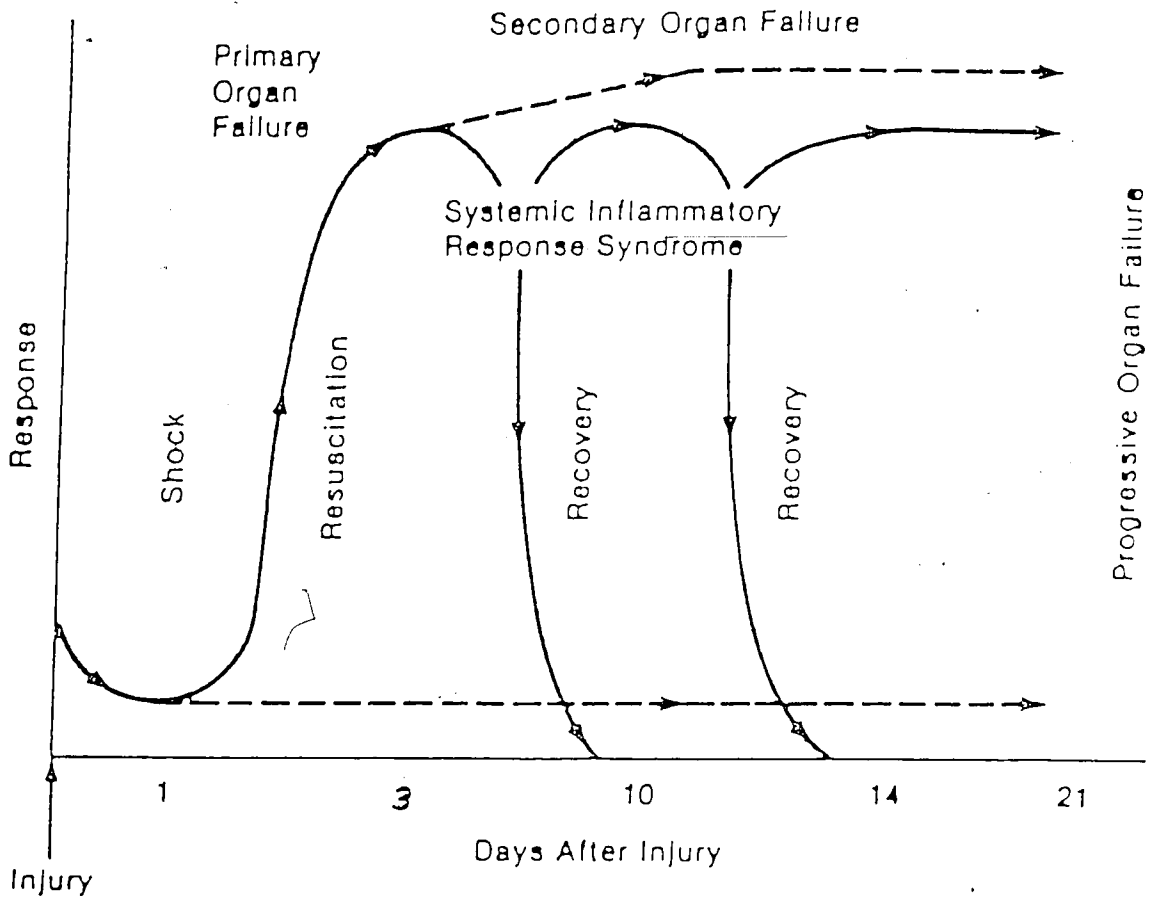
which can contribute to the onset of systemic inflammation (Livingston, 1993).

### **Systemic Inflammatory Response Syndrome**

Multiple organ dysfunction syndrome typically progresses from the systemic inflammatory response syndrome (SIRS). Clinically, SIRS presents as two or more of the following conditions: (1) temperature greater than 38 degrees Celsius or less than 36 degrees Celsius; (2) heart rate greater than 90 beats per minute; (3) respiratory rate greater than 20 breaths per minute or PaCO<sub>2</sub> less than 32 mm Hg; and, (4) white blood cell count greater than 12,000 / cu mm, less than 4,000 / cu mm (Bone et al., 1992). The response pattern that typically occurs consists of an inciting event, SIRS which peaks 3 to 4 days post-event. If the initial source is not controlled, or additional insults occur, SIRS may progress to clinical organ dysfunction, MODS, then death (Cipolle, Pasquale, Cerra, 1993) (see Figure 1, page 4).

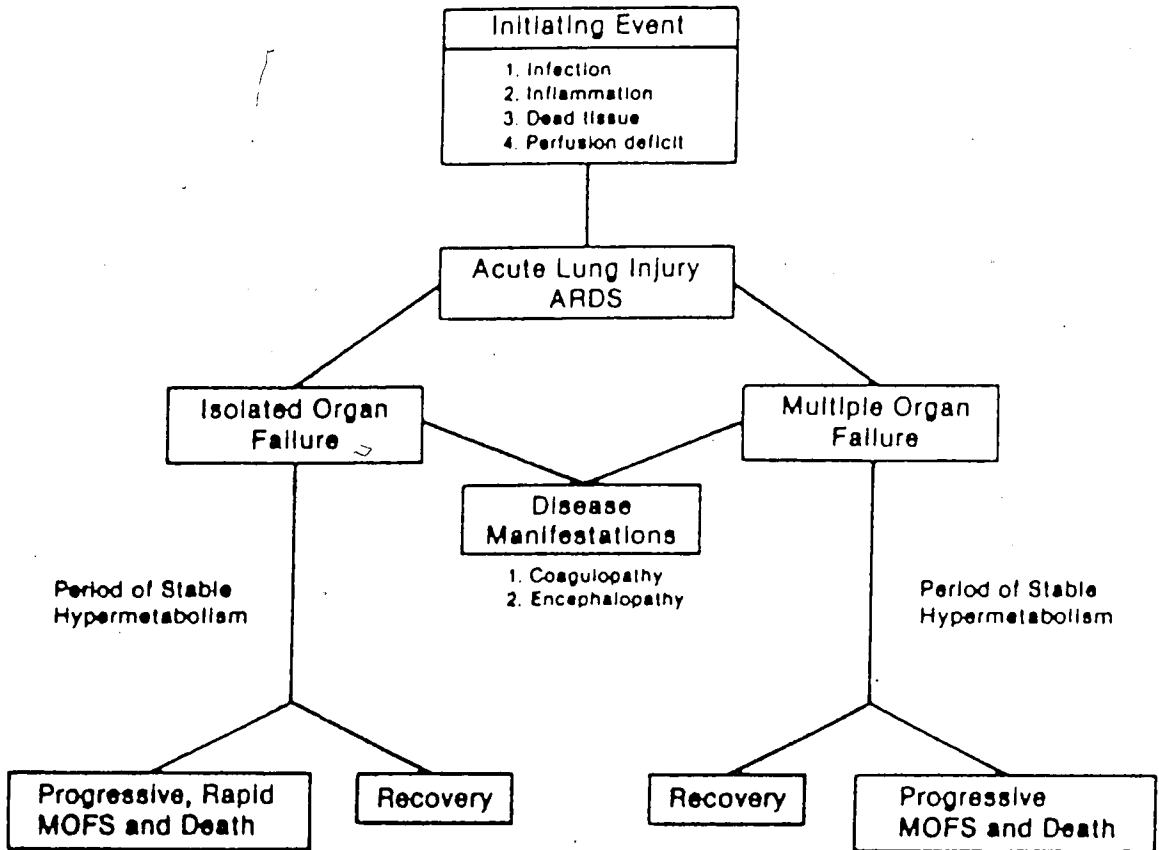
The initiating event may be septic shock, hypoperfusion (trauma or bleeding), inflammation (pancreatitis), or the presence of dead or injured tissue. If severe enough, the initial insult can lead to immediate death. If the patient survives the initial insult, he/she may recover or go on to develop SIRS. The inflammatory response generally follows one of two pathways (Cipolle et al., 1993) (see Figure 2, page 5). The first consists of a continued inflammatory response so called "stable hypermetabolism" which may be associated with the development of organ dysfunction if further complications occur. If these complications are properly

**FIGURE 1**



Inflammatory and organ failure responses to injury. In the spectrum of response leading to multiple organ failure, several phases of response can be identified: shock, resuscitation, inflammation, and progressive secondary organ dysfunction. The pathogenesis changes over time (Cipolle, Pasquale, Cerra, 1993).

**FIGURE 2**



Two general pathways of clinical response can be identified. In one, the clinical picture is dominated by lung dysfunction; in the other, by multiple organ dysfunction (Cipolle, Pasquale, Cerra, 1993).

confronted, SIRS can abate and the patient survives. The second pattern, however, is grave with the inciting event leading to the rapid development of multiple organ dysfunction. In this pathway, MODS does not abate but rather progresses to death.

### **Cytokine and Eicosanoid Responses**

Soft tissue injury from a traumatic insult, such as a crush or stress fracture, seems to be correlated with an early systemic inflammatory cytokine response. The peptide cytokines have numerous functions *in vivo*. There is evidence that they promote inflammation and initiate SIRS after an inciting event (Cipolle et al., 1993). However, some cytokine responses are protective and may promote survival. Cytokines are bioactive at extremely low concentrations and are often difficult to detect. These molecules are produced primarily by macrophages and lymphocytes, but also by endothelium, keratinocytes, and parenchymal cells throughout the body. They, therefore, differ in this respect from endocrine hormones (Demling et al., 1992).

It has been found that serum cytokine levels rise after acute trauma and hemorrhage (Baggiolini, Walz, Kunkel, 1989; Hechtman, Cybulsky, Fuchs, 1991; Meade, Shoemaker, Donnelly, Abraham, Jagels, Cryer, Hugli, Bishop, Wu, 1994; Rinaldo, Christman, 1990; Rot, 1992; Rush, Sori, 1988). The immediate response of specific cytokines to blunt injury, however, has not been clearly or consistently illustrated. Cytokines such as interleukin-6 (IL-6), and tumor necrosis factor (TNF) appear to be associated with infection, inflammation, and the evolution of MODS.

## **Interleukin-6**

The exact role of IL-6 is not known. This cytokine is probably released early in the cascade of host mediators after injury (Cipolle et al., 1993). Some studies illustrate that circulating IL-6, is increased immediately after blunt trauma in animals (Ayala, Wang, Chaundry, 1991; Cipolle, Pasquale, Shearer, Caldwell, Cerra, 1994; Pullicino, Carli, Elia, 1990) and humans (Hoch, Rodriguez, Abraham, 1993). This cytokine is released rapidly into the circulation in response to injury or infection, and, in particular, to circulating endotoxin. Interleukin-6 may be primarily beneficial to the host as it enhances immune function and hepatic, acute-phase, protein synthesis.

## **Tumor Necrosis Factor**

Tumor necrosis factor appears to be an important mediator in the pathogenesis of SIRS and MODS. Its production is stimulated by trauma, inflammation, burn, and endotoxin. Circulating levels of TNF are rapidly cleared, with a half-life of approximately 15 minutes. Although increases in TNF peak early, the response of this cytokine may persist due to the activation of secondary cascades such as the stimulation of endothelial cells or white cells release of oxidants and proteases (Demling et al., 1992). Unlike IL-6, there is evidence that suggests TNF levels do not rise after blunt trauma (Baggiolini et al., 1989; Rot, 1992; Rush, Sori, 1988; Stylanos et al., 1991). Other studies, however, report that after an endotoxin or bacterial challenge, TNF rather than IL-6 appears to rise and peak well before other potential mediators (Baggiolini et al., 1989; Deitch, 1992). In rats, rabbits, and baboons, TNF

levels increase within minutes after bacterial infusion (Fong, Moldawer, Shires, Lowry, 1990). Tumor necrosis factor also appears to attenuate the appearance of circulating IL-6 that is normally seen in severe bacteremia (Stylanois et al., 1991).

### **TxB2 and 6-Keto PGF<sub>1alpha</sub>**

Furthermore, eicosanoids such as thromboxane B2 (TxB2) and 6-keto-prostaglandin F<sub>1alpha</sub> (PGF<sub>1alpha</sub>), markers of endothelial activation, are thought to be involved in the immune response to trauma. The eicosanoid response to shock, however, varies among species and the relationship between cytokine peptides and eicosanoids is complex and variable (Cipolle et al., 1993). One study reports that these eicosanoids rise after blunt injury (Cipolle et al., 1994) whereas others do not (Butler, Wise, Halushka, 1983; Goldblum, Wu, Tai, 1986).

Cytokines and other markers of immune response that follow various sorts of traumatic insult are integral to normal homeostasis and to the response to injury. In moderate quantities, these proteins are involved in myelostimulation, activation of immune cells and reprioritization of metabolic processes to combat injury (Stylanois et al., 1991). Large quantities or prolonged production of these cytokines, however, may be detrimental and even fatal to the organism. Increases in IL-6 and PGF<sub>1alpha</sub> levels correlate with a high mortality rate (Demling et al., 1992), implicating they may be important in the development of MODS. Organ dysfunction, therefore, may result from an unchecked host response rather than from an unchecked inciting injury.

These experiments model a previous set performed at the University of

Minnesota Medical School which involved a femur-fracture degloving model and the use of four experimental groups (Cipolle et al., 1994). Initial experiments were conducted in perfusate void of white blood cells (WBCs), without hindlimb fracture, group 1 (-INJ/-WBC); perfusate void of WBCs with hindlimb fracture, group 2 (+INJ/-WBC); perfusate containing activated WBCs without hindlimb fracture, group 3 (-INJ/+WBC); perfusate containing WBCs with hindlimb fracture, group 4 (+INJ/+WBC). Tumor necrosis factor and TxB2 were measured in all four groups while  $\text{PGF}_{1\alpha}$  and IL-6 were measured in groups 3 and 4. Initial perfusions in the -WBC hindlimbs indicated no change in TNF release after injury. The TxB2 level increased during perfusion irrespective of injury. The  $\text{PGF}_{1\alpha}$  was elevated at 80 minutes in both groups 3 and 4, however at 20 minutes, these levels were higher in group 4 compared with group 3. The IL-6 level was significantly elevated at 80 minutes in group 4 but not in group 3. These initial experiments revealed that IL-6 is an important early mediator which is most-likely released directly from the endothelium of injured tissue that is well perfused.

Even though endothelial cells are probably somewhat activated by the preparation itself, blunt injury evokes the early release of IL-6 in the presence of WBCs (Cipolle et al., 1994). This leads to the experiment at hand which should further examine whether the rise in IL-6 after injury is dependent on injury or the presence of WBCs. The goal of these experiments, therefore, is to further delineate the early cytokine response to a fracture/crush injury.



## HYPOTHESES

Based on the previous experiments performed in the presence of WBCs, it is hypothesized that in the hindlimb perfused without WBCs:

- 1) IL-6 secretion increases as a result of blunt injury.
- 2) TNF secretion does not increase as a result of blunt injury.
- 3) TxB2 and PGF<sub>1alpha</sub> increase as a function of blunt injury, indicating endothelial cell activation. Although eicosanoids are augmented somewhat by the perfusion model itself, injury should further increase TxB2 and PGF<sub>1alpha</sub>.

By examining the role of these cytokines and eicosanoids in response to injury, therapeutic options may be created which are aimed at preventing or limiting the development of physiologic abnormalities early after injury that induce secondary organ failure. Multiple organ dysfunction syndrome presents the next, but surely not the last, obstacle that must be overcome in order to improve survival in the critically ill surgical patient (Deitch, 1992).

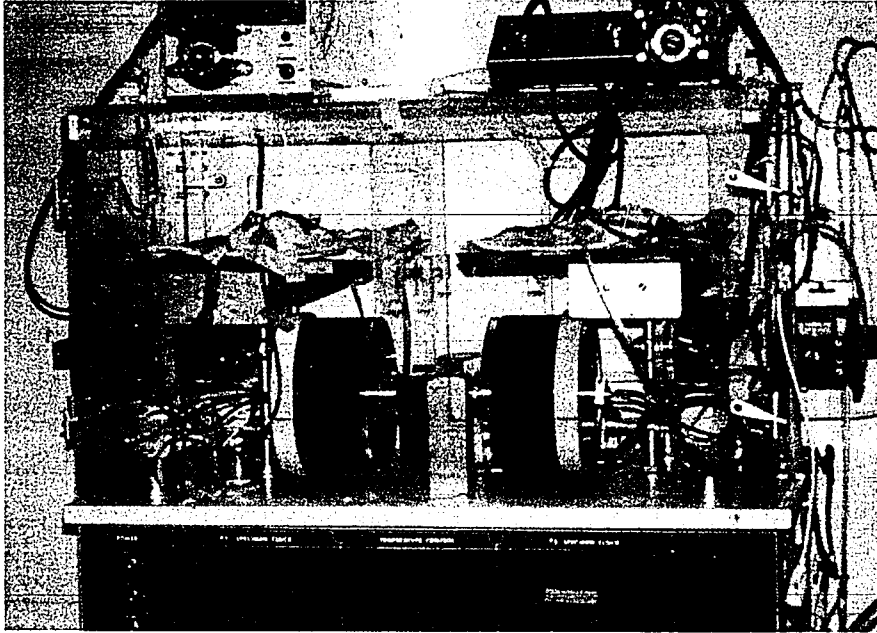
## METHODS

**Subjects:** Male Sprague-Dawley rats weighing 250 to 450 grams each were supplied by Charles River Laboratories (Wilmington, MA). Animals were housed in the Central Animal Facility and were maintained on a 12/12 light/dark cycle. They were offered water and rat chow ad libitum. All animals were anesthetized with 50 mg/kg sodium pentobarbital via an intraperitoneal injection and were supplemented with additional anesthetic, if needed. Animals were placed supine on a dissecting tray

located in the perfusion apparatus (see Figure 3, page 12) so that the hindlimbs were away from the operator and were administered a gas mixture of 95% Oxygen / 5% Carbon Dioxide (supplied by Blue Valley Gases) by face mask. The Plexiglas-encased chamber of the perfusion apparatus was maintained at 37 degrees Celsius throughout the duration of the experiment. The perfusion apparatus was custom built by the apparatus shop at Vanderbilt University (Tennessee). The unit allows for two animal perfusions simultaneously. It contains two dissecting trays, circular 500 ml rotating drums, a variable speed pump for each drum, oxygenator, bubble traps, and arterial and venous sampling ports (see Figure 3, page 12).

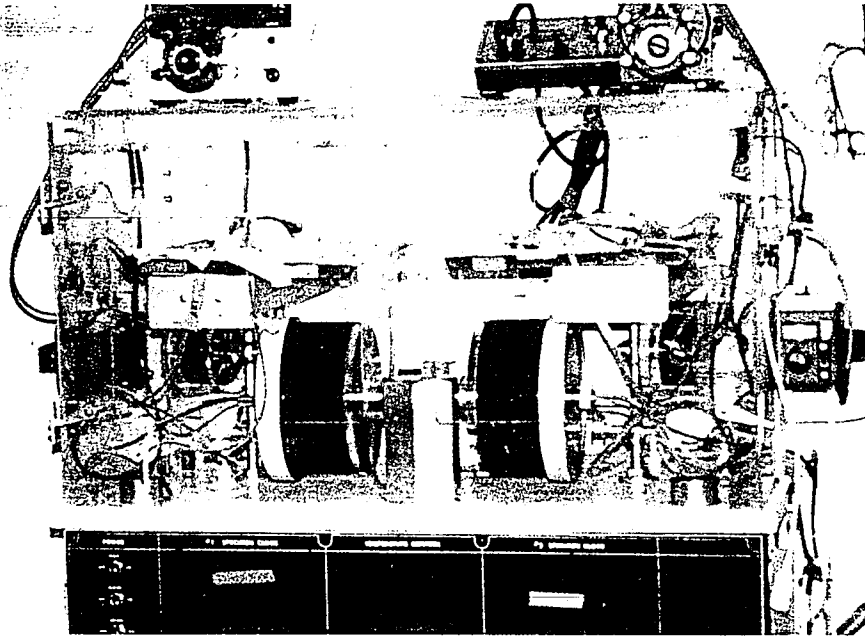
**Hindlimb Isolation:** The tail was tied with heavy silk. The animal was opened via a midline abdominal incision. The testicles were pulled intra-abdominally, ligated, and removed. The seminal vesicles, epididymal fat pads, and bladder were tied as a group and removed. The hypogastric vessels were exposed lateral to the base of the bladder and ligated bilaterally. The skin was dissected free from the external oblique muscle in the lower abdominal wall and the epigastric branches of the femoral vessels were ligated bilaterally. The arterial and venous supply to the external oblique muscle was then ligated. The proximal rectal and inferior mesenteric artery were ligated. The colon was transected and the peritoneal attachments

FIGURE 3



Perfusion apparatus designed by Vanderbilt University. All devices are housed in a Plexiglass unit with temperature controlled at 37 degrees Celsius.

FIGURE 3



Perfusion apparatus designed by Vanderbilt University. All devices are housed in a Plexiglass unit with temperature controlled at 37 degrees Celsius.

to the colon were divided up to the origin of the superior mesenteric artery. A silk tie was placed around the superior mesenteric and the celiac arteries. A silk tie was placed around the portal vein near its entrance to the liver. The animal was then heparinized via 0.2 ml heparin injection into the portal vein which was allowed to circulate for approximately 30 seconds before ligation of the mesenteric circulation. The animal was eviscerated. The iliolumbar, adrenal, and the renal arteries and veins were bilaterally ligated.

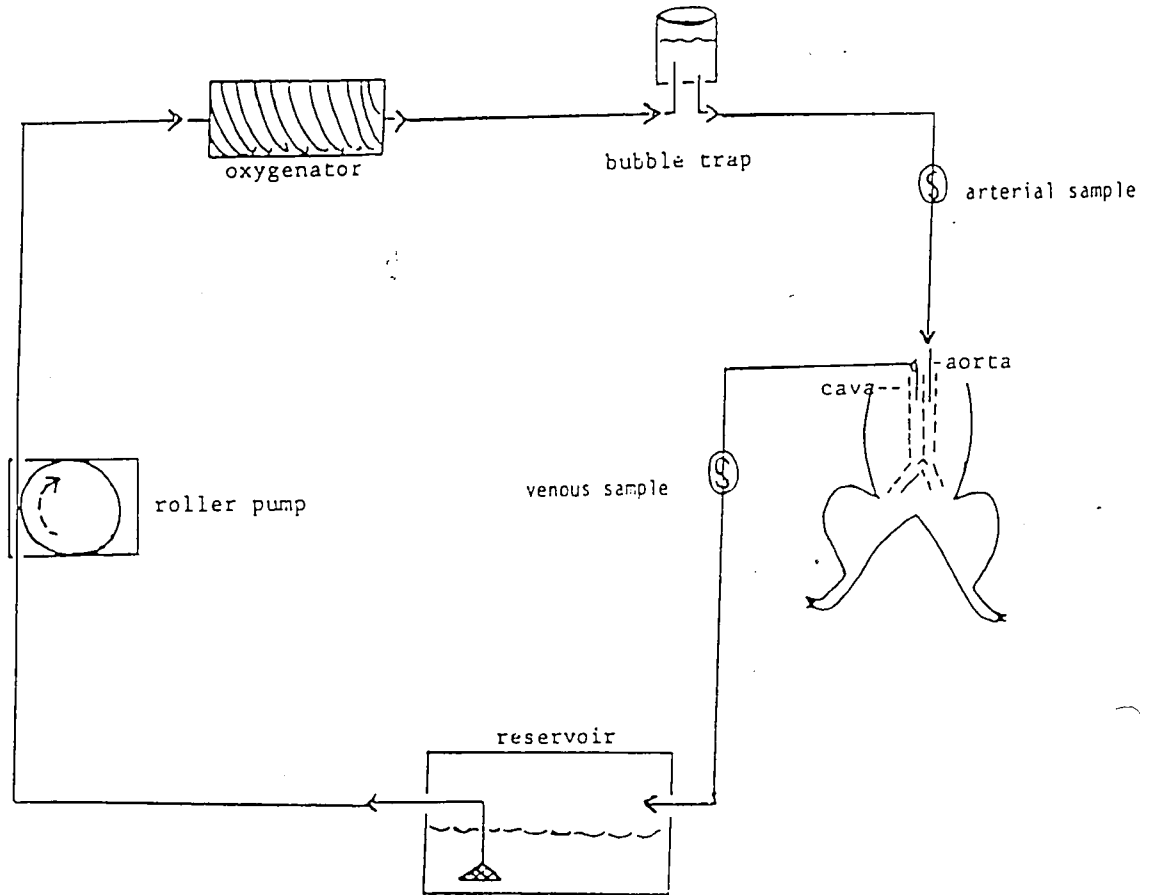
**Injury:** Animals in the "injured" group were administered a bilateral mid-femur fracture using two Kocher clamps. In addition, a 1x1 cm area of medial quadriceps muscle was crushed by a Kelley clamp for one minute.

**Cannulation:** The abdominal aorta and inferior vena cava were separated by blunt dissection. The aorta was then cannulated with an 18 gauge angiocatheter which was secured distally via a silk tie and perfusion was started at a flow of 10-12 ml/minute. The vena cava was cannulated using a 14 gauge catheter which was secured distally via silk tie. Both catheters were secured together with a large silk tie distally to assure that they were stable during the perfusion. The aorta and inferior vena cava were ligated proximal to the cannulation sites with a silk suture. The animal was then sacrificed via intracardiac injection of saturated potassium chloride. Flow rate was maintained at 10-12 ml/minute throughout the perfusion using a variable speed perfusion pump (supplied by Fisher, Pittsburgh, PA). The first five minutes of venous effluent was collected in a graduated cylinder in order to flush the animals blood volume and calculate the flow rate. This flush was discarded.

**Perfusion:** The animal was perfused with a modified Krebs-Henseleit buffer brought to a physiologic pH of 7.4. All chemicals for the buffer solution were reagent grade and were supplied by Sigma Chemical Company (St. Louis, MO). The buffer contained 10 mM glucose, 4 g/dl bovine serum albumin, washed, outdated, human red blood cells (supplied by Lehigh Valley Hospital Blood Bank, Allentown, PA), adjusted to a hematocrit of 30%, and a final concentration of 10% Travasol amino acid (supplied by Baxter Healthcare Corporation, Deerfield, IL). Perfusion was begun using 200 ml of perfusate in each reservoir drum. The perfusate was aerated with 95% oxygen / 5% carbon dioxide.

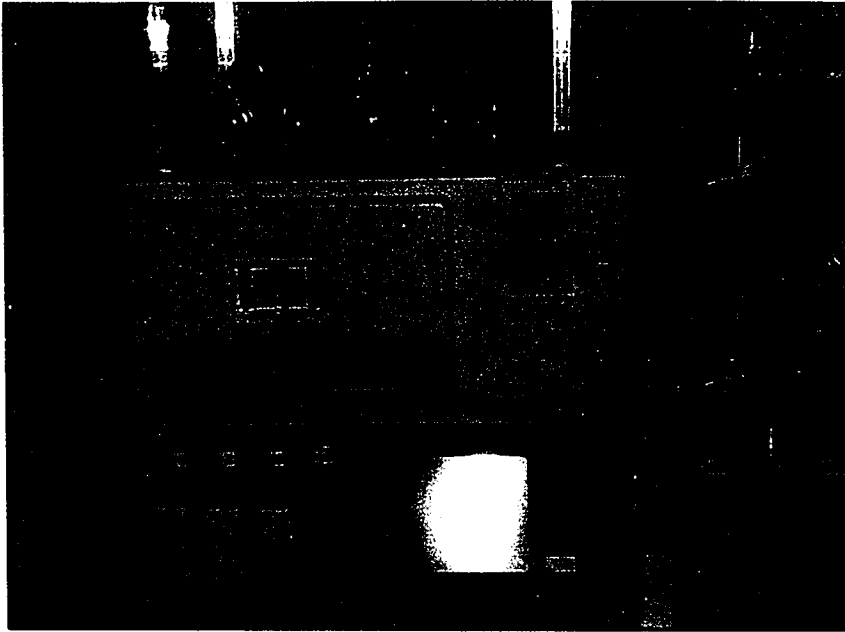
**Sample Collection:** After the animal was cannulated, the venous outflow from the first five minutes of perfusion was discarded in order to flush the hindlimb of endogenous blood and clotting factors. The venous outflow was then allowed to return to the drum, closing the system (see Figure 4, page 15). Arterial (0.5ml) and venous samples (5.0ml) were collected at 5, 20, and 80 minutes of perfusion. In addition, a baseline sample (5.0ml) was collected from the reservoir just prior to cannulating the animal. All samples were immediately placed on ice and arterial and venous blood gases were measured (Instrumentation Laboratory Blood-Gas Manager, see Figure 5, page 16) for determination of hindlimb oxygen consumption, and pH. Following collection of the 80 minute sample, one hindlimb was clamp-frozen with liquid-nitrogen, cut away, and stored at -70 degrees Celsius for future analysis of high energy phosphates, lactate, pyruvate, and water content. Venous samples were spun

**FIGURE 4**



A schematic of the isolated, perfused rat hindlimb preparation.

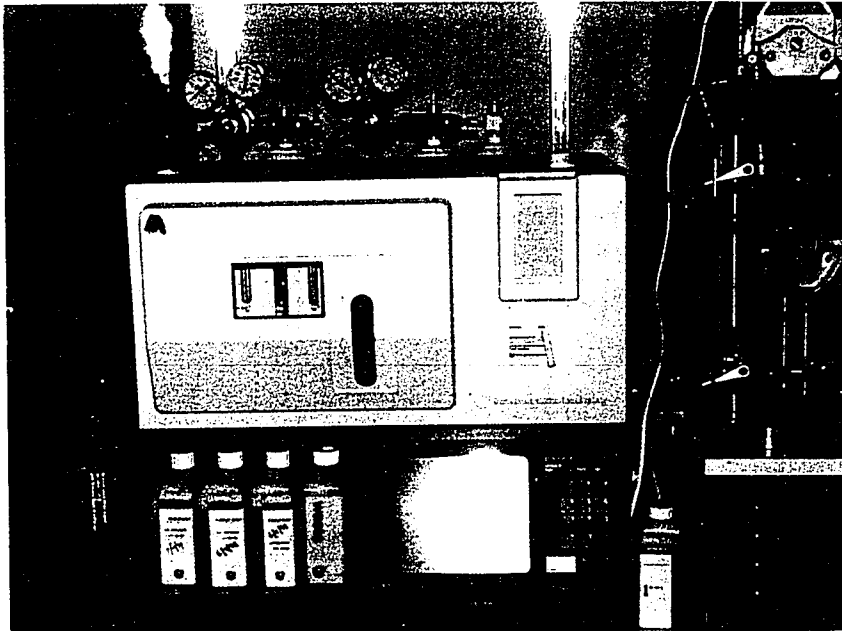
FIGURE 5



Blood-gas analyzer designed by Instrumentation Laboratories.



FIGURE 5



Blood-gas analyzer designed by Instrumentation Laboratories.

at 3800 rpm for 10 minutes at 0 degrees Celsius, aliquoted into multiple 0.2 ml samples and frozen at -70 degrees Celsius until analysis.

**Assays:** Venous samples were used to assess markers of metabolism, injury, and inflammation at each time point, 5, 20, and 80 minutes. Oxygen consumption and serial lactate concentrations were analyzed at each time point to assess the adequacy of limb perfusion. The venous samples were used to measure TNF and IL-6 (markers of early inflammation). Also, 6-keto-PGF<sub>1 $\alpha$</sub>  and TxB2 (markers of endothelial activation) were measured.

**Oxygen Delivery and Consumption:** Arterial and venous oxygen consumption were assessed using a blood gas analyzer (see Figure 5, page 16). Knowing the perfusate flow rate and hematocrit, oxygen delivery and consumption were calculated at each time point and corrected for 100g body weight.

**TNF:** This cytokine was measured with a cytotoxic bioassay using L929 murine fibroblasts. Cells were harvested and kept in 10% Fetal Calf Serum / Dulbecco's Modified Eagle's Medium (FCS/DMEM) (Sigma, St. Louis, MO) in 5% CO<sub>2</sub>. They were trypsinized, centrifuged, and plated in 96 well plates at approximately 50,000 cells per well per 0.01 ml in 10% FCS/DMEM and incubated for 46 hours. The assay was performed by developing a standard curve with serial dilutions of stock TNF (Genzyme, Cambridge, MA) in DMEM and adding 0.05 ml of standards (in triplicate) and 4 dilutions of perfusate samples using DMEM as diluent. Actinomycin D, 0.05 ml of 0.005 mg/ml was added to each well and plates were incubated at 37 degrees Celsius for 20 hours. Media was removed and cells were

washed with 0.2 ml of PBS per well. Cresyl violet staining was performed and cells were rewashed three times with PBS. Staining was read in the microplate reader at 590 nm and the weighted average of the 4 dilutions for each sample was calculated.

**IL-6:** This bioassay is based on the uptake of  $^3\text{HTdR}$  by dependent fibroblast cell lines. The B9 cell line was used to measure IL-6. Briefly, 0.1 ml of sample (4 dilutions) or standard was added to flat bottom wells. 0.05 ml of cells ( $0.4 \times 10^5$  cells/ml) were added to each well. Plates were incubated for 3 days at 37 degrees Celsius in 5% Carbon Dioxide. On day 3, cells were pulsed with  $^3\text{HTdR}$  (2uCi/well in 0.05 ml) for 6.5 hours, harvested and  $^3\text{HTdR}$  uptake measured.

**TxB2 and 6-keto  $\text{PGF}_{1\alpha}$ :** These eicosanoids were measured using commercial kits (Advanced Magnetics Inc., Cambridge, MA) by  $^3\text{H}$  RIA using magnetic, dextran coated charcoal to separate bound and unbound analyte. The assays used rabbit antibody,  $^3\text{H}$  tracer, and standard RIA separation and counting techniques.

**Statistical Analysis:** Approximately 8 animals were used per condition. All values are expressed as mean  $\pm$  SEM. Differences among groups, or among time points, were analyzed using repeated measures ANOVA. If differences between groups ( $p < 0.01$ ) were found, the data was further analyzed using a Student's Newman-Kuels post hoc multiple comparison analysis.

## RESULTS

There was no significant difference in oxygen consumption (Figure 6) or lactate levels (Figure 7) at any time point within any group indicating the hindlimbs were well-perfused.

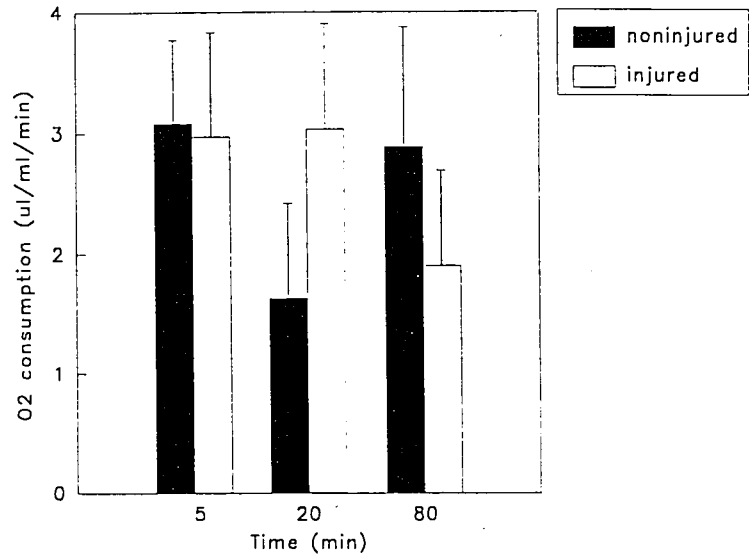


Figure 6. Oxygen consumption versus time of perfusion in each group. Oxygen consumption was corrected for rat weight by dividing by 100g body weight.

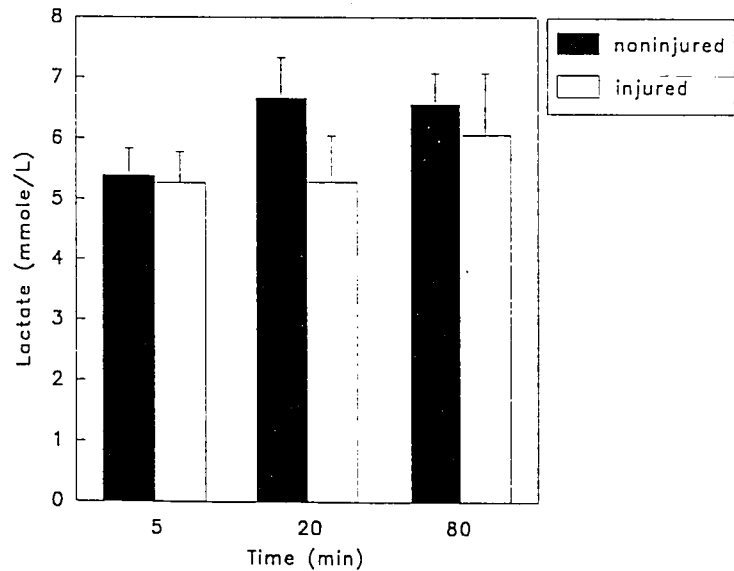


Figure 7. Lactate concentration in venous perfusate versus time of perfusion in each group.

Tumor necrosis factor was measured in both groups. Figure 8 shows that there was no significant change in TNF levels over the 80-minute perfusion in either group. The TNF levels were also checked in the baseline perfusate before cannulation and found to be no different than the values reported.

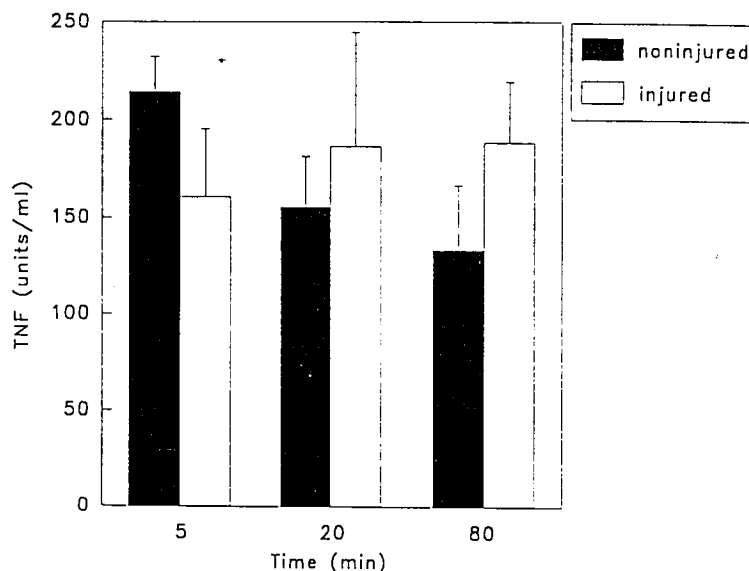


Figure 8. Tumor necrosis factor concentration in venous perfusate versus time of perfusion in each group.

As seen in Figure 9, IL-6 levels were significantly elevated at the 80-minute time point in both injured,  $F(2,18)=10.99$ ,  $p<0.01$ , and noninjured groups,  $F(2,12)=17.67$ ,  $p<0.01$ . The difference between injured and noninjured at 80-minutes was not significant.

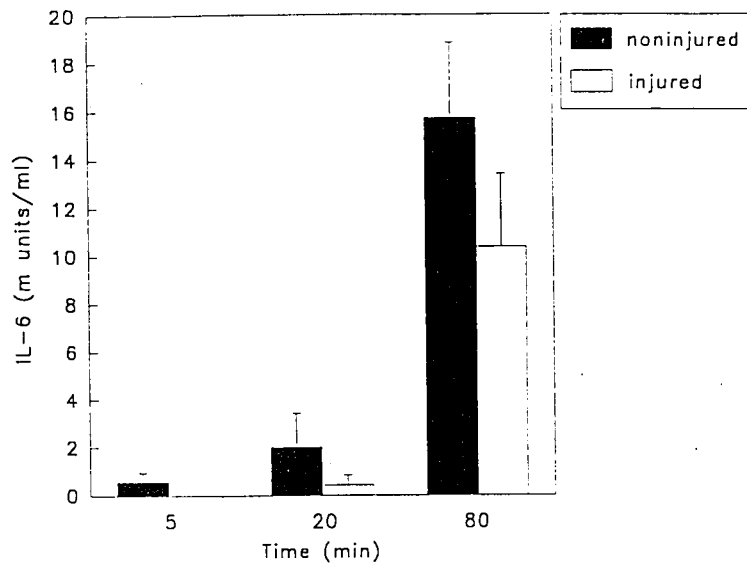


Figure 9. Interleukin-6 concentration in venous perfusate versus time of perfusion in each group.

In the injured condition, TxB2 levels were significantly increased at the 80-minute time point,  $F(3,26)=8.26$ ,  $p<0.01$  (Figure 10).

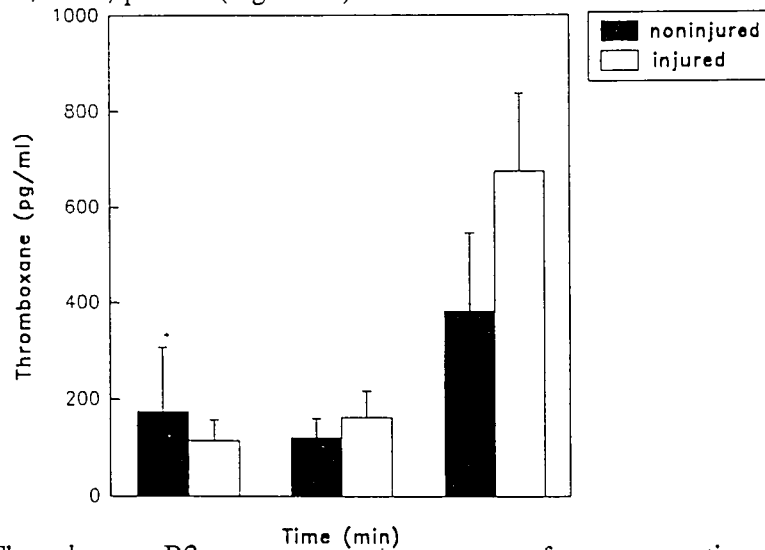


Figure 10. Thromboxane B2 concentration in venous perfusate versus time of perfusion.

Prostaglandin  $F_{1\alpha}$  levels increased in a step-wise manner in the control group but a similar pattern was not observed in the injured group (Figure 11). This increase, however, was not significant.

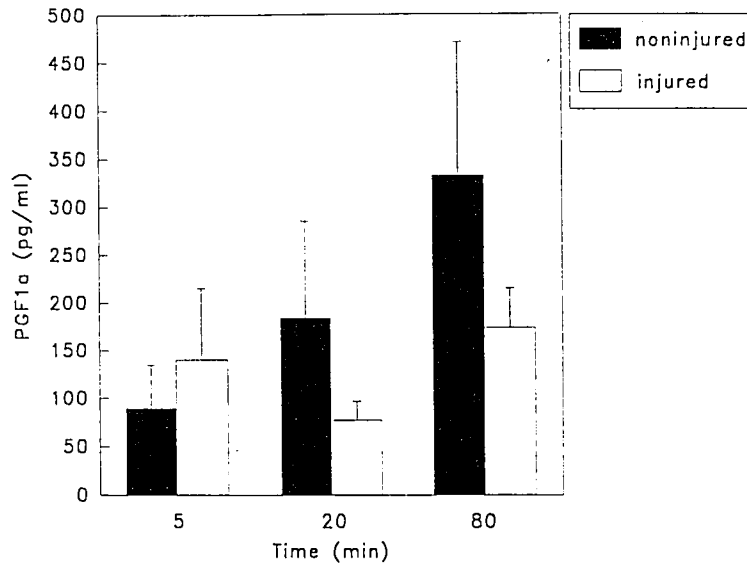


Figure 11. Prostaglandin  $F_{1\alpha}$  concentration in venous perfusate versus time of perfusion.

## DISCUSSION

These results are among the first to test the general hypothesis that significant soft tissue injury, in and of itself, contributes to the early onset of systemic inflammation. These experiments built upon those previously performed at the University of Minnesota Medical School (Cipolle et al., 1994) to further delineate the role of the white blood cell. The technique and model in these two set of experiments are the same. The model offers several advantages to general whole animal studies in that several factors that may contribute to the injury response in trauma were

controlled. That is, this model controls for flow rate, perfusion pressure, temperature, content of the perfusate, and the nature of the injury itself. Also, this model allows for an isolation of the injured limb from neuroendocrine inputs from other organs in the animal.

Several factors indicated that this preparation was stable and hindlimbs were adequately perfused. Oxygen consumption and lactate concentration were stable over time within groups. Lactate levels were similar to those reported previously in similar rat hindlimb perfusion models (Caldwell, Lacy, Exton, 1978; Cipolle et al., 1994; O'Connor, Scott, Mellick, 1982).

The crush fracture induced in these experiments did not augment hindlimb TNF release (Figure 8). It is possible that TNF release occurs early in the inflammatory response and was not detected by the 5-minute sample. This possibility is unlikely. The half-life of TNF is approximately 15 minutes therefore, it should have been detected by the 5 or even 20-minute samples. Also, baseline samples did not differ from the TNF levels in any group at any time point. It is possible, however, that TNF is released some time after the 80 minute time point of perfusion and the response was altogether missed.

It is more-likely that TNF is not an important mediator immediately after trauma. It has been reported that up to 24 hours post-injury, TNF levels did not rise in trauma patients with multiple injuries (Pullicino et al., 1990). Pigs sustaining hemorrhage and blunt thigh injury did not show significant increases in TNF up to 2 hours after injury (Stylanois et al., 1991). Finally, it has been shown in humans that



there are increases in TNF receptors after injury but no notable rise in TNF itself (Tan, Waxman, Scanelli, 1993).

The eicosanoid response was interesting. There was a great deal of variability in the thromboxane B2 assay suggesting that the results may not be indicative of the actual response. Thromboxane B2, a stable metabolite of thromboxane A2, and 6-keto Prostaglandin F<sub>1 $\alpha$</sub> , a stable metabolite of prostacyclin (PGI<sub>2</sub>), are generally thought to have opposing hemodynamic and hematologic action (Cipolle et al., 1994). Stimulated endothelial cells are an important source of these compounds (Cipolle et al., 1994; Guidet, Staikowsky, Offenstadt, 1992). It appeared that, in the absence of white blood cells, the preparation allowed for the greatest amount of TxB2 release from the endothelial cells in the injured condition (Figure 10). However, the amount of PGF<sub>1 $\alpha$</sub>  release from the endothelial cells was highest in the control condition (Figure 11). The response of this eicosanoid followed a stepwise progression but this was only seen in the control condition. In the injured condition, PGF<sub>1 $\alpha$</sub>  levels dropped at the 20-minute perfusion time-point but then rose. These results may indicate that the endothelium was activated by the preparation itself.

Figures 10 and 11 show the eicosanoid release patterns for the preparation performed in the absence of WBCs. It appears that the preparation itself activates the release of PGF<sub>1 $\alpha$</sub> , a prostacyclin precursor. Prostacyclin is generally thought of as a "good" prostaglandin, promoting vasodilation while inhibiting platelet aggregation. It appears that injuring the hindlimb shunts this prostacyclin response. Furthermore, TxB2 levels seem to be stable throughout the perfusion in the control animals.

However, when the hindlimb is injured, the release of this "bad" prostaglandin which promotes vasoconstriction and platelet aggregation is augmented. These results may be supporting the opposing action of these eicosanoids.

Several studies reveal IL-6 as an early mediator after trauma. It is released shortly after burn injury (Nijtsen, DeGroot, TenDiustt, 1989; Rodriguez, Miller, Remick, 1993), and elective surgery and trauma in humans (Pullicino et al., 1990). In this set of experiments, IL-6 was significantly increased at the 80-minute perfusion time point in the hindlimb preparation void of white blood cells (Figure 9). It is interesting that TNF release was not elevated significantly as was IL-6. Several studies, that examined TNF and IL-6, show similar results to those found in this set of experiments. While failing to report increases in TNF release, Pullicino et al. (1990) reported a significant increase in IL-6 within 4 to 6 hours after injury. Ayala et al. (1991) demonstrated an elevation in TNF 45 minutes after hemorrhage in rats, however, IL-6 levels were already elevated before hemorrhage. Hoch et al. (1993) reported augmented IL-6 levels without any detectable circulating concentrations of IL-1 or TNF after accidental injury in humans. The results from this series of experiments involve the same pattern: no significant increase in TNF secretion with increases in IL-6 concentration.

With adequate oxygen perfusion, blunt injury augmented IL-6 release in the isolated rat hindlimb perfused without WBCs. The model implemented in these experiments controlled for potential confounding variables to the inflammatory response. Furthermore, the injured limb was isolated from the rest of the circulation,

implying that this increase in IL-6 came directly from the hindlimb.

In the hindlimb perfused with WBCs (Cipolle et al., 1994), the cytokine response was enhanced even more so than that seen in the hindlimb perfused without WBCs. The 80-minute IL-6 levels were significantly less in the injured hindlimbs compared to injured hindlimbs perfused with activated WBCs,  $F(2,18)=10.99$ ,  $p<0.01$  (INJ-WBCs) versus  $F(2,18)=9.32$ ,  $p<0.01$  (INJ+WBCs). The actual IL-6 concentrations at 80-minutes were 10.34 milliunits/ml for the INJ-WBCs condition and 40.85 milliunits/ml for the INJ+WBCs condition.

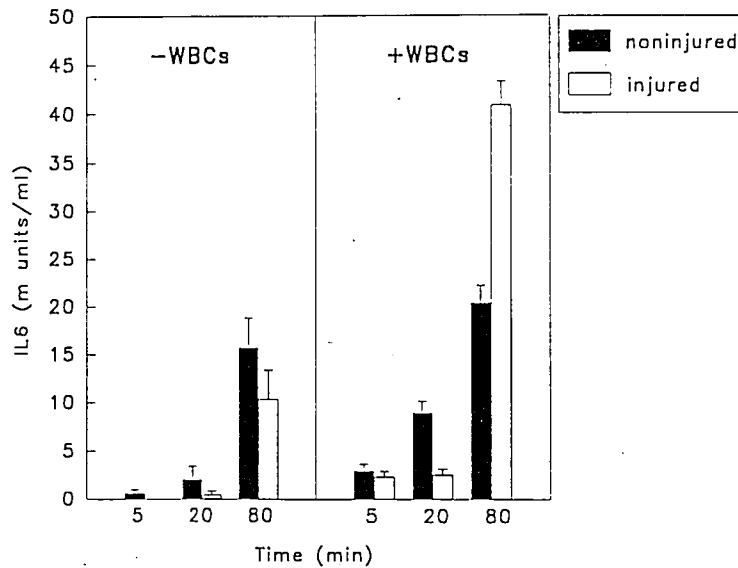


Figure 12. IL-6 concentration in perfusate void of WBCs and perfusate containing WBCs.

The only significant difference in these experiments is the type of injury administered to animals. The earlier set, perfused in the presence of WBCs, involved a less severe femur-fracture degloving injury whereas animals in these experiments experienced a mid-femur fracture with a one minute muscle crush injury. Most-interestingly, even with a less severe injury, experiments performed in the presence of WBCs had a

substantially greater amount of IL-6. Interleukin-6 release was augmented at 80-minutes of perfusion, however, blunt injury did not alter this response in isolated rat hindlimbs perfused without WBCs. Since IL-6 was markedly increased in injured hindlimbs perfused with WBCs, it can be concluded that despite activation of the endothelial cell and an eicosanoid release pattern seen in other forms of stress<sup>28</sup>, IL-6 release after blunt injury is dependent on the presence of WBCs.

In summary, the isolated, perfused rat hindlimb appears to be a good model for examining the early response to injury. It represents a stable, well-perfused preparation that is isolated from the rest of the circulation. However, the preparation itself may be responsible for endothelial injury, thereby activating the release of prostaglandins. Further experiments are needed to fully comprehend the role of the endothelial cell in response to blunt injury. Currently, the pathophysiology of ischemia-reperfusion injury is being studied using this same isolated hindlimb model.

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