Peripheral Bypass-Induced Pulmonary and Coronary Vascular Injury

Association With Increased Levels of Tumor Necrosis Factor

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Background. Although cardiopulmonary bypass is associated with systemic complement activation and neutrophil sequestration, it is unclear whether bypass-induced vascular injury is localized and dependent on organ ischemia. We hypothesized that other factors perhaps related to placement of a bypass circuit or to blood perfusion of a pump-oxygenator system may produce vascular injury caused by systemically circulating mediators. In dogs, we determined whether application of a systemic venoarterial bypass circuit with pump-oxygenator perfusion but without pulmonary or cardiac flow diversion (peripheral bypass) leads to vascular injury. Since several features of the postperfusion syndrome after bypass resemble sequelae of endotoxin exposure, we also measured circulating endotoxin and tumor necrosis factor levels.

Methods and Results. Anesthetized dogs underwent 2 hours of exposure to a pump-oxygenator with peripheral venoarterial bypass. We used a double indicator measurement of pulmonary and coronary vascular permeability (protein leak index [PLI]) as indexes of vascular injury. Compared with controls (n=7), the pulmonary PLI of dogs undergoing bypass (n=11) increased more than threefold (18.8±2.3 vs 63.3±7.6×10^(-4) min^-1; P<.05) and the coronary PLI increased more than twofold (P<.05). The rate of disappearance of intravascular radiolabeled protein increased threefold after bypass (disappearance t1/2, 241±35 vs 84±15 minutes, control vs bypass; P<.05), suggesting a generalized increase in vascular permeability. Circulating endotoxin was detectable in blood samples from 8 of 8 bypass animals (range, 0.24 to 4.56 ng/mL) compared with 2 of 5 controls (P<.05). Tumor necrosis factor levels increased significantly with bypass (6.7±3.8 vs 146.7±33.6 U/mL, baseline vs bypass; P<.05) and were only slightly and nonsignificantly increased in controls (7.0±4.4 vs 18.2±5.9 U/mL; P=NS). Peak tumor necrosis factor but not peak endotoxin levels correlated with pulmonary and with coronary protein leak. As expected, circulating complement (CH50) levels decreased significantly during bypass, reflecting systemic complement activation. However, the levels correlated poorly with the severity of vascular injury.

Conclusions. We conclude that peripheral pump-oxygenator bypass causes coronary and pulmonary vascular injury that is independent of blood flow diversion and is associated with the appearance of circulating levels of endotoxin and tumor necrosis factor, which may play a role in bypass-induced vascular injury. (Circulation 1993;88:726-735)

KEY WORDS • ischemia • bypass surgery • vessels • edema • arteries

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Despite improved operative mortality during cardiopulmonary bypass, postbypass organ dysfunction remains a persistent problem.1-5 Vascular injury, evident as increased permeability, altered vasoreactivity, and/or loss of the normal nonadhesive and nonthrombogenic vascular surface, may contribute to postbypass organ dysfunction.2,3,5,6 Although bypass is associated with significant complement activation and organ neutrophil sequestration that can contribute to vascular injury, it is unclear whether generalized postbypass injury occurs.9-15 Since blood flow to the heart and the lungs is markedly reduced during conventional bypass with cardiopulmonary blood flow diversion, subsequent cardiac and pulmonary dysfunction may reflect direct effects of flow diversion, organ ischemia, and reperfusion.1-3 However, associated cerebral,5 renal,4 and splanchnic4 dysfunction suggests that mechanisms other than local ischemia may play a role. Although vascular injury may play a role in postbypass organ dysfunction, previous studies using predominantly indirect methods of assessing vascular injury have produced conflicting results regarding its occurrence after bypass.9-17 In addition, prior studies of pulmonary and coronary vascular injury have not distinguished between the roles of local flow diversion and...
of other mechanisms leading to generalized vascular injury.

Complement activation that occurs with bypass could mediate bypass-induced organ dysfunction and vascular injury. However, several recent studies suggest that complement activation alone is not sufficient to cause vascular injury and that other factors, including local ischemia and neutrophil priming by other mediators, are necessary. A role for endotoxin in bypass-induced vascular injury seems possible, because several features of the “postperfusion” syndrome, including multiorgan dysfunction, circulating neutropenia with tissue neutrophil sequestration, eicosanoid production, lipid peroxidation, and complement activation, have been observed in sepsis or after the administration of endotoxin. In addition, Rocke et al and Karazumi and coworkers reported increases in circulating endotoxin levels in patients undergoing bypass. Thus, endotoxin and, therefore, tumor necrosis factor (TNF), an important mediator of endotoxin-induced vascular injury, could play roles in bypass-induced vascular injury.

We performed studies in dogs to determine whether peripheral placement of a pump-oxygenator in a systemic venoarterial circuit (peripheral bypass) causes pulmonary and coronary vascular injury independent of blood flow diversion and whether this is associated with increases in circulating endotoxin and/or TNF. As indexes of vascular injury, pulmonary and coronary vascular protein permeability were measured with a previously described double indicator radioisotope protein leak index (PLI). We found that peripheral bypass increased pulmonary and coronary vascular permeability with associated complement activation and increases in circulating endotoxin and TNF.

**Methods**

Mongrel dogs (n=17; weight, 23.7±2.4 kg) were preanesthetized with thiamylal (100 mg/kg), intubated, ventilated, and anesthetized with 0.5% intravenous chloralose (100 mg/kg bolus plus 20 mg·kg⁻¹·min⁻¹ infusion). Catheters were inserted into the pulmonary artery and aorta for pulmonary artery, pulmonary artery wedge (PAWP), and arterial pressure measurements, blood sampling, and drug administration. Respiratory rate and tidal volume were set to maintain physiological blood gases, and 2 cm of positive end-expiratory pressure was added. Temperature was monitored with an esophageal probe. Cardiac output and pulmonary blood flow were measured by the dye dilution method with indocyanine green dye using a Waters densitometer and an on-line computer.

To avoid the effects of cardiopulmonary blood flow diversion (and resultant cardiac and pulmonary ischemia), hypothermic cardioplegic arrest, and heparin-protamine interactions, a peripheral venoarterial bypass circuit was used. Cannulas for bypass were inserted into the femoral vein (22F) and artery (16F) and, when necessary for adequate pump flow, into the internal jugular vein as well. After insertion of the cannulas, heparin (100 U/kg) was administered and additional heparin given thereafter to maintain the activated clotting time at ≥400 seconds. After baseline measurements, femoral venoarterial bypass was begun by connecting the cannulas to a bypass pump-oxygenator circuit consisting of a large-diameter roller-head pump (Model 2000, Sarns, Inc), bubble oxygenator, and heat exchanger. The pump circuit was primed with warmed normal saline. Oxygen at 10 L/min was infused into the oxygenator. Pump flow was initiated at a flow rate of 100 mL·kg⁻¹·min⁻¹. Additional saline was given as necessary to maintain a mean blood pressure ≥90 mm Hg. To minimize the likelihood that our procedures could introduce exogenous endotoxin, we used new, disposable equipment for the bypass circuit, including cannulas and gas exchangers for each experiment, and intravenous solutions were all commercial human-grade preparations.

The experimental protocol used is outlined in Fig 1. Bypass was maintained for 2 hours. During this period, there was spontaneous cardiac activity, and the lungs were ventilated with room air. At the termination of bypass, residual blood in the extracorporeal circuit was returned to the animal and the cannulas were clamped and disconnected from the bypass circuit but left in place. No protamine was given. Gamma probes were then positioned over both lungs for the measurement of pulmonary vascular protein leak. Radiolabeled autologous protein and erythrocytes (RBCs) were injected into the femoral artery. External lung counts and blood samples were obtained during the next 60 minutes, after which the animals were killed with an overdose of pentobarbital. The lungs were clamped at the hilus and excised, and the heart was also removed. The heart and lungs were cleaned of excess blood, and tissue samples were taken for protein and RBC counts, histology, and gravimetric water content measurements, as outlined below. Central venous blood specimens for cell counts (hematocrit, leukocytes, platelets), total protein, complement (CH50), endotoxin, and TNF were taken before bypass (baseline), during early (30 minutes) and late (90 minutes) bypass, and 60 minutes after bypass.

**Assessment of Permeability**

The assessment of pulmonary and coronary vascular protein permeability using the double radioisotope PLI method has been described in detail previously. Briefly, the rate of accumulation of extravascular radio-
labeled protein in tissue is normalized for blood activity and perfused vascular surface by use of radiolabeled RBCs. This method allows a quantitative assessment of intravascular protein permeability and is a sensitive and specific index of vascular injury. Autologous radiolabeled transferrin (111mIn) and RBCs (99mTc) used in these studies were prepared in vitro as previously described.28,29

External measurement of pulmonary protein leak. External gamma scintillation probes (Picker International) modified to count two channels simultaneously are placed over the lungs to measure lung protein and RBC activity for 60 minutes after the injection of radiolabeled protein and RBCs. Blood samples are obtained every 15 minutes during the external measurements and counted in a gamma counter (Packard 5230 Auto Gamma Scintillation Spectrometer, Laguna Hills, Calif). The external probe protein counts are the sum of intravascular and extravascular activity. By use of the ratio: relative extravascular [EV] protein = (probe protein counts/blood protein counts)/(probe RBC counts/blood RBC counts), normalized lung protein activity represented by the relative EV protein can be calculated each minute and plotted against time. The slope of this line represents the rate of extravascular protein accumulation relative to regional blood protein activity. The slope of the line divided by the intercept at t = 0 (which reflects physical factors and initial probe field size affecting radioisotope counting) is the extravascular PLI (PLI), where PLI=(×10−9 min−1)=slope of relative EV protein vs time/(relative EV protein).

Tissue-based measurement of pulmonary and coronary protein leak. This method is analogous to the external measurement of pulmonary protein leak and has also been previously described in detail.29 In contrast to the external probe method, the tissue-based PLI is limited to a single time point for assessment of extravascular protein accumulation. Sixty minutes after injection, tissue biopsies were taken from lungs (upper, middle, and lower lobes) and the heart and consisted of 12 to 18 samples of lung (1 to 2 g each) from two or three sites of each lung region bilaterally and for the heart, 8 samples of 1 to 2 g each from each of four quarters of the left ventricle. A reference blood sample was drawn from the aorta. The radiolabeled protein and RBC activity of each tissue sample and of the reference blood sample were then determined. Extravascular protein that accumulated during the 60 minutes after intravascular injection was then calculated as follows: extravascular protein activity = total tissue protein activity intravascular protein activity, where intravascular protein activity = tissue blood weight×blood protein activity. Tissue blood weight was determined by use of the labeled RBC content of the tissue, and tissue intravascular and systemic hematocrit were assumed to be equal. Extravascular protein activity was normalized for the concentration of radiolabeled protein in the blood (blood protein activity) and for perfused vascular surface area (represented by tissue blood weight) and expressed as a tissue-based PLI (PLI), where PLI=(extravascular protein activity/blood protein activity)/tissue blood weight.

Systemic vascular permeability. The rate of disappearance of radiolabeled transferrin from the systemic vascular compartment was used to assess generalized protein leak. The radiolabeled protein content (per gram of plasma) of each of the blood specimens drawn at 15-minute intervals after intravenous injection of the labeled protein was measured. Using a single exponential analysis, the t1/2 of disappearance of the radiolabeled protein was calculated. Protein disappearance was compared with the t1/2 of disappearance of simultaneously administered radiolabeled RBCs.

TNF, Endotoxin, and Complement Measurements

Central venous blood specimens were collected in endotoxin-free sterile tubes (Gibco), the samples were centrifuged (20 minutes at 2500 rpm), and the plasma was removed and frozen immediately at −70°C. The samples were thawed once for endotoxin and TNF assays and again for complement measurements. The investigator performing the assays was blinded as to experimental groups and in vivo results. The total protein content (μg/mL) of each specimen was measured by the method of Bradford.32 Measured complement, endotoxin, and TNF levels were normalized for dilutional changes during bypass by use of the measured total protein content of each plasma sample according to the formula: normalized level = measured level/(measured sample protein content/baseline sample protein content). Samples from one control and one bypass animal were contaminated in storage and therefore were not assayed and are not included in the results.

TNF activity was measured using a modification33 of the cytotoxicity assay of Ruff and Gifford.34 Cultured L929 cells were seeded at a density of 5.5×10^4 cells/mL and incubated for 20 hours in Dulbecco’s modified Eagle’s medium (Whittaker Bioproducts, Inc, Walkersville, Md) supplemented with 10% heat-inactivated fetal calf serum, 0.29 mg/mL l-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin. After incubation, the old medium was removed. Fresh medium, 100 μL of sample (or standard), and 100 μL of 1.0 μg/mL actinomycin D (Sigma Chemicals, St. Louis, Mo) were added. The cells were reincubated for 20 hours, at which time L929 cytotoxicity was determined. The medium was removed, and 100 μL of a staining solution consisting of 0.1% crystal violet in 1% acetic acid was added for 15 minutes. The staining solution was removed, and the remaining cells were solubilized with 100 μL of 1% sodium dodecyl sulfate. Absorbance at 590 nm was measured with an automated microplate reader (Biotek, Winnuoki, Vt). The percent cytotoxicity was calculated from the measured absorbance. The concentration of TNF activity (units/mL) was derived by comparison to standards prepared with recombinant human TNF-α (Amgen Biologicals, Thousand Oaks, Calif).

Endotoxin (lipopolysaccharide) was measured with a quantitative chromogenic limulus lysate assay kit (OCL 1000, Whittaker Bioproducts) that we have previously used for plasma assays of endotoxin levels.2 The assay measures levels between 0.01 and 0.1 ng/mL lipopolysaccharide. Results were expressed as nanograms per milliliter.

Complement was measured as the total serum hemolytic complement (CH50) by methods previously described.20
TABLE 1. Hemodynamic Data: Baseline (Prebypass) and Final (60 Minutes Postbypass) Results

<table>
<thead>
<tr>
<th></th>
<th>PA (mm Hg)</th>
<th>PAW (mm Hg)</th>
<th>FA (mm Hg)</th>
<th>CO (L/min)</th>
<th>PVR (dyne·cm⁻²)</th>
<th>TSR (dyne·cm⁻²)</th>
<th>pHa</th>
<th>PacO₂</th>
<th>PacO₂</th>
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<tbody>
<tr>
<td>Baseline</td>
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<td></td>
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</tr>
<tr>
<td>Control</td>
<td>13.5±1.4</td>
<td>4.4±0.9</td>
<td>130±7</td>
<td>3.3±0.3</td>
<td>220±18</td>
<td>3322±229</td>
<td>7.40±0.17</td>
<td>34.1±1.7</td>
<td>66.1±1.7</td>
</tr>
<tr>
<td>Bypass</td>
<td>13.4±1.0</td>
<td>3.4±0.4</td>
<td>121±7</td>
<td>3.2±0.8</td>
<td>265±17</td>
<td>3166±174</td>
<td>7.37±0.029</td>
<td>32.0±0.9</td>
<td>69.2±0.0</td>
</tr>
<tr>
<td>Bypass+increased</td>
<td>13.7±2.3</td>
<td>4.1±1.2</td>
<td>120±5</td>
<td>3.3±0.6</td>
<td>236±14</td>
<td>2997±324</td>
<td>7.34±0.029</td>
<td>32.1±1.7</td>
<td>64.3±3.0</td>
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<tr>
<td>Final</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>13.9±6.1</td>
<td>5.1±0.6</td>
<td>129±5</td>
<td>3.1±0.2</td>
<td>231±24</td>
<td>3087±351</td>
<td>7.37±0.024</td>
<td>33.2±1.6</td>
<td>71.2±2.4</td>
</tr>
<tr>
<td>Bypass</td>
<td>16.7±3.0</td>
<td>6.6±0.8</td>
<td>92±11†</td>
<td>1.6±0.8†</td>
<td>597±32†</td>
<td>4499±499†</td>
<td>7.30±0.30</td>
<td>33.2±2.7</td>
<td>68.2±2.6</td>
</tr>
<tr>
<td>Bypass+increased</td>
<td>20.6±1.7†</td>
<td>14.3±1.3†</td>
<td>102±7†</td>
<td>1.8±0.4†</td>
<td>287±31</td>
<td>4787±584†</td>
<td>7.25±0.043</td>
<td>34.3±1.2</td>
<td>60.3±1.2</td>
</tr>
</tbody>
</table>

P<.05 vs baseline; †P<.05 vs control.

Tissue Water Content

Gravimetric tissue water corrected for residual blood content was determined. Lungs were excised, weighed, homogenized, and dried at 55°C in an oven for periods of 96 hours to 1 week until constant dry weight was achieved in each case. Aliquots of the homogenate were counted for labeled erythrocyte activity. Myocardial tissue was weighed, counted directly for labeled erythrocyte activity, and dried to constant weight. Tissue weights were corrected for residual blood content by comparison to a reference blood sample. Extravascular tissue water content was expressed as grams per gram bloodless dry tissue.

Histology

In three bypass and three control animals, a lower lobe of the lung was isolated (randomized right or left), the bronchus cannulated, and the lobe perfused with 10% formalin at 25 cm H₂O pressure for 24 hours. Tissue samples were stained with hematoxylin and eosin and examined by light microscopy for structural changes and the presence of neutrophils. Neutrophil sequestration was assessed by counting to determine the number of neutrophils falling on a superimposed grid within a high-power field. Counts were normalized for the number of alveolar intercepts per field. Five high-power fields from each of three sections per animal were counted.

Experimental Groups

Animals were divided into three experimental groups according to the protocol outlined in Fig 1. The control group (n=7) underwent a sham bypass procedure including femoral cannulation, systemic heparinization, and intravenous saline infusion comparable to the bypass circuit priming.

The bypass group (n=6) underwent 2 hours of femoral vein–femoral artery bypass (without cardiopulmonary blood flow diversion).

The bypass plus left atrial pressure (Pₐₐ) elevation group (n=5) underwent a left thoracotomy before bypass for insertion of a balloon catheter into the left atrium. The chest was closed, and the animals were allowed to stabilize, after which they underwent 2 hours of bypass. After bypass, during the last 30 minutes of the external permeability measurements, left atrial pressure was moderately increased by inflation of the balloon (PAWP, 12 to 15 mm Hg).

Statistics

Pulmonary and coronary PLIs, peak vs baseline levels, and baseline vs final hematological and hemodynamic values were compared by Student's t test. Time course comparisons of TNF and endotoxin levels within groups and of tissue water content among groups were done with an ANOVA with multiple comparisons. The presence vs absence of detectable endotoxin was compared by χ² analysis. Linear regression analysis was used for correlation comparisons. P<.05 was considered significant. Results are presented as mean±SEM.

Results

Peripheral venoarterial bypass without cardiopulmonary blood flow diversion was associated with hemodynamic and hematological changes summarized in Tables 1 and 2. These changes are comparable to those found by previous investigators examining full conventional bypass with cardiopulmonary flow diver-
Protein Leak Index

Bypass caused a greater than threefold increase in the external probe-based pulmonary PLI and more than a twofold increase in the tissue-based coronary PLI compared with controls (Fig 2). PLI data are summarized in Table 3. The tissue-based pulmonary PLI was increased 10-fold compared with control (0.46±0.13 vs 0.04±0.03; P<.05). Increases in the tissue-based pulmonary and coronary PLIs of individual animals were correlated (Fig 3). Consistent with a generalized increase in vascular permeability, radiolabeled protein disappeared from the systemic circulation more rapidly after bypass compared with control (t1/2 of disappearance, 241±35 vs 84±15 minutes, control vs bypass; P<.05). In contrast, the rate of disappearance of radiolabeled RBCs from the circulation after bypass was not different between groups (t1/2 of disappearance, 397±78 vs 373±67 minutes, control vs bypass; P=NS).

Tissue Water Content

Extravascular lung water was slightly increased after bypass (Table 3) despite a low PAWP (3.4±0.4 mm Hg) compared with control (4.4±0.9 mm Hg). When moderate elevation of left atrial pressure (mean, 13.7±2.3 mm Hg; range, 10.3 to 17.8 mm Hg) was induced, lung water increased more than 50% after bypass compared with control (Table 3). In previous studies in our laboratory, similar elevations of PAWP did not increase lung water in control dogs. In contrast, extravascular myocardial water content was not increased after bypass (3.31±0.09 vs 3.15±0.09 g/g bloodless dry tissue).

Endotoxin, TNF, and Complement Levels

Since there were no differences in TNF, endotoxin, or complement levels between the bypass and control groups, these data were combined and are presented as a single bypass group. Endotoxin was detectable in at least one plasma sample of two of five control and eight of eight bypass animals assayed (P<.05). In both control animals positive for endotoxin, the endotoxin was present in the baseline specimen and showed only minor subsequent increases.

Both uncorrected endotoxin levels and levels normalized for bypass-associated hemodilution increased during bypass (Fig 4). In the bypass group, three animals had low levels of endotoxin in the baseline phase, but in contrast to the control animals, all eight showed consistent substantial increases during the later, bypass phase of the study. However, there was variability in the peak endotoxin levels, which ranged from 2.26 to 7.30 ng/mL plasma, and peak values were noted in two animals in the early bypass sample, five in the later, and one in the final sample. As shown in Fig 4, TNF levels increased above baseline beginning early in bypass (146.7±33.6 vs 6.7±3.8 U/mL, peak bypass vs baseline; P<.05) and returned toward baseline by 60 minutes after bypass. TNF levels did not change from baseline in control animals (18.2±5.9 vs 7.0±4.4 U/mL, peak vs baseline; P=NS). As expected, total complement (CH50) levels decreased substantially during bypass (214±26 vs 44±26 U/mL, baseline vs minimum bypass; P<.05). Several animals had unmeasurable amounts of CH50 during bypass. The CH50 levels were unchanged in the control group (224±30 vs 214±24 U/mL, baseline vs baseline.
TABLE 3. Protein Leak Index and Tissue Water Data

<table>
<thead>
<tr>
<th></th>
<th>Lung</th>
<th>Heart</th>
<th>Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PLI_E (x10^-3 min^-1)</td>
<td>PLI_I</td>
<td>EVLW (g/g bloodless dry tissue)</td>
</tr>
<tr>
<td>Control (n=7)</td>
<td>18.8±2.3</td>
<td>0.04±0.03</td>
<td>3.13±0.07</td>
</tr>
<tr>
<td>Bypass (n=6)</td>
<td>69.5±15.6*</td>
<td>0.46±0.13*</td>
<td>3.56±0.19*</td>
</tr>
<tr>
<td>Bypass + increased P_LA (n=5)</td>
<td>55.8±8.9*</td>
<td>0.38±0.12*</td>
<td>4.71±0.27†</td>
</tr>
</tbody>
</table>

PLI_E, external protein leak index; PLI_I, tissue-based protein leak index; EVLW, EVMW, extravascular lung and myocardial water content, respectively; Prot, disappearance half-time of intravascular radiolabeled protein (111In transferrin) (values calculated from label activity per gram of plasma); RBC, disappearance half-time of intravascular radiolabeled (99mTc) erythrocytes. Values are mean±SEM. *P<.05 vs baseline; †P<.05 vs control.

vs sham bypass; P=NS). These changes in circulating levels were also evident using raw levels without normalization for dilutional changes.

Peak endotoxin and minimum CH50 levels correlated poorly with postbypass pulmonary (Fig 5) and coronary protein leak (data not shown). In contrast, peak TNF levels during bypass correlated with pulmonary and with coronary protein leak (Fig 6). The correlation was still evident using TNF levels that were not normalized for dilutional changes. TNF levels correlated poorly with endotoxin levels (data not shown).

Histology

Lung histology revealed an increased number of neutrophils in lung tissue of animals (n=3) after bypass compared with control (n=3) animals (0.2±0.2 vs 0.5±0.8 cells per alveolar intercept per high-power field). There was evidence of tissue injury, and in some cases significant interstitial edema was present in dogs subjected to bypass that was not observed in controls.

Discussion

These studies show that peripheral bypass causes pulmonary and coronary vascular injury in the absence of cardiopulmonary flow diversion and in association with the appearance of circulating endotoxin and TNF. The changes were substantial: after bypass, pulmonary vascular protein leak increased nearly fourfold, and coronary vascular protein leak increased more than twofold. In addition, there was a threefold increase in the escape rate of radiolabeled protein from the intravascular compartment, suggesting a generalized increase in permeability. The increase in pulmonary protein leak was comparable to that observed in our previous studies of canine endotoxin lung injury. The increase in coronary protein leak was comparable to those we have reported with myocardial ischemia-reperfusion injury.29 Lung, but not myocardial, water content was increased after bypass despite low hydrostatic pressures (PAWP), and lung water increased substantially after bypass with moderate left atrial pressure elevation, suggesting a physiologically significant increase in permeability. In previous studies from our laboratory, similar elevations of hydrostatic pressure did not increase lung water in animals with normal permeability.

Whether circulatory bypass with a pump-oxygenator causes vascular injury has been an unsettled question. Ratliff et al9 observed histological evidence of lung injury in dogs after bypass, as did Anyanwu et al14 in clinical studies, suggesting that pulmonary vascular injury occurred with bypass. However, Pitt and coworkers10 studied dogs undergoing 1, 2, or 3 hours of left heart bypass (with cardiopulmonary blood flow diversion) or right heart bypass (venovenous bypass without cardiopulmonary blood flow diversion) using transpulmonary metabolism of prostaglandin E_1 as an index of vascular function. Evidence of pulmonary vascular injury developed only after prolonged (3-hour) left heart bypass, and even then, only mild injury was observed. Using a similar method (transpulmonary serotonin extraction) to assess pulmonary vascular function, Dargent et al41 and later Gillis et al16 also found no evidence of vascular injury in patients undergoing bypass. However, measurements of transpulmonary metabolism of vasoactive compounds can be affected by changes in perfused surface area, rate of blood flow, and metabolism kinetics that complicate their interpretation.10,11,35

In contrast to these findings, experimental studies by Demling and coworkers13 and clinical studies by Byrick et al36 showed significant increases in lung water, which

![Graph showing that bypass-induced increases in pulmonary and coronary vascular protein leaks are correlated. Individual protein leak index (tissue-based) values are plotted for control, bypass, and bypass plus increased left atrial pressure (ILAP) groups. The pulmonary protein leak increased to a greater degree than did the coronary protein leak index.](http://circ.ahajournals.org/doi/abs/10.1161/01.CIR.93.4.731)
may reflect lung injury, after bypass. More recently, Braude et al,\textsuperscript{15} using a modification of the PLI methods used in our studies, found evidence of increased pulmonary vascular permeability in dogs undergoing full bypass with cardiopulmonary blood flow diversion. Although fewer studies have assessed coronary vascular injury after bypass, McDonagh et al\textsuperscript{17} used a first-pass indicator technique to assess coronary vascular permeability in dogs after cardiopulmonary blood flow diversion and found an increase in the albumin permeability–surface area product consistent with vascular injury.

Since blood flow is diverted from the cardiopulmonary circulation during conventional cardiopulmonary bypass, the heart and potentially the lungs are ischemic. Thus, clinical and previous experimental studies have not distinguished between direct localized injury caused by near total diversion of blood flow and indirect injury possibly caused by circulating mediators. In our studies, pulmonary and coronary vascular permeability increased after peripheral use of a pump-oxygenator without cardiopulmonary flow diversion. In our experiments, however, pulmonary blood flow was substantially decreased but remained at 50% of control values and was thus much greater than is seen with conventional cardiopulmonary bypass; it was probably sufficient to maintain nutrient flow to the lungs and thus did not represent clear ischemia. Also, although the venoarterial circuit we used reduces systemic venous return and pulmonary arterial blood flow, it augments systemic arterial flow so that bronchial and coronary flows are likely to be preserved, and these beds are probably not ischemic. This suggests an indirect, possibly humoral mechanism of vascular injury. Clinical postbypass dysfunction of organs not evidently ischemic,\textsuperscript{1,2} such as brain,\textsuperscript{4} kidney,\textsuperscript{3} and gut,\textsuperscript{17} could reflect indirect vascular injury.

Complement activation has been suggested as an important potential mediator of bypass-induced vascular injury.\textsuperscript{2,3} As in previous clinical and experimental studies of full bypass with cardiopulmonary blood diversion,\textsuperscript{3,7,8,10,13,15,16} we observed substantial complement activation, with decreased circulating leukocyte counts and histological evidence of pulmonary leukocyte sequestration. It is uncertain, however, whether complement activation alone is able to induce vascular injury. Henson and coworkers\textsuperscript{18} reported that complement activation failed to increase pulmonary vascular permeability in rabbits and suggested that complement activation alone, although necessary, was insufficient to cause vascular injury. Smedly et al\textsuperscript{19} found that in vitro stimulation of neutrophils with complement (C5a) or with formyl-methionyl-leucyl-phenylalanine caused little injury of cultured endothelial cells. However, both Henson and Smedly found that lipopolysaccharide (endotoxin) even in small amounts (1 to 10 ng/mL in vitro) greatly potentiated complement-mediated vascular injury. Bishop et al,\textsuperscript{20} studying pulmonary ischemia-reperfusion injury in rabbits, found that although systemic arterial
complement activation developed with even localized ischemia and reperfusion, vascular injury was not prevented by complement depletion.

We found that vascular injury with peripheral bypass was associated with circulating endotoxin and TNF. Further, the severity of vascular injury was correlated with peak TNF levels but not with peak endotoxin or minimum complement (CH50) levels. A potential role for endotoxin has also been suggested by reports of endotoxemia during bypass in patients.23,24 Also, several features of bypass postperfusion syndrome including multiorgan dysfunction, neutrophil sequestration, vascular injury, altered vasoreactivity, circulating eicosanoids, and tissue peroxidation resemble the sequelae of endotoxemia or sepsis.21,22,26,27,30,37 TNF is a potent polypeptide cytokine mediator of endotoxin-induced vascular injury and neutrophil activation25,26 capable of producing increased vascular permeability, neutrophil activation and adherence, organ leukocyte sequestration, neutrophil priming, and stimulation of endothelial interleukin-1 production, itself a cytototoxic cytokine.25,26,37,39 The TNF levels in our studies are comparable to levels that produce endothelial injury and neutrophil activation in vitro37–39 and to circulating levels present after endotoxin administration.27

Several problems arise in the interpretation of our studies. Our indicators of vascular permeability, the PLIs, are ratios of transvascular protein extravasation to tissue blood content. The denominator acts to minimize the effect of changes in vascular exchange area on protein transfer.28 However, this introduces the possibility that observed increases in leak index might reflect decreases in blood content, perhaps resulting from vasoconstriction, rather than increased protein efflux. To address this issue, we have compared our corrected indexes with measures of raw protein efflux without the blood content correction and find that this measure increased by 8.5-fold with bypass, similar to changes in the corrected indexes, indicating that the change in leak index was a result of increased protein efflux. Our measure of generalized vascular leak, the labeled protein disappearance half-time, is based on protein content per gram of plasma and could have been influenced by fluid infusions with hemodilution. However, this effect would be reflected in the change in RBC halftime, which was reduced by only 6% with bypass compared with a 65% decrease in protein half-time (Table 3). The difference in these decay curves suggests a significant effect on protein extravasation.

Finally, our finding of increased vascular leak in association with increased circulating levels of complement, endotoxin, and TNF does not indicate whether these agents mediate the observed changes in vascular permeability. Further studies using inhibitors or block-
ers will be necessary to clarify their roles. Our work also fails to indicate the source of and reasons for the observed increases in endotoxin with peripheral bypass. An important concern is that the endotoxin was an exogenous artifact of our preparation. However, control and bypass groups were handled identically, with the exception of placement of the bypass circuit in the latter. All of the bypass-related apparatus was sterile, unused, and disposable and thus an unlikely source of endotoxin. Similarly, in reported increases of circulating endotoxin levels in humans during cardiopulmonary bypass,23,24 an exogenous source also seems unlikely. The possibility that some contamination may have occurred is indicated by the finding of circulating endotoxin in two control animals, but these levels were low and failed to increase during the study, as did those in the bypass group. Thus, we believe that bypass did produce an increase in levels of endotoxin, which was probably of endogenous origin. Our study does not indicate a source of this increase in endotoxin, but several considerations point to the gut as a likely possibility. Increased splanchnic vascular permeability in dogs undergoing bypass has been demonstrated by Smith et al.16 Whether these changes result from splanchnic vasoconstriction in reflex response to altered
distribution of blood volume or flow during bypass or whether they reflect humoral effects produced by blood perfusion of the pump-oxygenator is unclear from work reported to date. Whatever the precise mechanism, the possibility that gut-derived endotoxin might contribute to postbypass organ dysfunction could have significant therapeutic implications.

In summary, peripheral circulatory bypass causes pulmonary and coronary vascular injury independent of cardiopulmonary blood flow diversion. This was associated with systemic complement activation, circulating leukopenia, organ neutrophil sequestration, and circulating endotoxin and TNF. The degree of bypass-induced vascular injury correlated with peak TNF levels. Thus, endotoxin and TNF may contribute to bypass-induced vascular injury and to postbypass organ dysfunction.

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