GRAM-NEGATIVE sepsis is one cause of noncardiac (primary) pulmonary edema in humans (Robin et al., 1972, 1973). Changes in the lung similar to those in the human disease can be produced in animals by infusing \textit{E. coli} endotoxin (Snell and Ramsey, 1969; Reeves et al., 1972, 1973). Although, by inference, endotoxin seems to increase lung microvascular permeability, there is little specific information about effects on permeability (Chien et al., 1964).

We showed earlier that infusing whole \textit{Pseudomonas} bacteria intravenously into unanesthetized sheep caused transient marked pulmonary hypertension followed by a long period of high flow of protein-rich lung lymph with stable pulmonary vascular pressures, indicating high exchanging vessel permeability (Brigham et al., 1974; Brigham et al., 1976b). Now we have infused \textit{E. coli} endotoxin into unanesthetized sheep and produced a similar biphasic reaction: transient pulmonary hypertension followed by a long period of high permeability (i.e., high flow of protein-rich lung lymph with stable vascular pressures). The endotoxin response was reproducible. The effects of endotoxin and \textit{Pseudomonas} on permeability were different in that endotoxin caused lymph clearance of large proteins less effectively than after \textit{Pseudomonas}. We conclude that \textit{E. coli} endotoxin in sheep causes a long period of increased lung vascular permeability and may have a greater effect on large solute pathways across microvessels than do \textit{Pseudomonas} bacteria. 

\textbf{Increased Sheep Lung Vascular Permeability Caused by \textit{Escherichia coli} Endotoxin}  
KENNETH L. BRIGHAM, RONALD E. BOWERS, AND JAMES HAYNES  

\textbf{SUMMARY} We infused \textit{Escherichia coli} endotoxin, 0.07-1.33 µg/kg, intravenously into chronically instrumented unanesthetized sheep and measured pulmonary arterial and left atrial pressures, lung lymph flow, lymph and blood plasma protein concentrations, and arterial blood gases. Endotoxin caused a biphasic reaction: an early phase of pulmonary hypertension and a long late phase of steady state increased pulmonary vascular permeability during which pulmonary arterial and left atrial pressures were not increased significantly and lung lymph flow was 5 times the baseline value. Lymph: plasma total protein concentration ratio during the late phase (0.76 ± 0.04) was significantly (P < 0.05) higher than during baseline (0.66 ± 0.03). The lymph response was reproducible. Lung lymph clearance of endogenous proteins with molecular radii (r) 35.5 to 96 Å was increased during the steady state phase of the reaction, but, as during baseline, clearance decreased as r increased. The endotoxin reaction was similar to the reaction to infusing whole \textit{Pseudomonas} bacteria, except that endotoxin had less effect on pressures during the steady state response and caused a relatively larger increase in lymph clearance of large proteins. We conclude that \textit{E. coli} endotoxin in sheep causes a long period of increased lung vascular permeability and may have a greater effect on large solute pathways across microvessels than do \textit{Pseudomonas} bacteria.
Methods

Experimental Preparation

We have published several descriptions of how we prepare sheep so that lung lymph can be collected and vascular pressures measured (Brigham et al., 1974; Brigham et al., 1975b; Staub et al., 1975; Erdmann et al., 1975; Brigham et al., 1976a, 1976b). Through three thoracotomies we put catheters into the left atrium, pulmonary artery, and the efferent duct from the caudal mediastinal lymph node. We ligated the tail of that node at the lower margin of the inferior pulmonary ligaments to eliminate systemic lymph (Staub et al., 1975), and we put catheters through neck vessels into the superior vena cava and thoracic aorta. Sheep recovered from surgery and had a stable flow of blood-free lymph by 3-5 days after surgery; then we made experiments.

Experimental Protocols

Throughout every experiment, sheep stood in a cage, unanesthetized, while we continuously recorded pulmonary arterial, left atrial, and aortic pressures, using strain gauges (Micron Instruments) and an electronic recorder (Hewlett-Packard). The zero reference for pressures was the level of the left atrium. We measured lung lymph flow by recording the amount accumulated in a graduated centrifuge tube each 15 minutes. We collected aortic blood each hour and pooled lymph each half hour and measured their protein concentrations.

Eight times in seven sheep, after a 1- to 2-hour stable baseline period, we gave intravenous infusions of \textit{E. coli} endotoxin (0.07 to 1.33 \textmu g/kg body weight) over 30 minutes using a constant rate infusion pump (Harvard Apparatus). In all experiments we used the same lot (number 3123-25) of endotoxin prepared according to the Westphal method by Difco Laboratories from \textit{E. coli} 0127:B8. The endotoxin was dissolved in 20 ml of sterile, pyrogen-free 0.89% NaCl solution immediately before the infusion was begun. After infusing endotoxin, we followed sheep until at least 2 hours of stable lymph flow and pressures were recorded (6-9 hours).

In four sheep we repeated infusions of the same dose of endotoxin 2 to 5 times over 1 to 2 weeks, allowing at least one day between infusions to test the reproducibility of the lung lymph response.

Protein Measurements

We measured total protein concentrations in lymph and blood plasma by a modified biuret method (Failing et al., 1960) with an automated system (AutoAnalyzer, Technicon Instruments); duplicate samples differed by less than 5%.

We separated proteins in blood plasma and lymph samples from steady state baseline and experimental periods by polyacrylamide gradient gel electrophoresis. The methods have been described (Brigham et al., 1975b; Brigham and Owen, 1975a; 1975b; Brigham et al., 1976a). We used 4-30% polyacrylamide gradient gels and consistently identified eight protein fractions in plasma and lymph. To estimate the effective molecular radius of each fraction, we calibrated the gels with five proteins with known free diffusion coefficients. From a standard curve of migration distance plotted against Einstein-Stokes radius for the five known proteins [published elsewhere Brigham and Owen, 1975a, 1975b], we estimated the radius of the eight plasma and lymph fractions.

Arterial Blood Gases and pH

We collected arterial blood samples anaerobically during steady state baseline and experimental periods and measured \textit{P}O\textsubscript{2}, \textit{PCO}2 and \textit{pH} using a blood gas analyzer (Instrumentation Laboratories).

Statistics

We tested the significance of differences between steady state baseline and experimental observations using a paired \textit{t}-test (Snedecor and Cochran, 1967). We also calculated means and standard errors for the steady state data and regression lines and correlation coefficients for the dose-response data.

Results

Figure 1 shows the pulmonary vascular response to \textit{E. coli} endotoxin infusion in a sheep. The reaction is similar to that we described earlier caused by infusing whole \textit{Pseudomonas} bacteria (Brigham et al., 1974; Brigham et al., 1976b). Shortly after the endotoxin infusion was begun, there was a large increase in pulmonary artery pressure, an increase in lymph flow, and a decrease in lymph-plasma
protein concentration ratio. Pulmonary artery pressure and lymph flow then decreased and the lymph:plasma protein concentration ratio rose. Four hours after the endotoxin infusion, lymph flow again increased, reaching a stable plateau while pulmonary vascular pressures were stable and the lymph:plasma protein concentration ratio was higher than during baseline. We define this long late period of high flow of protein-rich lymph as the steady state experimental period.

Table 1 summarizes the steady state data from baseline and experimental periods for the eight studies. Although pulmonary artery pressure tended to be higher and left atrial pressure lower than baseline in the experimental period, neither difference was significant. Lung lymph flow was on average 5 times higher than baseline during the experimental period, and the lymph:plasma protein concentration ratio was about 20% higher than baseline. Arterial Po2 tended to fall during the experimental period but, again, the difference from baseline was not significant.

The steady state experimental lymph flow response to endotoxin was quite reproducible. This is illustrated in Figures 2 and 3. Figure 2 shows the relationship between lymph flow response and endotoxin dose for the eight studies summarized in Table 1. Even though the studies were done in seven different sheep, there is excellent correlation between endotoxin dose and lymph response over a wide range of endotoxin doses. Figure 3 shows the steady state lymph response to repeated infusions of the same endotoxin dose in four sheep. The response is fairly reproducible in a given sheep for up to three infusions over the period of a week. As expected, with many repetitions over 2 weeks, endotoxin infusions have less effect (Petersdorf and Shulman, 1964).

The effects of endotoxin on steady state lung lymph flow as a function of estimated microvascular pressure are compared with previously reported effects of *Pseudomonas* infusion (Brigham et al., 1975; Bowers et al., 1977) and mechanically increased pressure (Bowers et al., 1976b) and for the endotoxin experiments. When lung vascular pressures are increased mechanically, there is a predictable fall in lymph:plasma protein concentration as lymph flow increases (Erdmann et al., 1975; Bowers et al., 1977). Large increases in lymph flow occur after *Pseudomonas* while lymph:plasma protein concentration tended to decrease slightly, but in six of the eight endotoxin studies, lymph:plasma protein concentration was 20% or more above baseline in spite of a 3- to 8-fold increase in lymph flow.

Table 2 summarizes the lymph:plasma concentration ratios for eight electrophoretically separated protein fractions during baseline and endotoxin experimental periods. The mean ratio was higher than baseline during the steady state period after endotoxin for all of the fractions except albumin for which the baseline ratio already was quite high (0.84).

A better idea of the relationship between lung

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**TABLE 1** Summary of Steady State Hemodynamic, Blood Gas, and Lymph Data

<table>
<thead>
<tr>
<th>Condition</th>
<th>No. of experiments</th>
<th>Body wt (kg)</th>
<th>Mean pressure (cm H2O)</th>
<th>Total protein concentration (g/dl)</th>
<th>Arterial blood (torr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>of sheep</td>
<td></td>
<td>Pulmonary artery Left atrium Lung lymph flow (ml/hr)</td>
<td>Lymph</td>
<td>Plasma</td>
</tr>
<tr>
<td>Baseline</td>
<td>8/7</td>
<td>31.7 ± 1.4</td>
<td>22 ± 1</td>
<td>1 ± 1</td>
<td>7.5 ± 0.6</td>
</tr>
<tr>
<td>Endotoxin</td>
<td>8/7</td>
<td>31.7 ± 1.4</td>
<td>31 ± 4</td>
<td>1 ± 1</td>
<td>35.9 ± 4.9</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM.

* Significantly different from baseline (*P < 0.05*).

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**Figure 2** Steady state lung lymph flow response to different doses of E. coli endotoxin in sheep. The eight studies in seven sheep are the same as those summarized in Table 1.
lymph protein flux and molecular size is given in Figure 6 where lymph clearance (flow × lymph:plasma concentration) is plotted as a function of molecular radius for the eight protein fractions. For comparison, the previously published data for Pseudomonas infusion (Brigham et al., 1976b) is shown, as well as the baseline and experimental data from the endotoxin studies reported here. Endotoxin, like Pseudomonas, caused a marked increase in clearance of all eight protein fractions. Also, as with Pseudomonas, there is a decline in protein clearance with increasing molecular radius, but the decline is less steep in the endotoxin studies. This difference between endotoxin and Pseudomonas is illustrated further in Figure 7 where clearance relative to baseline is plotted for each of the eight proteins as a function of molecular radius. Whereas Pseudomonas tended to cause similar increases in relative clearance for all of the protein fractions, endotoxin clearly caused a relatively larger increase in lymph clearance of the larger proteins.

Discussion

There is a sizable body of data supporting the assumptions that lung lymph flow and protein concentrations reflect exchanging vessel filtrate flow and protein concentration under steady state conditions in the lung (Staub, 1974; Nicolaysen et al., 1975; Vreim et al., 1976). If those assumptions are valid, then *E. coli* endotoxin increased lung microvascular permeability in our experiments. Endotoxin caused a long period of high lymph flow with little effect on pulmonary vascular pressures. Lymph flow increased on the average to five times baseline; whereas, when permeability is normal, increasing lung microvascular pressure by 15 mm H2O only doubles lymph flow (Fig. 4) (Erdmann et al., 1975; Bowers et al., 1977). Lymph:plasma protein concentration ratios were higher than baseline during the steady state endotoxin response. This differs from the effects of increasing pressure in lung microvessels with normal permeability. In that situation, lymph flow increases but lymph:plasma protein concentration ratio declines linearly with increasing lymph flow (Fig. 5) (Erdmann et al., 1975; Brigham and Owen, 1975a, 1975b). The high flow of protein-rich lung lymph with low pulmonary vascular pressures is explicable only by an increased leak of fluid and protein from lung exchanging vessels.

However, endotoxin did not produce gross leaks in exchanging vessel walls because it did not eliminate sieving of proteins less than 100 Å molecular radius. During the steady state period of high lymph flow, lymph clearance of plasma proteins 35.5 to 96 Å radius was increased dramatically from baseline, but clearance still decreased with increasing molecular size (Fig. 6). We have seen this same general pattern in every increased permeability state which we have produced (Brigham et al., 1974; Brigham and Owen, 1975b; Brigham et al., 1976a, 1976b). It must mean, as expected from theory (Brigham et
al., 1974; Blake and Staub, 1976; Harris et al., 1976), that large increases in fluid filtration can result from microvascular damage too trivial to preclude protein molecular sieving.

The endotoxin effect on lung vascular permeability was quite reproducible. The dose-response curve illustrated in Figure 2 suggests reproducibility of the lymph flow response among sheep. The responses of the same sheep to repeated endotoxin infusions shown in Figure 3 indicate good reproducibility in the same animal for up to three infusions over 7 days. This observation means that the experimental model should be good for studying the effects of various interventions on the permeability response.

The general course of the lung vascular reaction to endotoxin was similar to that we reported for infusions of whole Pseudomonas bacteria in sheep (Brigham et al., 1974; Brigham et al., 1976b). Both caused an initial phase of pulmonary hypertension followed by a long late phase of high vascular permeability. However, the endotoxin and Pseudomonas reactions do differ. Pulmonary vascular pressures were lower during the late phase with endotoxin than with Pseudomonas even though lymph flows were similar to those with Pseudomonas even though microvascular pressure was slightly lower. It also may be that there is a qualitative difference between the effects of endotoxin and Pseudomonas on lung exchanging vessels, endotoxin having a greater effect on large solute pathways [the "large pore" system (Blake and Staub, 1976; Harris et al., 1976)].

In animals, E. coli endotoxin increases permeability to fluid and protein in the systemic circulation (Chien et al., 1964) and causes edema in the lungs (Snell and Ramsey, 1969). E. Coli septicemia in humans can cause severe pulmonary edema with an increased leak of large molecules from the vascular space to lung edema fluid (Robin et al., 1972). Our studies document a reproducible increase in lung vascular permeability to fluid and proteins caused by E. coli endotoxin in sheep. Protein molecular sieving by exchanging vessels appears to persist during the period of increased permeability, but the relatively larger increase in lung lymph clearance of large proteins than of smaller ones suggests that

<table>
<thead>
<tr>
<th>Condition</th>
<th>Protein molecular radius (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>35.5 42 45 48 62 72 82 96</td>
</tr>
<tr>
<td>(8)</td>
<td>0.84 ±0.04 0.77 ±0.05 0.67 ±0.10 0.54 ±0.06 0.60 ±0.06 0.60 ±0.05 0.49 ±0.05</td>
</tr>
<tr>
<td>Endotoxin</td>
<td>0.82 ±0.01 0.83 ±0.04 0.98 ±0.13 0.73 ±0.04* 0.70 ±0.06 0.64 ±0.09 0.70 ±0.06</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM. * Significantly different from baseline (P < 0.05).
endotoxin may affect predominantly large solute pathways across microvessels. These findings are consistent with reported data for animals (Snell and Ramsey, 1969; Reeves et al., 1972) and humans (Robin et al., 1972) and implicate increased pulmonary vascular permeability as an important lesion in pulmonary edema accompanying gram-negative sepsis.

References
Increased sheep lung vascular permeability caused by Escherichia coli endotoxin.

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