Group B Streptococcal Sepsis in the Piglet: Effects of Fluid Therapy on Venous Return, Organ Edema, and Organ Blood Flow

Michael A. Bressack, Neil S. Morton, and John Hortop

We investigated the physiologic effects of normal saline versus 5% albuminated saline fluid resuscitation on 10-12-day-old piglets infected with group B streptococci for four hours. After intravenously receiving $1 \times 10^8$ bacteria/kg over 45 minutes, one group was untreated while the two fluid-treated groups received enough intravenous fluid to maintain the baseline cardiac output. An increase in the resistance to venous blood return was the major limitation to cardiac output. The resistance nearly quadrupled in the untreated piglets as shown by a 50% decrease in cardiac output with a nearly doubling of the driving pressure for venous return (mean circulatory pressure was normal and atrial pressures decreased by 70%). In both fluid-treated groups, resistance doubled as shown by an unchanged cardiac output with a doubling of the driving pressure (mean circulatory pressure increased by 50%) and atrial pressures remained at baseline. Blood volume was 9% below control in both fluid-treated groups. Much more crystalloid (155 ml/kg) than colloid (58 ml/kg) was necessary to maintain baseline cardiac output; this resulted in a 36% decrease in the plasma protein oncotic pressure of the former group and a 15% increase in the oncotic pressure of the latter group. Organ edema formation (ileum, pancreas, kidney, adrenal gland, lung) occurred only in the saline-treated animals. We conclude that increased resistance to venous return was the primary cause of shock in our model and that this can be effectively treated by giving enough intravenous fluid to elevate the mean circulatory pressure. However, if the plasma protein oncotic pressure is also lowered (saline group), organ edema results. (Circulation Research 1987;61:659-669)

Group B β-hemolytic streptococcus (GBS) is the most common infectious cause of neonatal mortality. There is an incidence in neonates of 1-4/1,000 live births with a case-fatality ratio of 25-50%. Because the disease can rapidly progress with eventual death in spite of early treatment with appropriate antibiotics, other forms of therapy have been investigated. For example, Rojas et al., Runkle et al., and Meadow et al. have evaluated indomethacin and tolazoline in various animal models of GBS infection. Since fluid resuscitation is basic to the treatment of septic shock, we wished to see if crystalloid versus colloid fluid therapy might differentially affect cardiovascular function or organ edema. To assess these two processes, we treated 10-12-day-old piglets, infected with GBS for 4 hours, with enough normal saline or 5% albuminated saline to maintain baseline cardiac output. As opposed to most clinicians, who focus on cardiac factors as the only determinants in shock, we found that the major factor limiting cardiac output in all infected animals was an increased resistance to venous blood return. Cardiac output, which fell markedly in the untreated piglets, could be brought back to normal and organ blood flow improved with either type of fluid therapy if enough fluid was given to elevate the blood volume and the upstream driving pressure for venous return (mean circulatory pressure).

There has long been a controversy over the differential effects of crystalloid versus colloid fluids on organ edema, especially pulmonary edema, in septic shock. We found organ edema (ileum, pancreas, kidney, adrenal gland, lung) only in the crystalloid-treated animals, possibly due to a significant decrease of plasma protein oncotic pressure in association with the elevation of mean circulatory pressure.

Materials and Methods

Forty 10-12-day-old male Landrace piglets (3.24 ± 0.66 kg) were evenly divided between 4 groups ($n = 10$); age and weight were comparable in all groups: group I (control): surgically prepared, uninfected, and untreated; group II: surgically prepared, infected but untreated; group III: surgically prepared, infected, and treated with normal saline (0.9% sodium chloride); and group IV: surgically prepared, infected, and treated with 5% human albuminated saline.

Surgical Preparation

After being pretreated with ketamine hydrochloride (4 mg/kg i.m.), the piglets were weighed, intubated, then anesthetized with 0.5-0.75% halothane and 50% nitrous oxide. We ventilated the animals with a piston-type respirator (model 101, New England Medical Instruments) at an FIO$_2$ of 35%, peak inspiratory pressure of 14-16 cm H$_2$O, and a rate necessary to keep the Pco$_2$, 35-45 torr and the pH 7.35-7.45. During the experiments, we did not make further respirator changes.

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Through cutdowns, we placed 3F polyethylene catheters (Clay-Adams, Inc.) in the midabdominal aorta, the right atrium, and the inferior vena cava. Via a left thoracotomy, we inserted a 3F polyethylene catheter into the left atrium and a 3F double lumen thermodilution probe (American Edwards Laboratories) 3 cm into the pulmonary artery. Postoperatively, the pericardium was left open and the ribs loosely reaposed. Halothane and nitrous oxide were discontinued, and the animals were allowed to recover for the next hour. During the recovery and experimental periods, the piglets were kept supine at a core temperature of 38–39° C (heating pad and lamp) and maintained on intravenous fentanyl (4 µg/kg) and pancuronium (0.1 mg/kg) every 45–60 minutes. To maintain animal comfort, the fentanyl dosage and interval were selected by its ability to maintain blood pressure and heart rate during the recovery and baseline periods at a similar level to that during halothane anesthesia.

**Preparation of Bacteria**

Group B β-hemolytic streptococcus was isolated from an infected human neonate (Royal Victoria Hospital, Montreal). So that each experiment would involve comparable bacteria, we plated the organism on many blood agar plates, then stored the bacteria in multiple glycerol vials at −70° C. For each experiment, a separate vial was thawed, inoculated into 50 ml of Todd-Hewitt broth (BBL Microbiology Systems), and were incubated at 37° C for 6 hours. The infected broth was poured into 500 ml of Todd-Hewitt broth (pH 7.8–8.0) and agitated at 37° C for 16 hours so that the bacteria could reach their log phase of growth. We washed the bacteria twice by centrifuging the bacteria at 2,000 rpm (4° C), pouring off the supernatant, and adding sterile normal saline. We quantified the final bacterial suspension by serial dilution plates and confirmed bacterial purity by blood agar plate inoculation and identification.

**Experimental Protocol**

After 1 hour of baseline measurements, we followed all 4 groups for 4 experimental hours. Group I was not infected. Animals in groups II, III, and IV received the bacterial suspension intravenously over 45 minutes (6 ml of approximately 6 × 10^6 bacteria/ml). Group II animals were not treated. During the entire 4-hour experimental period, groups III and IV received enough intravenous fluid therapy (prewarmed to body temperature) to maintain the baseline cardiac output. We infused the fluid in increments of 10 ml/kg given over 5 minutes at 10-minute intervals for a cardiac output below baseline. Group III received normal saline (Abbott Laboratories), and group IV received 5% human albuminated saline. The albuminated saline was prepared by diluting 25% human albumin (Cutter Biological) in normal saline. Small samples of the 5% albuminated saline (7.5 ml/kg × 2) were injected into 3 nonexperimental piglets to confirm a lack of any toxic effects.

During the 4 hours of the experiment we continuously monitored aortic (Ao), pulmonary artery (PA), left atrial (LA), and right atrial (RA) pressures (Bentley Trantac model #800 transducers, Beckman Dynograph Recorder R-611). Cardiac output (CO) was measured by computer (model COM-1, American Edwards Laboratories) using thermodilution in triplicate every 10–15 minutes (1 ml of iced D5W). We measured hourly aortic Po2, Pco2, and pH corrected for body temperature (Radiometer, Copenhagen), hemoglobin and hematocrit (Coulter Electronics), arterial lactate (enzymatic UV-method, Boehringer Mannheim), total plasma protein (Biuret method, Astra-8), plasma albumin (Brom cresol green, COBAS B10), and blood bacterial colony counts (serial dilution plates). Bacterial purity was confirmed with blood agar inoculation and identification.

Derived values included cardiac index (CO/kg weight), systemic vascular resistance (Ao—RA pressure/Cl), pulmonary vascular resistance (PA—LA pressure/Cl), and plasma protein oncotic pressure (πmv) (calculated from total protein and albumin).

**Blood Volume Measurements**

Before each experiment began we mixed 10 ml of the piglet's blood with 50 µCi of 51Cr (Merck Frost Co.) for 30 minutes, added vitamin C, washed the cells twice, and reconstituted the cells in normal saline. After intravenously injecting a known quantity of the labelled red cell suspension into the piglet at hour 3 of the experiment, we sampled blood every 10–15 minutes for 1 hour, counted the blood samples and a reference sample (the original suspension) in a gamma counter, measured the hematocrit on the blood and reference samples, and calculated red blood cell volume by extrapolating the intravascular dilution of 51Cr back to time zero. Total blood volume (V) was derived using the animal's hematocrit and correcting for differences between whole body and venous hematocrit.6

**Radionuclide-Labelled Microsphere Injections**

Organ blood flow was determined according to the method of Heymann et al.7 We injected approximately 3 × 10^5 111Ce-labelled microspheres (3M, diameter 13.5 ± 0.5 µm) at time zero and 85Sr-labelled microspheres (3M, diameter 13.4 ± 0.5 µm) at time 3½ hours into the left atrium over 30 seconds. A reference sample was withdrawn (Harvard pump) from the aortic catheter, starting 10 seconds before the injection and continued for 70 seconds at a rate of 1.5 ml/min. In two animals from each group, 85Nb-labelled microspheres were also injected into the right atrium at 3½ hours; this confirmed the absence of a significant right-to-left shunt.

**Postmortem studies**

After 4 hours of the experiment, we acutely stopped the heart with a lethal left atrial injection of potassium chloride. Within 7 seconds of cardiac arrest, we measured the mean circulatory pressure (Pmc) from
the right atrial pressure plateau and corrected for any differences between aortic and atrial pressures.\textsuperscript{10,12}

\textbf{Histology}

The left lung hilum was clamped at an end-expiratory pressure of 5 cm H\textsubscript{2}O and a 4-mm wide section of the left lower lobe was placed into buffered formalin. Four-millimeter wide sections of liver, left ventricle, diaphragm, pancreas, adrenal gland, ileum, kidney, cerebral cortex, and hippocampus were fixed in buffered formalin. All sections were stained with HPS (hemalum, phloxine, saffron) and examined by light microscopy.

\textbf{Organ Blood Flow}

Most of the lung, liver, brain, diaphragm, kidney, heart, pancreas, adrenal gland, and distal ileum were cut into sections, placed into counting vials, weighed, and counted along with the reference blood samples in a gamma counter (Beckman Biogamma II). Counting window widths were selected to give maximum efficiency as well as good separation for each isotope. Although a relatively narrow range was chosen for each isotope, organ counts were corrected for isotopic interference. Organ blood flow was calculated using the reference sample method\textsuperscript{3} and expressed as milliliters per minute per gram dry, bloodless tissue to eliminate any effects due to differences in organ blood or edema content. Organ blood flow equals

\[ \text{organ radioactivity} \times \text{reference sample withdrawal rate} \div \text{reference sample radioactivity} \]

The homogeneity of the microsphere distribution was verified by comparing the results between kidneys and cerebral hemispheres. More than 400 microspheres were verified in each tube counted. Blood flows were expressed in absolute terms and also in reference to the cardiac index.

\textbf{Organ Wet/Dry Weight Ratio}

After counting the tissue samples in the gamma counter, the tissue was dried to a constant weight in a 70\textdegree C oven (Fisher, model 106G). We calculated the ratio of wet/dry organ tissue weight exclusive of blood by the method described by Pearce et al\textsuperscript{4} using the \textsuperscript{51}Cr-labelled red blood cells injected premortem and 10 ml of blood withdrawn, weighed and counted at the time of death. Plasma showed insignificant \textsuperscript{51}Cr activity. No significant differences in organ blood volume were found between groups. There was no need to correct tissue weights for the microspheres because the weight of the spheres was less than 0.005\% of the total dry weight.

\textbf{Organ Albumin Content}

Keeping the tissues cool at all times, we weighed, diluted with normal saline (buffered with Tris to pH 8), then homogenized (Polytron-Brinkman homogenizer PT 10-35, generator PTA-10 TF) 0.5--2.0-g samples of the organs studied, excluding the adrenal cortex. We ultracentrifuged the tissue (7,500 rpm \times 15 minutes), collected the supernatant, resuspended the pellet, ultracentrifuged again (15,000 rpm \times 20 minutes), and collected the supernatant. A third extraction showed insignificant albumin content. The two supernatants were combined and analyzed for porcine albumin content using Mancini’s technique of radial immunodiffusion.\textsuperscript{13} Rabbit anti-porcine albumin antibody (Cooper Biomedical) was used for the diffusion plates and porcine albumin (fraction V, 97\% purity, Sigma Chemical Co., St. Louis, Mo.) for the standards. For each organ and animal, porcine albumin content was corrected for tissue blood albumin content (from the \textsuperscript{51}Cr assay on that organ and the hematocrit and plasma albumin concentration) and reported as milligrams albumin per gram bloodless wet tissue weight.

\textbf{Statistics}

All data are reported as mean ± SD. For comparisons of repeated measurements within groups, a repeated measures analysis of variance was used. A one-way analysis of variance was used for comparisons between groups at each time period. The means were compared post hoc by the Tukey test.\textsuperscript{16} Significance is at a \( p<0.05 \).

\textbf{Results}

Three animals from group II did not survive the experiment and are not included in the results; the 10 animals reported in group II all survived the 4-hour experiment. All other groups had 100\% survival. There were no significant differences between groups in the number of bacteria injected intravenously (1.1 ± 0.5 \times 10\textsuperscript{9}kg) or the blood bacterial colony counts (5.1 ± 5.0 \times 10\textsuperscript{7}ml) during the experiment.

\textbf{Circulatory Measurements}

Mean aortic pressures (Figure 1) remained at baseline during the entire 4 hours in all groups except for a brief elevation in the fluid-treated groups during the...
bacterial infusion and a downward but insignificant trend in the infected, untreated group. In groups II, III, and IV, the mean pulmonary artery pressure (Figure 1) greatly increased (3 times baseline) during the bacterial infusion then plateaued at approximately twice baseline during the rest of the experiment. Left and right atrial pressures (Figure 2) decreased in the infected, untreated group but did not change significantly in the other groups.

Cardiac index (Figure 3) was maintained at baseline values (except briefly during the bacterial infusions) in all except the infected, untreated animals; the latter group maintained a low cardiac output during the entire study with a slow fall to 50% baseline by 4 hours. Heart rate did not significantly change in any of the groups.

Associated with the above changes, the systemic vascular resistance (SVR) in group II slowly rose from 1½ to 2 times baseline (Figure 4) as the cardiac output fell during the study but did not significantly change in the other groups with stable cardiac outputs except briefly during the bacterial infusion. On the other hand, pulmonary vascular resistance (PVR) increased to 5 times baseline (Figure 4) in group II and 2½ to 3 times baseline in the fluid-treated groups; the increase in group II was significantly larger than in groups III or IV.

The normal saline-treated animals required 155 ± 45 ml/kg of fluid during the 4-hour study while the 5% albuminated saline-treated animals required 58 ± 13 ml/kg (Table 1). This resulted in an elevation in blood volume (V) of approximately 13% and in mean circulatory pressure of 50% in both fluid-treated groups; the increase in group II was significantly larger than in groups III or IV.

Injections showed the absence of significant right to left shunting in all groups (insignificant 99mTc counts in systemic organs). There were no significant changes in aortic $P_{CO_2}$. All infected groups developed a metabolic acidosis with a fall in pH and an increase in arterial lactate levels. The untreated animals had significantly more metabolic/lactic acidosis than the fluid-treated animals.

**Protein and Hematologic Measurements**

Total protein and albumin concentrations decreased significantly by 12% in group II and by 31% in group III; the difference between these two groups was also significant (Table 3). In group IV, the total protein concentration did not change, but the albumin concentration increased by 31%. The Hct fell by 13% in group II and 36% in group III but increased by 15% in group IV. The hematocrit and hemoglobin concentrations increased by 7% in group II and decreased by 10% in groups III and IV.

**Blood Gas and Lactate Measurements**

Aortic $P_{O_2}$ decreased by approximately 15% in all infected groups (Table 2). Right atrial microsphere injections showed the absence of significant right to left shunting in all groups (insignificant 99mTc counts in systemic organs). There were no significant changes in aortic $P_{CO_2}$. All infected groups developed a metabolic acidosis with a fall in pH and an increase in arterial lactate levels. The untreated animals had significantly more metabolic/lactic acidosis than the fluid-treated animals.
organ blood flow was increased to brain (30%), heart (33%), and adrenal glands (58%) but still decreased to 60% less (2.16±0.94 versus 4.10±1.00), the pancreas (11%) (Figures 5 and 6).

In group II, the absolute organ blood flows significantly decreased in all organs at 3'/2 hours. When expressed in reference to the animals' cardiac index, organ blood flow was increased to brain (30%), heart (33%), and adrenal glands (58%) but still decreased to 60% less (2.16±0.94 versus 4.10±1.00), the pancreas (11%) (Figures 5 and 6).

Group III had comparable organ blood flows; the only change from baseline was an absolute and relative (expressed per cardiac index) decrease in blood flow to the liver (hepatic artery) (26%), pancreas (22%), and ileum (11%) (Figures 5 and 6).

**Postmortem Findings**

There was a minimal amount of ascites in group IV and a moderate amount in group III. Light microscopic examination of the organs showed diffuse hepatic vacuolization in all infected groups. The only other organ showing consistent light microscopic changes was the lung. There was moderately more interstitial edema in the saline-treated group compared with the other groups. The other organs in group III with increased wet/dry weight ratios did not appear grossly edematous.

**Discussion**

GBS sepsis is the most common infectious cause of neonatal mortality. 1 Our animal model resembles many of the features of the human disease. Although precise measurements have not been published, the human neonate clinically develops a low cardiac output with high pulmonary and systemic vascular resistances and metabolic acidosis. 2 This is unlike the septic older child and adult who often have a high cardiac output and low systemic vascular resistance. Like the human neonate, our untreated piglets (group II) had a 50% decrease in cardiac output with a twofold increase in systemic vascular resistance, a fivefold increase in pulmonary vascular resistance, and significant metabolic acidosis.

Human neonates with severe sepsis can have blood colony counts of >10^9/ml. 20 Our piglets developed 5 x 10^8 bacteria/ml of blood after receiving 1 x 10^10 bacteria/kg. Our cardiovascular results are quite similar to the piglet study by Runkle et al 21 except for the maintenance of an elevated systemic vascular resistance and normal blood pressure in our animals; the difference may be due to Runkle's higher bacterial dosage (4 x 10^9 bacteria/kg) and our inclusion of only the survivors.

Most animal and human studies of septic shock examine endotoxin or gram negative bacteria. The few studies done with GBS 21,22 have shown hematologic, pulmonary, and immunologic responses quite similar to endotoxic shock. Rojas et al 22 relate this to a circulating

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**Table 1. Vascular Measurements**

<table>
<thead>
<tr>
<th>Group</th>
<th>Intravenous fluid (ml/kg)</th>
<th>Blood volume (ml/kg)</th>
<th>Mean circulatory pressure (torr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>...</td>
<td>78.1 ± 4.0</td>
<td>7.14 ± 0.88</td>
</tr>
<tr>
<td>Group II</td>
<td>...</td>
<td>71.2 ± 4.1</td>
<td>6.86 ± 0.94</td>
</tr>
<tr>
<td>Group III</td>
<td>155*</td>
<td>86.6* ± 45</td>
<td>10.20* ± 3.5</td>
</tr>
<tr>
<td>Group IV</td>
<td>58*</td>
<td>90.0* ± 58</td>
<td>10.78* ± 5.5</td>
</tr>
</tbody>
</table>

*Significant difference (p < 0.05) compared with group I.

**Table 2. Blood Gas and Lactate Measurements**

<table>
<thead>
<tr>
<th>Group</th>
<th>(P_0_2) (torr)</th>
<th>(P_0_2) (torr)</th>
<th>pH</th>
<th>Lactate (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>126±120</td>
<td>39±39</td>
<td>7.41±7.40</td>
<td>12.1±9.2</td>
</tr>
<tr>
<td>Group II</td>
<td>122±104</td>
<td>38±41</td>
<td>7.43±7.23*</td>
<td>10.7±5.5</td>
</tr>
<tr>
<td>Group III</td>
<td>121±17</td>
<td>38±41</td>
<td>7.41±7.29*</td>
<td>11.2±3.8</td>
</tr>
<tr>
<td>Group IV</td>
<td>122±107*</td>
<td>38±41</td>
<td>7.41±7.31*</td>
<td>12.2±26.6</td>
</tr>
</tbody>
</table>

Numbers to the left are measurements at baseline; those to the right are after 3'/2 hours of the experiment. *Significant difference (p < 0.05) compared with group I after 3'/2 hours of the experiment; †significant difference (p < 0.05) from the baseline value within the same group.
In fact, output by maintaining an adequate preload.

versally accepted as basic treatment of all types of properties in the bacterial cell wall of GBS.

Toxin while Fenton and Strunk find endotoxin-like 21

21 phenomenon of decreased venous return has been

Moreover, the vasculature, 30

emphasized the importance of venous return

Shubin and treatment of early GBS shock. In 1972, Weil and

experiments emphasize the importance of venous

Cardiac output is actually determined -

Cardiac output - aM factors include cardiac preload, afterload, and

If cardiac output was decreased primarily due to

primary problem was inadequate venous blood return.

uted somewhat to our hemodynamic abnormalities,

33

shock.” Although Weil’s evidence for decreased ve-

in septic shock when they coined the term “distributive

Although there is disagreement about -aM

These large veins include central veins such as the vena

Guyton explained the concepts of venous return in

1973. 12 Because of their large capacitance, veins are the major determinants of venous return (systemic much more than pulmonary). The small veins/vevules are the principle sites of the intravascular reservoir, and the large veins are the primary sites of resistance to venous return; this is an oversimplification since all types of veins have components of capacitance and resistance. These large veins include central veins such as the vena cavae, which are passively affected, and peripheral large and medium-sized vessels, which can be passively affected or responsive to circulatory vasoactive mediators and autonomic stimulation. 32 Venous return (equivalent to cardiac output in a closed loop circuit) equals

\[
Pmc - PA \over Rv
\]

where Pmc is mean circulatory pressure, PA is atrial pressure, and RV is resistance to venous return (this normally is quite similar to venous resistance since the intravascular reservoir is mostly in the small veins/vevules). Pmc is the intravascular pressure measured when the heart has been stopped and the blood in the circulation has been redistributed so that pressures everywhere in the circulation are exactly equal. 11,17

During active circulation Pmc is felt to be the pressure

Table 3. Protein and Hematologic Measurements

<table>
<thead>
<tr>
<th></th>
<th>Total protein (g/dl)</th>
<th>Albumin (g/dl)</th>
<th>pmv (cm H2O)</th>
<th>Hct (%)</th>
<th>Hb (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>4.14 ± 0.14</td>
<td>2.43 ± 0.24</td>
<td>15.5 ± 1.5</td>
<td>36.5 ± 3.6</td>
<td>10.8 ± 1.0</td>
</tr>
<tr>
<td>Group II</td>
<td>4.19 ± 0.36</td>
<td>2.39 ± 0.21</td>
<td>15.6 ± 1.6</td>
<td>35.5 ± 3.9</td>
<td>10.7 ± 1.4</td>
</tr>
<tr>
<td>Group III</td>
<td>4.18 ± 0.29</td>
<td>2.62 ± 0.32</td>
<td>15.6 ± 1.5</td>
<td>36.6 ± 3.2</td>
<td>10.9 ± 1.2</td>
</tr>
<tr>
<td>Group IV</td>
<td>4.32 ± 0.43</td>
<td>2.29 ± 0.22</td>
<td>15.8 ± 1.6</td>
<td>36.4 ± 3.3</td>
<td>11.1 ± 1.2</td>
</tr>
</tbody>
</table>

Numbers to the left are measurements at baseline, those to the right are after

hase of the experiment; *significant difference (p < 0.05) compared with group I after 3 hours of the experiment; †significant difference (p < 0.05) from the baseline value within the same group.

Table 4. Organ Wet/Dry Weight Ratio†

<table>
<thead>
<tr>
<th></th>
<th>Ileum</th>
<th>Pancreas</th>
<th>Liver</th>
<th>Lung</th>
<th>Adrenal</th>
<th>Kidney</th>
<th>Brain</th>
<th>Heart</th>
<th>Diaphragm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group II</td>
<td>5.618</td>
<td>4.698</td>
<td>4.140</td>
<td>5.234</td>
<td>5.367</td>
<td>5.513</td>
<td>4.858</td>
<td>4.471</td>
<td></td>
</tr>
<tr>
<td>Group IV</td>
<td>5.503</td>
<td>4.641</td>
<td>4.172</td>
<td>5.222</td>
<td>4.638</td>
<td>5.541</td>
<td>4.733</td>
<td>4.420</td>
<td></td>
</tr>
</tbody>
</table>

*Significant difference (p < 0.05) compared with group I.
†Results are expressed as wet bloodless tissue/dry bloodless tissue weight.
within the intravascular reservoir and is the upstream
driving pressure for venous return\(^1\)\(^,\)\(^2\),\(^3\),\(^4\); atrial pressure
\((P_A)\) is the downstream pressure. \(Pmc\) is a function of
the blood volume and the elastic properties of the
circulation

\[
\frac{V - V_o}{C}
\]

where \(V\) is the total intravascular blood volume, \(V_o\) is
the unstressed vascular volume (the volume contained
within the vascular reservoir when the mean circulatory
pressure is atmospheric), and \(C\) is the vascular com-
pliance \((V_o\) and \(C\) characterize vascular elasticity).

\(R_v\) is greatly affected by the distribution of blood
flow (determined by artery/arteriolar function). The
systemic vasculature can be divided into vascular beds
with short time constants (striated muscle, kidney) and
long time constants (hepatosplanchnic). Flow through
vascular beds with short time constants will decrease
resistance and therefore improve venous return while
flow through vessels with long time constants will
increase resistance and, therefore, interfere with ve-
 nous return.\(^5\)

Applying these concepts to our infected, untreated
animals, there was no difference from the control group
in \(Pmc\) and a 70% decrease in \(P_A\). Therefore, the very
low venous return (50% decrease in cardiac output) in
this group, in association with a driving pressure
\((Pmc - P_A)\) that is nearly doubled, can be explained by
an \(R_v\) that is 3-4 times the control (total systemic
vascular resistance was only doubled); moreover, the
normal \(Pmc\) with a 9% depression in \(V\) compared with
the control group supports an increase in the elastic
characteristics of the circulation \((V_o\) and/or \(C\) are
decreased).\(^1\)\(^,\)\(^7\) However, the decreased blood volume in
group II may be erroneous due to a significant
difference between large-vessel and whole body he-
matocrit in conditions where there is marked
vasoconstriction.\(^1\)\(^,\)\(^7\) The increased resistance to venous
return occurred despite a redistribution of blood flow
away from the hepatosplanchnic bed (long time
constant).\(^1\)\(^,\)\(^7\) The causes of the increased \(R_v\) in
group II were possibly active constriction of the peripheral large
and medium-sized veins, hemocoagulation (from hypo-
 volemia), and increased vascular resistance (arterio-
constriction can increase the capacitance of proximal
arteries). Another major cause of the increased \(R_v\) in
 group II was a passive elastic recoil of the large veins
resulting from the low blood flow.

Compared with the control group, both fluid-treated
groups had a 50% elevation in the \(Pmc\) associated with
baseline venous return (cardiac output) and \(P_A\). There-
before, the doubling of the driving pressure (Pmc—Pa) necessary to normalize venous return can be explained by a doubling of Rv (total systemic vascular resistance remained at baseline); moreover, the 50% elevation in Pmc can be explained by the 13% elevation in V; vascular elasticity (Vo and C) may or may not have been altered. Thus, in order to overcome the increase in Rv, we needed to elevate Pmc by increasing V. Active constriction of the peripheral veins seems to have been the primary cause of the elevated Rv since there was no component of arterioconstriction (systemic vascular resistance was normal), hyperviscosity (the hematocrit was actually decreased), or passive elastic recoil (the cardiac output was normal). If ascites (moderate in group III) were an important cause of the increase in venous resistance, the measured determinants of venous return (V, Pmc) would have been different in groups III and IV. Moreover, group II showed an increase in Rv without any ascites. Another hypothesis to explain the increased Rv relates to blockage of small veins/venules by microvascular agglutination and/or clotting during the bacteremia; Rv would increase from a combination of loss of vascular surface area and passive elastic recoil of the large veins distal to the plugging. However, there was no clinical evidence for a consumptive coagulaopathy (no bleeding from the surgical sites), no increase in systemic vascular resistance secondary to arteriolar occlusion, and except for a slight decrease in the hepatoplasticchnic bed, no decrease in organ blood flow; significant small vein/venule occlusion should adversely affect organ blood flow. Venous return may have been helped by the redistribution of blood flow away from the hepatoplasticchnic bed and by the hemodilution resulting from hypervolemia.

In the above analysis, we approached the circulation as a single-circuit model rather than separating the systemic and pulmonary systems; the two vascular beds showed the same changes in atrial pressures, and there were no significant differences in organ blood volume between groups. Because the systemic compliance is so much larger than pulmonary compliance, any analysis is strongly weighted in favor of the systemic bed. Moreover, our values for Pmc and Rv are average values for the whole body, weighted in proportion to each vascular segment’s capacitance. A limitation in our methods is that we made only one measurement of mean circulatory pressure and blood volume; our analysis is, therefore, most applicable to the final hour of the experiment.

In spite of an increased resistance to venous return impairing adequate venous return (cardiac output), both normal saline and 5% albuminates saline were equally able to normalize cardiac output and atrial pressure through the elevation of blood volume and Pmc (increased upstream driving pressure). However, much more crystalloid (155 ml/kg) than colloid (58 ml/kg) was needed; moreover, the plasma protein oncotic pressure was much lower in the crystalloid group (Table 3). This lower oncotic pressure contributed to the excess extravascular fluid formation in the ileum, pancreas, kidney, lung, and adrenal glands (Table 4). Conversely, the slightly increased oncotic pressure in the colloid-treated group possibly prevented organ edema.

The heated debate concerning the differential effects of crystalloid versus colloid fluids on organ edema (especially pulmonary edema) in septic shock has resulted in conflicting experimental results. Starling initiated and Landis and Pappenheimer completed the description of fluid filtration across the capillary membrane: Qf = Kf(Pmv—Ppmv)—σf (σf = plasma protein oncotic pressure, and Ppmv is interstitial protein oncotic pressure).

It is unlikely that we had a significant permeability change in our animals (increased Kf, decreased σf) because of the absence of edema formation in the colloid group (normal wet/dry weight ratios) and the low albumin content of the edematous organs in the crystalloid group.

Compared with the control group, calculated values for Pmv were decreased by 13% in group II, 36% in group III, and increased by 15% in group IV. Although a decrease in plasma protein oncotic pressure is quite important in determining net fluid filtration (lymph flow increases), pulmonary edema does not occur if only Pmv is altered. On the other hand, Guyton has shown that decreased Pmv lowers the edema threshold in the lung when Pmv is increased.

An elevation of Pmv seems very likely in our fluid-treated animals in spite of baseline aortic and atrial pressures. One explanation is a decrease in the pre/post capillary resistance ratio. This decrease occurs in all forms of shock due to the dilatory effects of local factors such as hypoxia, acidosis, and adenosine on arterioles (autoregulation). It has specifically been seen in septic shock. In groups III and IV, a decreased pre/post capillary resistance ratio can be inferred from the normal total systemic vascular resistance with a doubling of the resistance to venous return. In group II, resistance to venous return quadrupled with only a doubling of total systemic vascular resistance.

Since the location of the venous reservoir (Pmc) is in the venules, our values for mean circulatory pressure are probably a close approximation to Pmv. Thus, the 50% elevation in Pmc in groups III and IV is strongly supportive of a significant increase in Pmv.

Therefore, the organ edema found in group III was probably due to an increase in Pmv (Pmc was elevated 50%) and a decrease in Pmv (36%). Group IV did not have organ edema in spite of an elevation in Pmc possibly because of a slight increase in Pmv (15%) and the tissue edema safety factors such as increased lymph flow and interstitial pressure (Ppmv) and dilution of interstitial protein (Ppmv). In spite of a slightly low Pmv (13%), group II had no edema because of a
normal Pmc (measured only at the end of the study) and a probable decrease in capillary surface area secondary to high vascular resistance and low organ blood flow. Our analysis is limited by an absence of data for rPmv and Ppmv. Moreover, we did not look for edema in the skin, subcutaneous tissue, or striated muscle (except the diaphragm); these tissues are the site of most of the body’s interstitial fluid and protein.19

Why should organs such as the ileum, pancreas, kidney, lung, and adrenal glands become edematous while the diaphragm, heart, liver, and brain remain unaffected? Although our experiment does not directly examine this complex question, differences relate to local factors such as the pre/post capillary resistance ratio, interstitial compliance, lymphatic drainage, and an overflow system.48

Because of the kidney’s unique system of fluid movement, Starling forces operate across two capillary beds, the glomerulus for filtration and the peritubular capillary for reabsorption. Although there may be an increase in Pmv within the peritubular capillaries related to an increased resistance to venous return, the renal edema in the saline-treated group was probably primarily due to the low plasma protein oncotic pressure within the peritubular capillaries. Using micropuncture techniques, investigators have observed decreased salt and water reabsorption due to low peritubular capillary oncotic pressure.49,50

Many of the other findings in our experiments have been seen by other investigators.22,51-55 For all infected groups, we found an extremely large increase in PVR followed by a more moderate response (Figure 4). These early pulmonary vascular changes have been blocked by cyclooxygenase inhibitors.23,51 The rise in SVR in association with a low cardiac output in our infected, untreated group has been seen by Runkle et al8 and is what is clinically seen in human neonates. With enough fluid therapy to normalize cardiac output, the SVR normalized (Figure 4).

Organ blood flow measurements during septic shock have shown tremendous variability in the literature51-55; this may be due to differences in species and experimental conditions. In our infected, untreated group (group II), we observed relative sparing of well-autoregulated organs such as the brain, heart, and adrenal glands (Figures 5 and 6); blood flow in these organs was actually increased when adjusted for changes in cardiac index. Blood flow to the kidney and diaphragm changed in parallel with the cardiac index. On the other hand, hepatosplanchnic organs such as the liver, ileum, and pancreas were poorly perfused, even when adjusted for changes in cardiac index. Since the vessels of these organs normally have the longest time constants,64 redirecting blood flow away from them improves venous return. Organ blood flow in group II may have been worsened by the hemodilution resulting from hypovolemia.

Both forms of fluid resuscitation equally improved organ blood flow with a normalization of all flows except the hepatosplanchnic. Thus, the amount of organ edema found in the saline-treated group did not interfere with total organ blood flow. Blood flow was probably benefited in groups III and IV by the hemodilution resulting from hypervolemia.

We have emphasized the importance of increased resistance to venous return as the primary cause of shock in our model; this increase was proportionally much larger than any increase in total systemic vascular resistance. Excluding studies in dogs whose hepatic veins are uniquely sensitive,6 our experiment is the first to document elevated Rv in septic shock. In our experiment, the abnormalities of the vasculature dominated over any cardiac dysfunction. Venous return is of secondary importance when the primary problem is cardiac contractility or afterload; this might occur if the experimental circumstances were different, i.e., a more prolonged or intense bacteremia. Moreover, increased resistance to venous return is probably not found in hyperdynamic shock.

To obtain adequate venous return in our study we needed to increase the Pmc by increasing blood volume; crystalloid or colloid were equally effective. The consequence was a tendency for organ edema in the animals with both a large elevation of Pmc and depression of rPmv (group III). Although many investigators have looked for pulmonary edema,6 we are the first to look at edema in other organs during fluid resuscitation in septic shock. In spite of the fact that the amount of edema was small (<20%), not detected histologically (except in the lung), and did not alter total organ blood flow, it could impair cellular O2 delivery and metabolic function35 and perhaps adversely affect the microcirculation. Moreover, the amount of edema could appreciably increase over time if the abnormal Starling forces were to continue with or without a contributory permeability change. Colloid therapy is, therefore, reasonable in order to prevent organ edema during early phase of GBS shock. This conclusion would have no relevance after significant permeability changes occur because of major alterations in the Starling forces.

Another approach to therapy would be to reverse the increased resistance to venous return with agents that preferentially dilate the peripheral large and medium-sized veins (decrease Rv) without appreciably decreasing vascular elasticity (increase C, Vo) (small veins/venules). If this could be done, much less intravascular fluid therapy would be required. Since changes in Pmc and rPmv would be small, there should not be increased organ edema with crystalloids or colloids. A third approach would be to discover and eliminate the mechanism causing the increased resistance to venous return. This may involve a reflex pathway or a circulating vasoactive mediator. Finally, therapy against microvascular agglutination and/or clotting may be appropriate if small vein/venule blockage is significant.

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