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Microvascular Changes Explain the "Two-Hit" Theory of Multiple Organ Failure

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Objective

The objective was to determine intestinal microvascular endothelial cell control after sequential hemorrhage and bacteremia.

Summary Background Data

Sepsis that follows severe hemorrhagic shock often results in multiple system organ failure (MSOF) and death. The sequential nature of this clinical scenario has led to the idea of a "two-hit" theory for the development of MSOF, the hallmark of which is peripheral vasodilation and acidosis. Acute bacteremia alone results in persistent intestinal vasoconstriction and mucosal hypoperfusion. Little experimental data exist to support the pathogenesis of vascular dysregulation during sequential physiologic insults. We postulate that hemorrhagic shock followed by bacteremia results in altered microvascular endothelial cell control of dilation and blood flow.

Methods

Rats underwent volume hemorrhage and resuscitation. A sham group underwent the vascular cannulation without hemorrhage and resuscitation, and controls had no surgical manipulation. After 24 and 72 hours, the small intestine microcirculation was visualized by *in vivo* videomicroscopy. Mean arterial pressure, heart rate, arteriolar diameters, and A1 flow by Doppler velocimetry were measured. Endothelial-dependent dilator function was determined by the topical application of acetylcholine (ACh). After 1 hour of *Escherichia coli* bacteremia, ACh dose responses were again measured. Topical nitroprusside was then applied to assess direct smooth muscle dilation (endothelial-independent dilator function) in all groups. Vascular reactivity to ACh was compared among the groups.

Results

Acute bacteremia, with or without prior hemorrhage, caused significant large-caliber A1 arteriolar constriction with a concomitant decrease in blood flow. This constriction was blunted at 24 hours after hemorrhage but was restored to control values by 72 hours. There was a reversal of the response to bacteremia in the premucosal A3 vessels, with a marked dilation both at 24 and 72 hours. The sequence of hemorrhage and *E. coli* resulted in a progressive enhanced reactivity to the endothelial-dependent stimulus of ACh in the A3 vessels at 24 and 72 hours. Reactivity to endothelial-independent smooth muscle relaxation and subsequent vessel dilation was similar for all groups.

Conclusions

These data indicate that there is altered endothelial control of the intestinal microvasculature after hemorrhage in favor of enhanced dilator mechanisms in premucosal vessels with enhanced constrictor forces in inflow vessels. This enhanced dilator sensitivity is most evident in small premucosal vessels. This experimental finding supports the premise that an initial pathophysiologic stress alters the subsequent microvascular blood flow responses to systemic inflammation. These changes in the intestinal microcirculation are in concert with the "two-hit" theory for MSOF.

Since its initial description in 1975,[1] the syndrome of multiple system organ failure (MSOF) has been recognized as a common cause of morbidity and death in the surgical intensive care unit after traumatic injury. Several theories for the etiology of this syndrome have been proposed.[2] Currently, a popular theory is the "two-hit" concept of MSOF.[2–5] This model of MSOF holds that a series of sublethal pathophysiologic insults (hits) combine to initiate

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the hemodynamic and metabolic state characteristic of MSOF.[6] The first hit is usually hemorrhage, associated with trauma or major surgery, but it could also be a thermal injury, soft-tissue necrosis, or invasive infection. The first insult is believed to result in a priming of certain aspects of the inflammatory response (*i.e.*, macrophages, neutrophils). The initial changes might also lead to a relative immunosuppressive state by impairing certain aspects of T-cell function,[7–9] thus rendering the host more susceptible to a secondary challenge. Once a second insult stimulates these primed or suppressed cells, an inappropriate inflammatory response is activated. The usual trigger for the second "hit" is thought to be infectious but might instead be a hypoxic or hypotensive event. Stimulated macrophages might initiate the cytokine cascade (interleukin-1, tumor necrosis factor, and interleukin-6) unopposed by downregulation, and stimulated neutrophils may lead to oxidant damage. According to the two-hit theory, this enhanced inflammatory response leads to further tissue damage, hemodynamic compromise, and organ dysfunction.

Although there is little direct clinical evidence, experience suggests that the two-hit model makes sense. Patients often develop MSOF remote from the initial insult. Often this is preceded by sepsis, hypotension, or hypoxia.[2,3] Levels of proinflammatory cytokines are frequently elevated in these patients and are associated with the development of MSOF.[10–14] In animal models, blockade of these proinflammatory mediators can abrogate some of the pathophysiologic changes associated with sepsis,[15] but to date human studies with a variety of "antimediators" have been disappointing.[16,17] Recent studies have suggested that the addition of certain therapies at the time of the initial hit might prevent some of the subsequent inflammatory responses.[18–20]

The clinical hallmark of sepsis associated with the initial stages of MSOF is the hyperdynamic cardiac response and a marked decrease in peripheral vascular tone.[2] Excessive production of nitric oxide has been implicated in these vascular responses.[21,22] Nitric oxide (NO) is produced by two classes of enzymes: a constitutive NO synthase and an inducible NO synthase. Both are present in vascular endothelial cells and thus are involved in vascular control. Proinflammatory mediators, which are initiated by the first hit and hypoxia and reoxygenation, are potent stimulators for the production of inducible NO synthase. Thus, the first hit, in addition to priming inflammatory cells, may lead to an enhanced ability of endothelial cells to produce NO. Once activated, endothelial cells could alter vascular tone and the response to a second hit, thus mediating organ blood flow redistribution. A redistribution of nutrient blood flow has been demonstrated in the liver and kidney in several animal models of injury and sepsis,[23–26] and NO has been implicated in some of these phenomena.[24,27] A role for the endothelial cell in priming and dysfunction after trauma or sepsis has been investigated. However, these studies have used aortic rings[28] and have not studied the primary resistance arterioles in the microcirculation.

Acute bacteremia in normal rodents appears to impair both endothelial-dependent and smooth muscle function in the intestinal microcirculation.[29] However, the two-hit concept of microvascular endothelial cell priming has not been investigated. The hypothesis for this study is that hemorrhagic shock and resuscitation alters microvascular endothelial cell control in the intestine to a subsequent bacteremia. We believe these changes contribute to the hyperdynamic cardiovascular state noted during the initial developmental stages of MSOF.

METHODS

Hemorrhage Protocol

Male Sprague-Dawley rats (180 to 220 g) were acclimated for 2 weeks in an approved animal-care facility before protocol entry. Animal care and experimentation conformed to National Research Council guidelines. Food but not water was withheld overnight before initiation of the hemorrhage. Under sodium pentobarbital (50 mg/kg given intraperitoneally) anesthesia, the femoral vein was cannulated under sterile conditions. While breathing spontaneously, animals then underwent a fixed-volume hemorrhage by the initial withdrawal of 2.5 ml blood (more than 10 minutes) into a syringe that had been rinsed with heparin (1000 units/ml). An additional 0.5 ml blood was withdrawn over the next 10 minutes, with no other intervention for the ensuing 40 minutes. Sixty minutes after the start of hemorrhage, resuscitation was begun with the shed blood (3 ml)

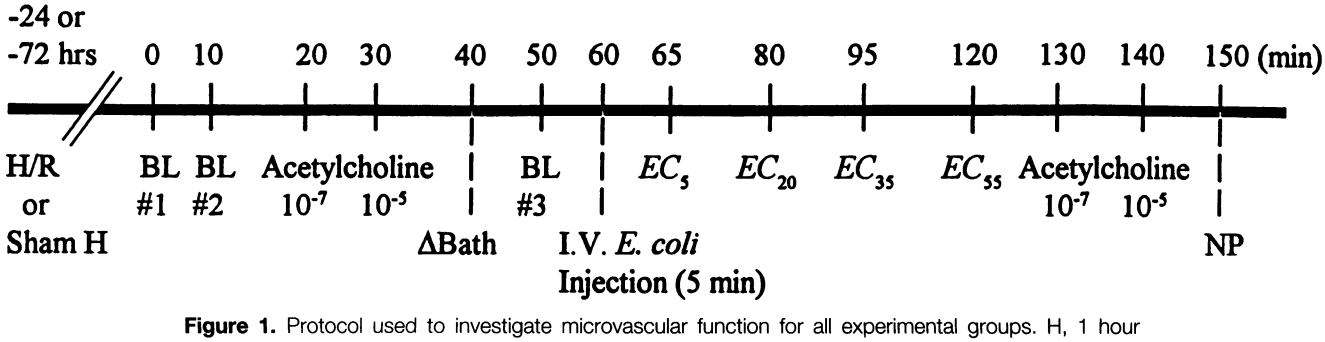

Figure 1. Protocol used to investigate microvascular function for all experimental groups. H, 1 hour hemorrhage; R, resuscitation; NP, sodium nitroprusside; ACh, acetylcholine; BL, baseline; *EC, E. coli.*

Table 1. BASELINE MEASUREMENTS*

Variable	Control (n = 5)	Sham *E. coli* (n = 6)	24 hr *E. coli* (n = 5)	72 hr *E. coli* (n = 6)
A1 diameter (μm)	87.5 ± 3.1	83.5 ± 5.5	75.9 ± 5.0	88.7 ± 6.9
A3P diameter (μm)	15.5 ± 1.1	11.7 ± 0.5	13.7 ± 0.6	11.8 ± 0.7
A3D diameter (μm)	7.2 ± 0.3	7.9 ± 0.5	6.5 ± 0.2	7.5 ± 0.6
A1 flow (nL/sec/100 g)	11.1 ± 0.6	9.5 ± 1.9	7.5 ± 1.7	12.3 ± 2.0
Cardiac output (mL/min/100 g)	72.8 ± 2.4 (n=3)	81.3 ± 7.2 (n=3)	—	77.4 ± 8.6 (n=4)
Mean arterial blood pressure (mmHg)	113.0 ± 2.0	106.2 ± 2.3	112.2 ± 1.9	105.1 ± 1.6

* Data are mean ± standard error of the mean. There were no differences between groups by analysis of variance and Tukey-Kramer Honestly Significant Difference test. Sample sizes for groups are indicated under column headings for all measurements except cardiac output where the sample sizes are indicated beneath the measured values.

plus two volumes of saline (6 ml) over 60 minutes. The cannula was removed, the femoral vein ligated, and the skin incision closed. Sham hemorrhage rats were prepared in a similar fashion, but no blood was withdrawn or saline delivered during this 2-hour period. All animals were then allowed to recover with free access to food and water.

General Animal Preparation

Twenty-four or 72 hours after hemorrhage and resuscitation, the rats were anesthetized with intraperitoneal urethane (800 mg/kg) and chloralose (60 mg/kg). Supplemental doses of urethane (25% of the initial dose) were given subcutaneously every 30 minutes, as needed, to maintain a surgical plane of anesthesia throughout the experiment. During preparation and experimentation, the body temperature was monitored with a rectal probe and regulated at 37° ± 0.5°C, using a back heating pad and a temperature feedback controller. A tracheostomy was performed and the animals spontaneously breathed room air. The left femoral vein and artery were cannulated for the infusion of bacteria and hemodynamic monitoring of mean arterial pressure and heart rate. An aortic thermistor was placed at the aortic root via right carotid artery cannulation and the right jugular vein was cannulated to allow measurement of cardiac output (CO).[30] Transpulmonary thermodilution CO measurements were made by the bolus injection of 40 μl of room-temperature saline into the jugular vein. Measurements were done in triplicate for each experimental time point and averaged.

Microcirculation Preparation

A standard small intestine preparation was used, as previously described.[31–33] Briefly, the terminal ileum was exteriorized through a right paramedian incision with an intact neurovascular supply and suspended in a tissue bath over an optical port. The tissue bath was continuously monitored and maintained at physiologic temperature (37°C ± 0.5°C), pH (7.40 ± 0.05), PO_2 (30 to 50 mm Hg), and pCO_2 (35 to 45 mm Hg) with a modified Krebs solution (25.5 mM $4NaHCO_3$, 112.9 mM NaCl, 4.7 mK KCl, and 2.55 mM CaCl $2H_2O$). Isoproterenol hydrochloride was added to decrease peristalsis at a concentration (1×10^{-8} g/ml) 1000 times less than that that affects small intestinal microvascular diameters or reactivity.[31]

Animals were then transferred to the stage of a triocular microscope and positioned for transillumination of the ileal segment through the optical port. The images were transmitted by a closed-circuit videocamera to a monitor and videocassette recorder through a lens system. Microvascular anatomy was defined according to branch order. First-order arterioles (A1) are inflow vessels that originate from the

Table 2. MAXIMUM PERCENT CHANGE FROM BASELINE FOLLOWING *E. COLI* INFUSION*

Variable	Control (n = 5)	Sham *E. coli* (n = 6)	24 hr *E. coli* (n = 5)	72 hr *E. coli* (n = 6)
A1 diameter	−12.3 ± 1.9	−15.2 ± 1.6	−8.7 ± 3.5†	−16.8 ± 3.6
A3P diameter	−9.94 ± 2.7	−12.4 ± 2.4	15.9 ± 11.2†	10.5 ± 7.2†
A3D diameter	−6.1 ± 1.8	−7.6 ± 4.1	3.6 ± 4.1†	20.6 ± 3.5†
A1 flow	−21.5	−18.9	−17.1	−31.7
Cardiac output	12.0 (n = 3)	12.6 (n = 3)	—	3.6 (n = 4)

* Data are mean maximum percent change from baseline values observed during the post-*E. coli* period (A1, A3P, and A3D diameters are expressed as mean ± standard error of the mean). Sample sizes for groups are indicated under column headings for all measurements except cardiac output where the sample sizes are indicated beneath the measured values.

† $p < 0.05$ *vs.* Sham *E. coli* by analysis of variance and Tukey-Kramer Honestly Significant Difference test.

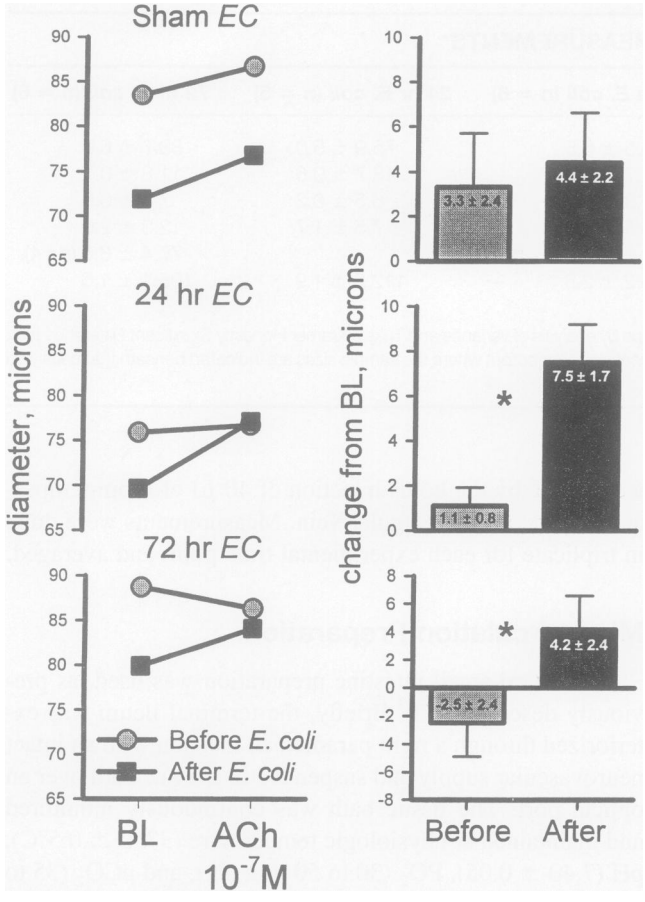

Figure 2. A1 arteriolar diameters before and after intravenous *E. coli* infusion in response to the application of ACh to the small intestine (circle, before *E. coli*; square, after *E. coli*). The bar graph on the right shows the change in diameter in microns from the appropriate baseline value. *p < 0.05 before *E. coli vs.* after *E. coli* by paired t test.

mesenteric arcade arteries. They penetrate the outer muscular layers of the bowel into the submucosa, where they give rise to second-order arterioles (A2), which run longitudinally in the submucosa. Third-order arterioles (A3) are right-angle branches of A2 that supply the mucosa as terminal central villus arterioles. Arteriolar diameters were measured using on-line video calipers. Ten to 15 multiple diameter measurements were taken for each experimental point. Centerline red blood cell velocity was measured on-line with an optical Doppler velocimeter, and microvascular blood flow was calculated by the equation: Flow = (V/0.6) × (R^2) (0.0001). Only vessels exhibiting resting vascular tone and vasomotion were included in the analysis.

Bacteremia Model

Acute bacteremia was induced by the infusion of live *Escherichia coli* (6×10^{-8} colony-forming units per 100 g body weight over 5 minutes), which has consistently produced a hyperdynamic state with elevated cardiac output in previous experiments.[26,29,34]

Experimental Protocol

The general experimental timeline is shown in Figure 1. One hour was allowed for hemodynamic and microvascular equilibration from the surgical preparation. After the equilibration period, baseline measurements were made on all animals. At baseline and each experimental time point, mean arterial pressure, CO, heart rate, A1, A3 proximal, and A3 distal vessel diameters, and A1 flow were measured. Animals were considered stable when measured parameters were within 10% of the initial baseline readings. Before and 1 hour after bacteremia, endothelial-dependent microvascular function was measured by the topical addition of acetylcholine (ACh) to the tissue bath for a final bath concentration of 10^{-7} M and 10^{-5} M. Sodium nitroprusside (10^{-4} M), a direct NO donor, and vascular smooth muscle dilator were added to the tissue bath of all preparations at the conclusion of each experiment to determine dilator capacity. Five minutes after the addition of each of these dilator agonists, all measured variables were recorded and the change from the preagonist baseline was calculated. These calculated changes allow

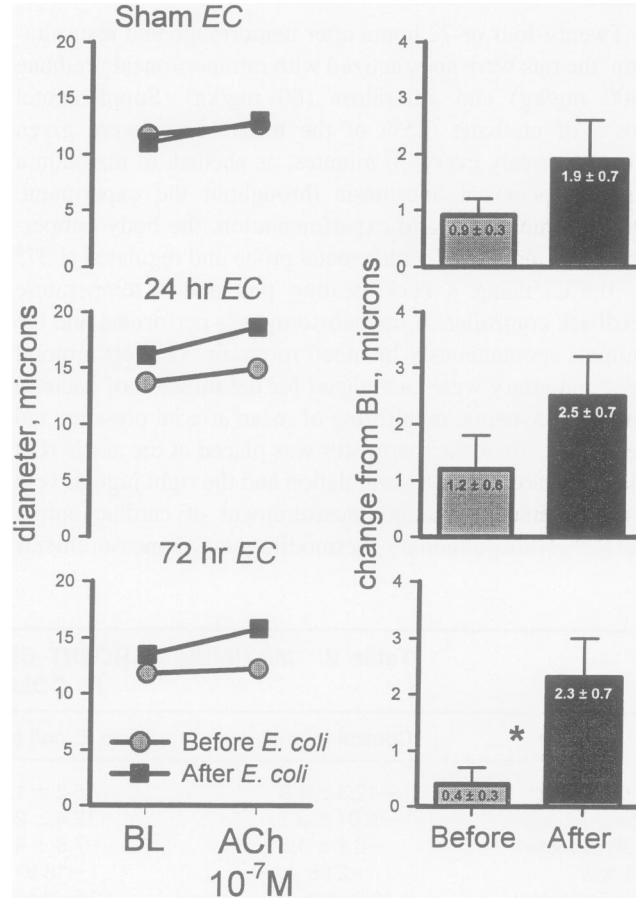

Figure 3. Proximal A3 arteriolar diameters before and after intravenous *E. coli* infusion in response to application of ACh to the small intestine (circle, before *E. coli*; square, after *E. coli*). The bar graph on the right shows the change in diameter in microns from the appropriate baseline value. *p < 0.05 before *E. coli vs.* after *E. coli* by paired t test.

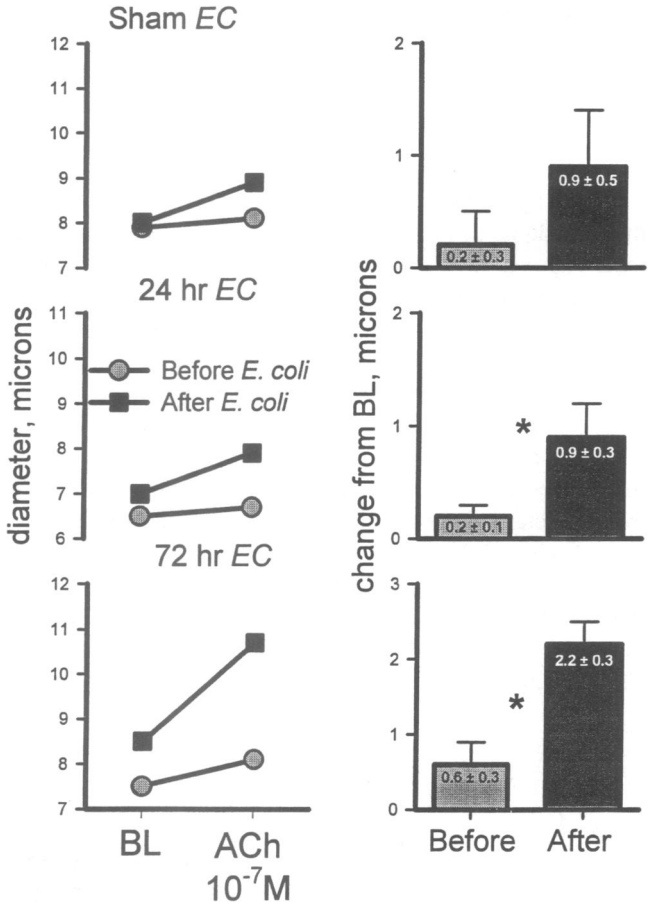

Figure 4. Distal A3 arteriolar diameters before and after intravenous *E. coli* infusion in response to application of ACh to the small intestine (circle, before *E. coli*; square, after *E. coli*). The bar graph on the right shows the change in diameter in microns from the appropriate baseline value. *p < 0.05 before *E. coli vs.* after *E. coli* by paired t test.

microvessel reactivity to be compared among the experimental groups.

Experimental Groups

Animals were divided into four experimental groups. For each animal within each group, microvascular function was measured before and after bacteremia, allowing each animal to serve as its own control. The first group (n = 5) were naïve animals that had no hemorrhage manipulation before microvascular study. Group II (n = 6) underwent sham hemorrhage and microvascular study 72 hours later. Groups III and IV underwent hemorrhage and resuscitation followed by microvascular studies at 24 hours for group III (n = 5) and 72 hours for group IV (n = 6).

Data Analysis and Statistics

To control for differences in starting diameters and resting tone between groups, the response to bacteremia was measured as the maximum percent change from the prebacteremic baseline: (maximum diameter change − baseline diameter)/(baseline diameter) × 100. The response to ACh was determined by measuring the actual diameter change from the pre-ACh baseline (Change = Baseline diameter − ACh diameter). All data are presented as mean ± SEM. Differences among groups were determined by analysis of variance, and differences from baseline values were determined by analysis of variance for repeated measures. When differences were found, *post hoc* comparisons were made using the Student–Newman–Keuls test. A paired Student's t-test was used to compare diameter changes after ACh stimulation. The null hypothesis was rejected at $p < 0.05$.

RESULTS

Preliminary studies of the hemorrhage protocol demonstrated that a 3-ml blood withdrawal resulted in a mean arterial pressure of about 50% of baseline. Resuscitation with shed blood and 6 ml of normal saline then returned mean arterial pressure to baseline or above. Throughout the study with both preliminary and study animals, hemorrhage

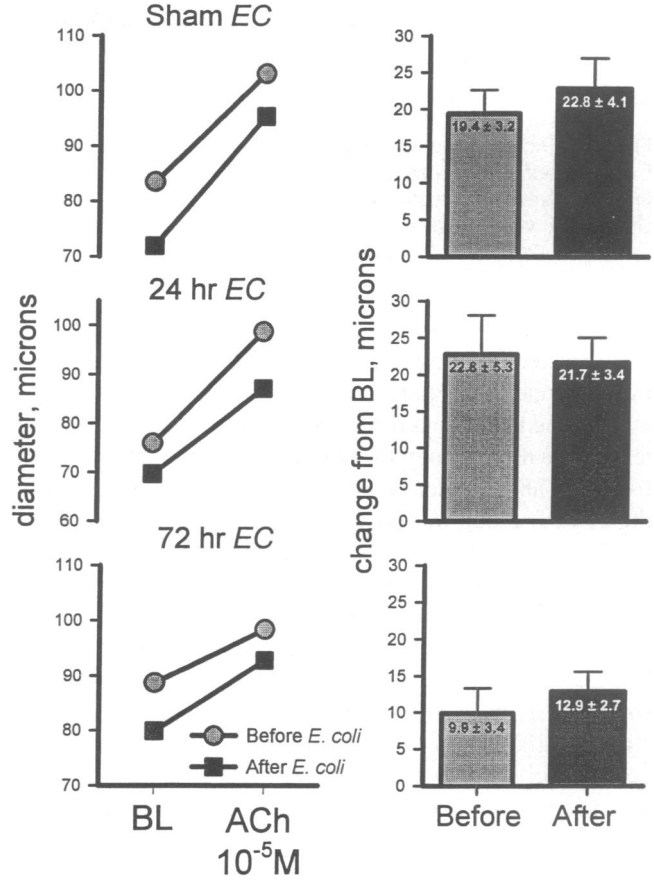

Figure 5. A1 arteriolar diameters before and after intravenous *E. coli* infusion in response to application of ACh to the small intestine (circle, before *E. coli*; square, after *E. coli*). The bar graph on the right shows the change in diameter in microns from the appropriate baseline value. *p < 0.05 before *E. coli vs.* after *E. coli* by paired t test.

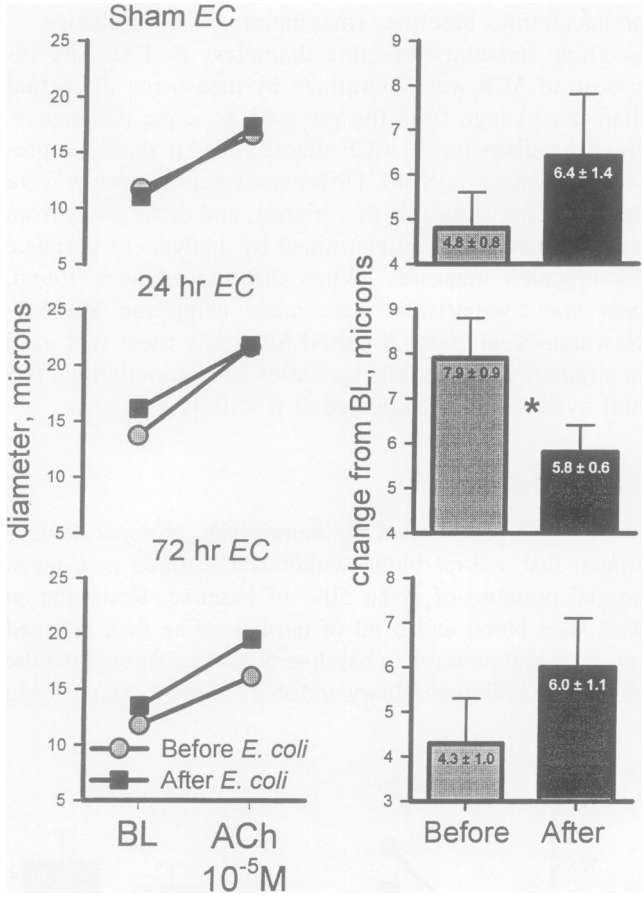

Figure 6. Proximal A3 arteriolar diameters before and after intravenous *E. coli* infusion in response to application of ACh to the small intestine (circle, before *E. coli*; square, after *E. coli*). The bar graph on the right shows the change in diameter in microns from the appropriate baseline value. *$p < 0.05$ before *E. coli vs.* after *E. coli* by paired t test.

resulted in a mortality rate <10%. These deaths invariably were related to anesthetic administration from secondary intraperitoneal bleeding.

Baseline hemodynamic values and microvascular diameters on the day of study for the four experimental groups are listed in Table 1. Because of thermistor malfunction, CO for the 24-hour group was not measured. The diameter and flow for the A1 vessels 24 hours after hemorrhage tended toward constriction with a concomitant decrease in flow, although these differences did not reach statistical significance. Table 2 lists the diameter and hemodynamic changes from baseline that occurred with *E. coli* bacteremia. In the control and sham hemorrhage groups, bacteremia resulted in vasoconstriction at all levels of the microcirculation and a resultant decrease in A1 flow. In contrast, bacteremia resulted in a significant vasodilation of both the A3 proximal and A3 distal vessels 24 and 72 hours after hemorrhage. Also, the A1 constrictor response was blunted in the 24-hour bacteremic group. Flow in the A1 vessels was reduced in all groups. CO was increased with bacteremia in animals where a measurement could be obtained.

The absolute diameter values after the addition of the endothelial-dependent vasodilator ACh before and after bacteremia are shown in Figures 2 through 7. The diameter changes for each vessel level are represented by the bar graphs in each figure. There was a significant change in the vascular reactivity of A1 vessels after bacteremia in both the 24- and 72-hour groups *versus* the sham group. This change in reactivity was caused by a depressed response in endothelial dilator function after hemorrhage. Postbacteremic reactivity was similar for all three groups. A similar shift in vascular reactivity to bacteremia was noted for the proximal A3 microvessels in the 72-hour group. As in the A1 vessels, this change was due primarily to a depressed vascular response before bacteremic stimulation. In the distal A3 vessels, increased reactivity was noted for both the 24- and 72-hour groups. However, these differences were caused by the increased dilator response after bacteremia rather than the prebacteremic depression in reactivity that was noted in the A1 and A3 proximal vessels.

After sodium nitroprusside was added to the tissue bath, the maximum vessel diameters achieved for all anatomic vessel levels were similar in all four experimental groups. This indicates that the maximum dilator capacity of intes-

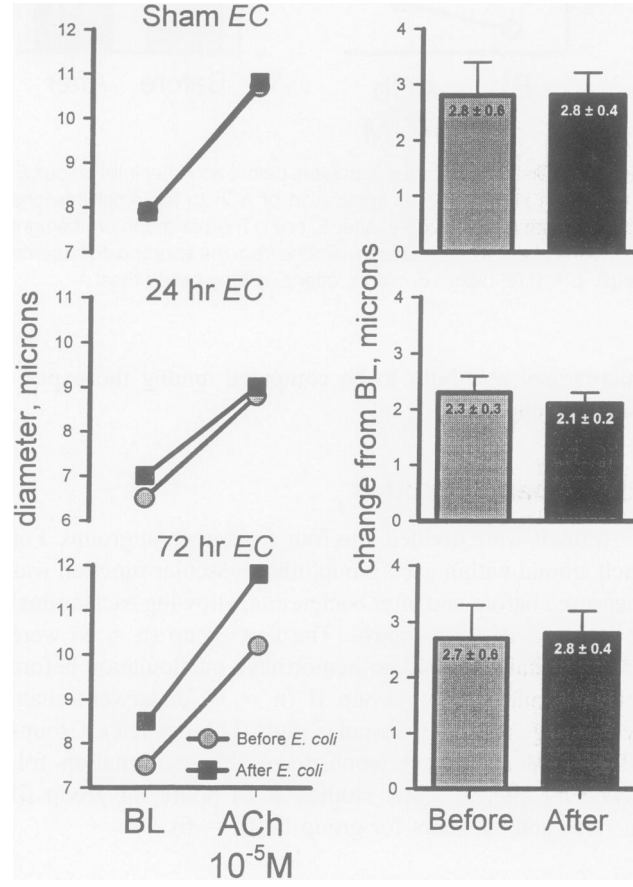

Figure 7. Distal A3 arteriolar diameters before and after intravenous *E. coli* infusion in response to application of ACh to the small intestine (circle, before *E. coli*; square, after *E. coli*). The bar graph on the right shows the change in diameter in microns from the appropriate baseline value. *$p < 0.05$ before *E. coli vs.* after *E. coli* by paired t test.

Table 3. SUMMARY OF CONSTRICTION/ DILATION BALANCE TO *E. COLI*

Variable	Sham *E. coli* (n = 6)	24 hr *E. coli* (n = 5)	72 hr *E. coli* (n = 6)
A1 diameter	Constriction	Small constriction	Constriction
A3P diameter	Constriction	Small dilation	Small dilation
A3D diameter	Constriction	Small dilation	Dilation

tinal microvessels is not altered after hemorrhage and bacteremia.

DISCUSSION

The two-hit theory for the development of MSOF emerged from the clinical observation that multiple, sequential insults are the most common scenario leading to MSOF, rather than a single event.[35] The fact that the initial signs of a sustained systemic inflammatory response during MSOF occur over time and are separated from the initial injury lends credibility to this theory. Initial experimental studies focused on the pathophysiologic inflammatory state during MSOF.[36,37] Recent studies have focused on the temporal development of the syndrome.[38] Neutrophils have been shown to be a key effector in the development of MSOF through a priming mechanism.[39,40] Depression in cellular immunity[41] and increased cytokine activation after hemorrhage caused by priming[42] are also consistent with the two-hit theory.

Previous studies from our laboratory focused on the intestinal microvascular responses to acute hemorrhage or bacteremia. Hyperdynamic bacteremia results in vasoconstriction and mucosal hypoperfusion[32] in the rat small intestine. This response is mediated, in part, by both prostaglandins[34] and NO[26,29] and can be obviated by treatment with pentoxifylline[43] or mucosal glucose exposure.[44] A similar intestinal vasoconstrictive response is noted during hemorrhage,[45] mediated primarily by alpha-adrenergic activity.[46] The extent to which these acute constrictive reactions persist or prime endothelial function over time has received little attention. During experimental peritonitis, liver blood flow is reduced[23] and endothelial-dependent relaxation is impaired, as shown by aortic vascular ring studies.[28] However, microvascular endothelial function during chronic infection has not been studied.

The current model attempts to simulate the two-hit theory of MSOF. *In vivo* videomicroscopy of the intestinal microcirculation was used to assess endothelial-dependent vasodilation after a moderate nonlethal hemorrhage and subsequent bacteremia. The vascular responses are summarized in Table 3. As noted in our previous studies, bacteremia in the normal animals in group I caused a consistent vasoconstriction of all microvessels and an associated decrease in blood flow. This vasoconstrictive response was unchanged in the sham hemorrhage animals in group II. However, bacteremia resulted in dilation of both the proximal and distal premucosal A3 vessels in groups III and IV, when hemorrhage had occurred 24 or 72 hours before the onset of sepsis. In addition, the constrictor response in the A1 vessels at 24 hours was blunted. By 72 hours, the magnitude of the constriction had returned to that noted in the sham animals.

The mechanism for this reversal in the bacteremic response noted after hemorrhage appears to be an augmentation over time of endothelial-dependent dilator systems. As noted in Table 4, which summarizes the microvascular dilator reactivity to the endothelial-dependent agonist ACh, a significant increase in vascular endothelial-dependent responsiveness was noted in the premucosal distal A3 vessels after bacteremia. This increase in vascular reactivity appears to be caused by a greater sensitivity to the dilator agonist through the release of NO. The change in A1 inflow vessel reactivity in the 24- and 72-hour animals appears to be caused by an increase in constrictor mechanisms rather than a depressed reaction to ACh after hemorrhage. This increase in constrictor force is overcome by bacteremic stimulation of dilation.

Microvascular tone in the intestines is a balance between constrictor and dilator forces. The factors that balance these two opposing forces vary by vascular levels and stimuli. The concept of a shift in anatomic site for microvascular balance during bacteremia has been demonstrated for hypertension.[47] The new data from this study demonstrate the dynamic nature of this balance, which fits a model for two-element control in which each opposing force can react to a given external stimulus and at the same time respond to the counterforce. Our data demonstrate this concept in Tables 3 and 4: constriction

Table 4. VASCULAR REACTIVITY FOR DILATION

Variable	Sham *E. coli* (n = 6)	24 hr *E. coli* (n = 5)	72 hr *E. coli* (n = 6)
A1 diameter	Same	Decreased reactivity before *EC*	Decreased reactivity before *EC*
A3P diameter	Same	Same	Increased reactivity after *EC*
A3D diameter	Same	Increased reactivity after *EC*	Increased reactivity after *EC*

EC = E. coli.

in A1 vessels was depressed at 24 hours after hemorrhage, but at 72 hours constriction balance had recovered; at the same time, dilation reactivity in A3 vessels progressed throughout our experimental protocol.

Although this study focused on endothelial-dependent vasodilation, it has unmasked other constrictor forces in the A1 vessels that appear to be stimulated with hemorrhage. Based on our previous studies,[47] adrenergic activity after hemorrhage appears to be a major factor in this augmented constrictor response, which persists for several days.

Overall, our data show that there is a shift in microvascular control for both dilation and constriction after hemorrhage. This shift is dependent on both time and anatomy, with the largest augmentation of endothelial-dependent dilation occurring at 72 hours in the distal premucosal vessels and increased constriction influence in A1 inflow vessels. The prolonged vasodilatory changes that were unmasked by hemorrhage in our two-hit model contribute to the decrease in peripheral vascular resistance noted during the initial stages of hyperdynamic sepsis and MSOF.

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Discussion

DR. MARK A. MALANGONI (Cleveland, Ohio): Dr. Laws, Dr. Copeland, Members, and Guests. I'd like to thank Dr. Garrison for asking me to discuss this paper. He provided me with a very concise and well-written manuscript in plenty of time to review.

Multiple organ failure is a syndrome, that is, a group of signs and symptoms that occur together, the exact cause for which is still unknown. It's a difficult topic for us to conceptualize and relate to a clinical situation and to extrapolate the results to treatment of patients.

I'd like to summarize Dr. Garrison's observations. First, he showed that hemorrhage alone or bacteremia alone results in constriction of the intestinal microcirculation at all levels. However, when these two insults occur in sequence, there's constriction of the A1 diameter, but a paradoxical increase in A3 vessels that is more obvious in distal arterials.

The uniform vasoconstriction restricts entry of white blood cells to small vessels, and velocity is changed somewhat, but minimally. With the second hit theory, there is a distal vasodilation with resultant increased sequestration of white blood cells that may have been primed by the first insult and then activated due to changes in the local environment. These white cells may then exert a more damaging local effect that results in the systemic response that we see as multiple organ failure.

Thus, the Louisville group has added to the body of literature suggesting that the gut may be the so-called motor of multiple organ failure. How does that relate to clinical of your patients and mine? Clearly, with hemorrhage we give fluids to try and overcome the vasoconstriction. With bacteremia, again fluids are given and antibiotics as well to try and combat infection and hopefully reverse the pathophysiologic effects that are associated with this problem.

With hemorrhage and then subsequent bacteremia, again fluid and antibiotics, but now perhaps other therapies are needed to alter the local effects demonstrated by this elegant study.

I only have one question for the authors. Have you tested your hypothesis further by trying to manipulate this model to elucidate the explanation for these observations?

Some suggestions would be depletion of leukocytes, binding of complement, blockade of receptors, inhibition of nitric oxide synthesis, and evaluation of other substances that affect endothelial function such as CD-18 antibodies and antibodies to the various selectins.

I'd like to commend you and your colleagues on another fine study that inserts another piece into the puzzle of multiple-organ-system failure syndrome.

Thank you. [Applause]

DR. LEWIS M. FLINT, JR. (New Orleans, Louisiana): Thank you, Dr. Laws and Dr. Copeland. I only have three points that I'd like to make.

The first falls into the category of picking and nit because all of us — all the authors on the paper and myself were taught microcirculation physiology by Dr. Patrick Harris, who drilled into us day after day that you can't talk about sensitivity or reactivity without constructing a dose-response curve, a piece of data that is missing from the paper. I wonder if we can really call it that. I suppose since this is an audience of surgeons and not microcirculation physiologists that it probably doesn't matter that much.

The next point I'd like to make has to do with the origin of the vasodilator stimulus. You've made a case for this being an endothelial-dependent vasodilator mechanism, although I don't believe we can be absolutely certain that the acetylcholine acts solely on the endothelial cells.

And I wonder if there are other cells in the area that might be producing substances which are vasodilator in nature, for instance, various white cells and so on. And this follows Dr. Malangoni's observation about the potential for migration of activated leukocytes into the local area of the microcirculation under study. I wondered if you'd comment on that.

The one area that has always bugged me trying to impute an effect of the intestinal microcirculation on the general cardiovascular picture that we see in patients who are bacteremia is the fact that, sure, we may have lowered resistance in the small mucosal blood cells — small mucosal blood vessels, but overall the flow to the gut is diminished. And so if the overall flow to the gut is diminished, how does the decreased resistance in the microcirculation contribute to the general circulatory pattern that you see in patients who are septic?

We all understand that the intestine has a dual blood supply. Part of it goes to the smooth muscle of the intestinal wall, and part of it goes to the mucosa, and we've concentrated on the mucosal part of this blood flow. And I wonder if we might ought to begin to consider studying the smooth muscle microcirculation and skeletal muscle microcirculation in a remote site to get a more complete understanding of how the altered microvascular control may contribute to the total circulatory picture in patients and animals who are septic.

Thank you very much. [Applause]

DR. DAVID A. SPAIN (Closing Discussion): Thank you, Dr. Laws and Dr. Copeland.

Dr. Malangoni, some people have postulated some other thera-