Leukocytes Are Required for Increased Lung Microvascular Permeability after Microembolization in Sheep

Michael R. Flick, Azriel Perel, and Norman C. Staub

SUMMARY We studied the effects of uneven pulmonary artery obstruction by microemboli on steady state transvascular fluid and protein exchange in normal and leukopenic sheep. We measured pulmonary artery and left atrial pressures, cardiac output, lung lymph flow, and lymph plasma protein concentrations. Sheep were made profoundly leukopenic by administration of intra-arterial mechlor-ethamine hydrochloride (0.4 mg/kg, two doses) and colchicine (0.1-0.2 mg/kg, anesthetized sheep only). In anesthetized sheep, we injected glass beads 200 μm in diameter via the right atrium to raise pulmonary vascular resistance to 2-3 times baseline values. With normal levels of circulating leukocytes, sheep developed an increased protein-rich lymph flow from the lung characteristic of increased permeability edema. Leukopenic sheep had a significantly attenuated response after embolization for equivalent degrees of vascular obstruction. In unanesthetized sheep, we continuously infused air bubbles 1 mm in diameter via the right atrium to raise pulmonary vascular resistance to about 2 times baseline values. Each sheep served as its own control. With normal circulating leukocyte levels, there was an increase in protein-rich lymph flow from the lung during embolization. When the air infusion ended, the sheep recovered to the baseline condition in 24 hours. We induced emboli with the same amount of air when the sheep were profoundly leukopenic; lymph and protein flow from the lung were significantly less for equivalent degrees of obstruction. We conclude that circulating leukocytes are essential for the microvascular injury that results in increased permeability in the lungs of sheep after microembolization.

Methods

Surgical Preparation

Acute Experiments

We have reported in detail the basic sheep preparation that permits collection of nearly pure lung lymph (Staub et al., 1975; Ohkuda et al., 1978). We used 14 yearling female lambs weighing 27-43 kg (average, 35 kg). At preliminary surgery (thiopental sodium, 20-30 mg/kg, iv for induction, halothane for maintenance), we made a left thoracotomy and implanted polyvinyl catheters directly into the pulmonary artery and left atrium. A 2F-thermistor (Kimray Medical Associates, Inc.) was attached to the pulmonary artery catheter to be used later for the determination of cardiac output by thermodilution. After surgery, we allowed the animal to recover for 4 days. We then arbitrarily assigned animals to one of four groups: control, leukopenia, emboli, or leukopenia-emboli. During the next 6 days, we injected all sheep with intra-arterial gentamicin [2-3 mg/(kg × d)] and chloramphenicol [25-35 mg/(kg × d)].

On the day of the experiment, each sheep was anesthetized (thiopental sodium 20-30 mg/kg iv for induction, halothane for maintenance), intubated and ventilated (50% oxygen, 50% nitrogen). Through a right thoracotomy we cannulated the efferent duct of the caudal mediastinal lymph node and ligated and resected the distal portion of the node caudal to the pulmonary ligament. We also
placed polyvinyl catheters in the right atrium via an external jugular vein for injection of glass beads and fluid for thermodilution cardiac output, and in the descending aorta via a carotid artery to record systemic arterial pressure. The animal was placed on its back for the duration of the experiment.

We connected the pulmonary artery, left atrial, and aortic catheters to strain gauges (Statham P23G, Gould-Statham Instruments, Inc.) and recorded pressures continuously on a direct-writing polygraph (Model 7, Grass Instr. Co.). We determined cardiac output on a computer (#3500 COC, Kimray Medical Associates, Inc.) by the thermodilution technique, injecting 5 ml of 5% dextrose in water at room temperature into the right atrium three times every 15 minutes. We used the mean value of data obtained from these injections to calculate pulmonary vascular resistance as mean pulmonary artery minus mean left atrial pressure divided by mean cardiac output.

We collected lung lymph in heparinized graduated test tubes, recorded flow every 15 minutes and collected the lymph every 30 minutes, together with a simultaneous heparinized blood sample for determination of total plasma protein and albumin concentration (Erdmann et al., 1975). Arterial blood samples were taken every 60 minutes for determination of PaO₂, PaCO₂ and pH (Radiometer, Copenhagen). We counted leukocytes and platelets in plasma and lymph every 60 minutes in a hemacytometer by phase contrast microscopy.

We maintained these sheep throughout the experiment with 0.75–1.5% halothane anesthesia. We adjusted the inspired oxygen fraction and minute ventilation to maintain PaO₂ >100 mm Hg and PaCO₂ < 40 mm Hg. During the experiment the animals were anticoagulated with intermittent heparin injections.

Chronic Experiments
We used four female lambs weighing 27–39 kg (average weight, 32 kg). These lambs also were prepared in two stages. We anesthetized and ventilated the animal in the same way as for the acute experiments. At the left thoracotomy, in addition to inserting catheters directly into the pulmonary artery and left atrium, we placed an ultrasound flow cuff (Parks Electronics Lab) around the main pulmonary artery for later determination of cardiac output by Doppler shift. After surgery, we allowed the animal to recover for 7 days. The second operation consisted of the two right thoracotomies already described for acute experiments, but was concluded by bringing the lymph catheter to the side and closing the chest (Staub et al., 1975). We then placed catheters in the descending aorta through the right carotid artery and in the right atrium through the right external jugular vein. Attached to the right atrial catheter was a small (PE-60 Intramedic, Clay-Adams) catheter through which we later infused air. Air infused through this catheter into plasma gave bubbles about 1 mm in diameter. The animal was allowed to recover for 5–7 days before experiments began.

During experiments, we attached the pulmonary arterial, left atrial, and aortic catheters to strain gauges (model MP-15, Micron Instruments, Inc., or model P23 ID, Gould-Statham Instruments, Inc.) at the level of the left atrium and recorded pressures continuously on the direct-writing polygraph. We determined cardiac output using an ultrasonic flowmeter (model 806A, Parks Electronics Lab) and continuously recorded it on the polygraph. We calculated pulmonary vascular resistance every 15 minutes.

Lung lymph was collected and analyzed as described for the acute experiments. We collected plasma every 60 minutes for total protein and albumin determinations. At the same interval, we took arterial blood for analysis of PaO₂, PaCO₂, and pH, and counted circulating leukocytes and platelets. During experiments, the sheep were anticoagulated with intermittent heparin injections.

We housed the chronically instrumented sheep in mobile metabolic cages. The animals were awake, unrestrained, breathing room air spontaneously, and had free access to food and water during experiments. To maintain stability, we did not permit the sheep to lie down during studies.

Leukocyte Depletion
Acute Experiments
We injected the sheep with mechlorethamine hydrochloride, 0.4 mg/kg (Merck, Sharp & Dome), intra-arterially on the 6th day and again on the 3rd day prior to the experiment. On the day of the experiment, we gave colchicine (Eli Lilly & Co.), 0.1–0.2 mg/kg intra-arterially, 3 hours prior to cannulation of the lymphatic duct.

Chronic Experiments
We injected these sheep with two doses of mechlorethamine hydrochloride following the same regimen as for the acute experiments. We did not give colchicine in the chronic experiments.

Experimental Procedure
Acute Experiments
A. Controls: In four sheep, after lung lymph flow and hemodynamics became stable, we made no interventions but monitored all variables for 8 hours.

B. Leukopenia: In two sheep, made leukopenic, we did not achieve successful lymph cannulation but left the efferent lymph duct to drain freely into the open chest. We monitored all the other variables for 8 hours.

C. Microemboli: In four sheep, after a stable 2-hour baseline period, we injected silicone-coated glass microspheres 200 μm in diameter into the right atrium in 1- to 3-g increments over 30–60
minutes until pulmonary vascular resistance increased to about three times the baseline value. We monitored all the variables for 6 hours, including 3-4 hours of a new steady state.

D. Microemboli and Leukopenia: In four leukopenic sheep, after a stable 2-hour baseline period, we induced microemboli as in group C.

At the conclusion of every experiment, we killed the sheep by clamping the hila and removing the lungs and measured extravascular lung water, using a single marker (hemoglobin) for the residual blood mass in the lung (Flick et al., 1979) and correcting for the weight of glass beads recovered in the lung.

Chronic Experiments

A. Controls: Each sheep was monitored for 10 hours, during which we recorded all variables.

B. Air Emboli: After a 2-hour stable baseline period, we infused room air continuously through the small right atrial catheter. We chose an air dose for each sheep sufficient to raise pulmonary vascular resistance to about twice the baseline value. Air infusion doses ranged from 0.046 to 0.064 ml/(min \( \times \) kg). Each animal received the same amount of air each time emboli were given. We infused air for 4 hours, then monitored the animal for 4 hours of recovery.

C. Leukopenia: In these experiments, we observed the immediate effects of mechlorethamine hydrochloride injection. After a 2-hour baseline, we gave the mechlorethamine as an intra-arterial bolus (0.4 mg/kg), then watched the sheep for the next 4-6 hours.

D. Air Emboli and Leukopenia: We observed the effects of air emboli in the leukopenic sheep 3 days after the first dose of mechlorethamine and again 3 days after the second dose (6 days after the first dose). In two of these sheep, we also gave air emboli 21 and 49 days after the first dose of mechlorethamine as circulating leukocytes returned.

Statistics

In the acute experiments, we compared the baseline and the final 2-hour steady state periods (postembolic) within each group using a paired Student’s t-test, and between groups using an unpaired Student’s t-test. Between the microemboli (group C) and microemboli and leukopenia (group D) groups, we also compared the regression lines of lymph protein flow as a function of pulmonary vascular resistance by analysis of covariance.

In the chronic experiments, we compared the 2-hour baseline and embolic steady state periods within and among experiments, using analysis of variance for single factor experiments having repeated measures of the same elements (Winer, 1971). We compared the regression lines of lymph protein flow as a function of pulmonary vascular resistance by analysis of covariance.

The data are expressed as the group means ± 1 sd. In all statistical tests, we accepted \( P < 0.05 \) as indicating statistical significance. The size of each group is small (n = 4), but in every group, each animal responded in the same way.

Results

Acute Experiments

A. Controls: There were no significant variations in hemodynamic or lung fluid balance measurements over the 8 hours of observation. The group data are not included in Table 1 since similar control data have been published before (Ohkuda et al., 1978; Binder et al., 1979, 1980). Extravascular lung water in these sheep averaged 3.8 ± 0.4 g/g dry lung, which is in the normal range for our

### Table 1 Effects of Leukopenia on Pulmonary Hemodynamics and Lung Fluid Balance after Microembolization in Anesthetized Sheep (Acute Experiments)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Pulmonary hemodynamics</th>
<th>Lung fluid balance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Circulating leukocytes (10⁶/mm³)</td>
<td>Pulmonary arterial pressure (cm H₂O)</td>
</tr>
<tr>
<td>A. Normal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline*</td>
<td>4</td>
<td>12.9</td>
</tr>
<tr>
<td></td>
<td>± 2.5</td>
<td>± 3.2</td>
</tr>
<tr>
<td>Microemboli</td>
<td>13.1</td>
<td>32.5</td>
</tr>
<tr>
<td></td>
<td>± 3.4</td>
<td>± 6.9(s)</td>
</tr>
<tr>
<td>B. Leukopenia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>4</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>± 0.1(ss)</td>
<td>± 3.3</td>
</tr>
<tr>
<td>Microemboli</td>
<td>0.1</td>
<td>34.0</td>
</tr>
<tr>
<td></td>
<td>± 0.1(ss)</td>
<td>± 4.3(s)</td>
</tr>
</tbody>
</table>

Results are expressed as average ± sd. (s) = statistically significant at \( P < 0.05 \) by paired t-test between baseline and microemboli; (ss) = statistically significant at \( P < 0.05 \) by unpaired t-test between normal and leukopenia groups.

* 2-hour baseline period and 2-hour steady state after microemboli.
laboratory. Frozen lung sections showed no evidence of interstitial or alveolar edema.

**B. Leukopenia:** The two animals had stable hemodynamic measurements and arterial blood gases over the 8 hours of observation (data not included). Extravascular lung waters in these sheep were 4.2 and 4.4 g/g dry lung. These are in the upper normal range for our laboratory. Frozen lung sections showed no evidence of interstitial or alveolar edema.

**C. Microemboli:** The time course of one experiment is shown in Figure 1. The summary data are shown in Part A (Normal) of Table 1. Following the administration of enough glass beads in these four sheep (0.4 ± 0.03 g/kg) to raise pulmonary vascular resistance to about three times the baseline value, lymph flow more than doubled (114% average increase) without a change in lymph:plasma protein concentration ratio. The platelet count fell slightly (17%), but we did not detect any change in circulating leukocyte count or in the hematocrit. After embolization, peak inspiratory airway pressure rose, PaO₂ fell and PaCO₂ rose, and respiratory acidosis began to develop.

Terminally, extravascular lung water averaged 4.3 ± 0.3 g/g dry lung. We saw interstitial edema (in the form of peribronchovascular cuffs) in every animal; there was no evidence of alveolar edema.

**D. Microemboli and Leukopenia:** The time course of one experiment in a leukopenic sheep is shown in Figure 2. The data are summarized in Part B (Leukopenia) of Table 1. There were no significant differences in the baseline period data between normal and leukopenic sheep except in circulating white blood cell count. White blood cell count in the normal sheep averaged 12.9 ± 2.5 X 10³ cells/mm³ (40% polymorphonuclear leukocytes) during the baseline period and had been stable for the 7 days prior to the experiment. Leukopenic sheep, beginning with circulating white blood cell counts the same as normal sheep, had only 0.2 ± 0.2 X 10³ cells/mm³ (all lymphocytes) on the day of the experiment. Platelet count, hematocrit, and arterial blood gases were the same in normal and leukopenic sheep in the baseline period.

Following the administration of enough glass beads (0.5 ± 0.3 g/kg) to the leukopenic sheep to raise pulmonary vascular resistance to more than three times baseline values, lymph flow increased by 94% on the average. This increase is not statistically significant. There was a significant fall in lymph:plasma protein concentration ratio, and total protein flow out of the lung in lymph was less in the leukopenic sheep than in the normal ones. The effects of emboli on the protein flow in normal and leukopenic sheep are compared in Figure 3. The figure shows the data for each normal and leukopenic sheep. Three of the four leukopenic sheep show less increase in protein flow after embolization. The group linear regressions of lymph protein flow (Qprt) in mg/h on pulmonary vascular resistance (PVR) in cm H₂O/liter per min for each group are:

- **Normal:** Qprt = 55PVR + 235 (r = 0.57)
- **Leukopenia:** Qprt = 13PVR + 227 (r = 0.46)
Lymph Protein Flow (mg/hl)

FIGURE 3 Comparison of lung protein flow (lymph flow x protein concentration) as a function of the pulmonary vascular resistance in the acute experiments. Each of the four pairs of steady state data before and after emboli are shown for the normal (filled circles) and the leucopenic (unfilled circles) sheep.

The difference in slope is statistically significant.

Comparing postembolization steady states, pulmonary vascular resistance was 75% higher in leucopenic sheep compared to normal ones. After embolization, the circulating platelet count fell by 27% in the leucopenic sheep (not different from the fall seen in normal sheep). There was no change in circulating leukocyte count or in the hematocrit. Arterial blood gases were not different from normal sheep.

Extravascular lung water in the leucopenic sheep averaged 4.8 ± 0.6 g/g dry lung. We saw interstitial edema (in the form of peribronchovascular cuffs). There was no grossly evident alveolar edema.

Chronic Experiments

A. Controls: There were no significant variations in hemodynamic or lung fluid measurements over the 10-hour observation period (data not included in Table 2).

B. Air Emboli: The time course of one experiment is shown in Figure 4 (broken lines). The summary data from these experiments are shown in Part A (Normal) of Table 2. In the 2-hour baseline period, data for these sheep did not differ from measurements made in the control experiments. During the administration of air for 4 hours at a rate [0.058 ± 0.0085 ml/(min X kg)] sufficient to raise pulmonary vascular resistance to about 3 times the baseline value, lung lymph flow quadrupled without a change in lymph:plasma protein concentration ratio. There were no changes in circulating leukocyte count, platelet count, or hematocrit. Arterial blood showed a significant fall in Pao2 from baseline (97 ± 7 to 71 ± 14 mm Hg), but pH and Paco2 were not changed significantly from baseline values.

C. Leukopenia: The time course of the acute effects of mechloretamine injection is shown in Figure 5. There were no changes over the 4-hour observation period in hemodynamics, lung lymph variables, arterial blood gases, or counts of formed elements in the blood (data not included in Table 2).

D. Air Emboli and Leukopenia: The time course of an experiment 6 days after the first nitrogen mustard injection is shown in Figure 4 (solid lines), and the data from all four such experiments are summarized in Part B (Leukopenia) of Table 2. Circulating leukocytes had fallen by 94% from baseline values (from 9.2 ± 2.0 X 103 to 0.6 ± 0.2 X 103 cells/mm3 and from about 50% polymorphonuclear

### Table 2 Effects of Leukopenia on Pulmonary Hemodynamics and Lung Fluid Balance after Air Embolization in Unanesthetized Sheep (Chronic Experiments)

<table>
<thead>
<tr>
<th>Condition</th>
<th>No.</th>
<th>Circulating leukocytes (10³/mm³)</th>
<th>Pulmonary arterial pressure (cm H₂O)</th>
<th>Left atrial pressure (cm H₂O)</th>
<th>Cardiac output (liters/min)</th>
<th>Pulmonary vascular resistance (cm H₂O/liter per min)</th>
<th>Lymph flow (ml/hr)</th>
<th>Lymph protein concentration (lymph:plasma)</th>
<th>Lymph protein flow (mg/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Normal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>4</td>
<td>9.2 ± 2.0</td>
<td>22.2 ± 4.0</td>
<td>6.8 ± 0.7</td>
<td>6.8 ± 1.7</td>
<td>2.5 ± 1.3</td>
<td>5.1 ± 0.7</td>
<td>0.72 ± 0.06</td>
<td>202 ± 52</td>
</tr>
<tr>
<td>Microemboli</td>
<td></td>
<td>10.4 ± 4.9</td>
<td>40.5 ± 6.0</td>
<td>−0.2 ± 0.2</td>
<td>6.0 ± 0.9</td>
<td>7.3 ± 0.7</td>
<td>20.9 ± 96</td>
<td>0.05 ± 0.05</td>
<td>817 ± 66</td>
</tr>
<tr>
<td>B. Leukopenia</td>
<td>4</td>
<td>0.6 ± 0.2</td>
<td>18.0 ± 6.4</td>
<td>1.2 ± 0.2</td>
<td>4.6 ± 0.9</td>
<td>3.7 ± 0.6</td>
<td>4.6 ± 0.6</td>
<td>0.65 ± 0.12</td>
<td>137 ± 47</td>
</tr>
<tr>
<td>Baseline</td>
<td></td>
<td>0.6 ± 0.2</td>
<td>31.9 ± 6.4</td>
<td>0.4 ± 0.2</td>
<td>3.7 ± 0.7</td>
<td>8.9 ± 0.7</td>
<td>8.3 ± 0.58</td>
<td>0.59 ± 0.28</td>
<td>238 ± 58</td>
</tr>
<tr>
<td>Microemboli</td>
<td></td>
<td>0.6 ± 0.2</td>
<td>31.9 ± 6.4</td>
<td>0.4 ± 0.2</td>
<td>3.7 ± 0.7</td>
<td>8.9 ± 0.7</td>
<td>8.3 ± 0.58</td>
<td>0.59 ± 0.28</td>
<td>238 ± 58</td>
</tr>
</tbody>
</table>

Results are expressed as average ± SD. (s) = statistically significant at P < 0.05 by analysis of variance between baseline and microemboli; (ss) = statistically significant at P < 0.05 by analysis of variance between normal and leukopenia states.

* 2-hour baseline period and 2h steady state during microemboli.
Figure 4: Comparative time course of two chronic embolization experiments in a chronic (unanesthetized) sheep. During the infusion period the animal was embolized with room air at the rate of 0.06 ml/(kg × min). A: Broken lines are experiment when circulating leukocyte concentrations were normal. B: Solid lines are for the same animal when leukopenic.

leukocytes to 100% lymphocytes). Circulating platelets had fallen by 37% and hematocrit had risen by 15%. Plasma total protein was lower, principally because plasma globulins were lower. Cardiac output was reduced, but pulmonary artery and left atrial pressures and pulmonary vascular resistance were not different.

During embolization with air at the same rate as in the experiments when circulating leukocyte counts were normal, pulmonary vascular resistance increased to about 2.5 times baseline values. Lymph flow increased in each animal from the baseline steady state, but the increase averaged only 80% and was markedly less than in the normal condition. Lymph:plasma protein ratios did not change significantly. Protein flow from the lung was significantly lower than when the sheep were normal. The paired protein flow responses to emboli in each normal and leukopenic sheep are compared in Figure 5. The rate of increase of lung total protein flow is reduced for each animal when leukopenic as compared to its control response. The group linear regressions of lymph protein flow on pulmonary vascular resistance are:

Normal: $Q_{prt} = 109PVR - 27 \quad (r = 0.97)$
Leukopenia: $Q_{prt} = 19PVR + 70 \quad (r = 0.81)$

The difference in slope is statistically significant.

There were no changes in circulating leukocyte and platelet counts or hematocrit during air embolization in the leukopenic sheep. $P_{aO_2}$ was 87 ± 12 mm Hg in the leukopenic embolized sheep and was not significantly less than in the baseline period. This was in contrast to the large decrease in $P_{aO_2}$ during embolization in the normal state. $P_{aco_2}$ and pH did not change significantly.

We also did experiments while circulating leukocytes were falling, on day 3 after the initial dose of mechlorethamine when circulating leukocyte counts were depressed by 70% below baseline values. In each experiment, the lung lymph and protein flow response to air emboli was decreased by 50%.

In two sheep, we also did experiments 3 weeks and 7 weeks after the initial dose of mechlorethamine, when circulating leukocytes were recovering toward baseline values. At 3 weeks, leukocytes were at 20% of baseline values and air emboli resulted in 50% of the rise in lung lymph and protein flow seen while the animals were normal. At 7 weeks, although the leukocyte counts were still only at about
50% of baseline values, the air emboli caused increases in lung lymph and protein flow that were greater than the original response.

**Discussion**

Our results show that, in sheep, most of the increase in microvascular permeability seen after microembolization of the lung with glass beads or air bubbles is caused in some way by circulating leukocytes. Our sheep model, which allows the separation of high pressure edema from permeability edema (Brigham et al., 1974; Erdmann et al., 1975), was stable in the acute experiments for at least 8 hours and in the chronic experiments for at least 10 hours. Injection into the pulmonary circulation, of either siliconized glass microspheres 200 μm in diameter (acute sheep) or air bubbles 1 mm in diameter (chronic sheep) until pulmonary vascular resistance was 2-3 times baseline levels resulted in the high lymph flow-high protein flow we previously have shown to be characteristic of permeability edema (Brigham et al., 1974). Since microvascular pressures were probably increased in the over-perfused open circulation, a high pressure type of edema (decreased lymph protein concentration) (Erdmann et al., 1975) coexisted with the permeability defect.

In chronically instrumented animals, when we stopped embolizing the lung with air, lymph and protein flow out of the lung increased to levels higher than during embolization (Fig. 4). This was at a time when vascular pressures were decreasing as the obstructing air was absorbed. We attribute these increases to perfusion of areas of vasculature previously obstructed, with the result being an increase in surface area for fluid and protein flow across the damaged vascular endothelium. This is further evidence that the main site of increased fluid and protein leakage is in open perfused vessels of the microcirculation (Ohkuda et al., 1978).

We depleted circulating leukocytes in 13 acutely studied sheep with a combination of mechlorethamine and colchicine. Only six of them successfully completed the regimen and the experiment. Three animals died from sepsis; in four others we could not obtain a stable 2-hour baseline period, which is an absolute requirement in our laboratory. These unacceptable animals had low cardiac outputs, high pulmonary vascular resistances, and progressively rising lung lymph flow.

Because we were concerned about the combined effects of leukocyte depletion, anesthesia, and surgery, we repeated the experiments in the chronic, unanesthetized sheep to compare the results. We were able to maintain the chronically instrumented animals in better general health during their leukocyte depletion (using mechlorethamine alone). Mechlorethamine had no acute toxic effects on lung fluid and protein balance or hemodynamics, and there was no evidence that it affected lymphatic function (Fig. 5). In both acute and chronic experiments, circulating leukocytes were depressed markedly (to less than 10% of original values) by the depletion regimen.

When we injected microemboli into the pulmonary circulation of leukopenic animals, fluid and protein flow out of the lung increased, but to a lesser extent (for equivalent degrees of obstruction) than in animals with normal circulating leukocyte counts. The modest increase in lymph flow with lower protein is compatible with high pressure edema. We expected this, since pressure in the open, perfused microcirculation probably was increased. In the chronic, leukopenic animals, when we stopped infusing air, there was a significant but small increase in lung lymph and protein flow as the vascular bed reopened and surface area increased. This suggests there is some microvascular injury. The air emboli may injure the endothelium directly, but such injury is small compared to that occurring when the animal has normal circulating leukocyte levels.

We found no significant differences in lung water among the four acute experimental groups. The two leukopenic control sheep had lung water at the upper limit of normal for our laboratory (Erdmann et al., 1975). This is not too surprising; mechlorethamine is a powerful cytotoxic agent. The leukopenic sheep had no histological evidence of interstitial edema. It is possible that any extra lung water was intracellular. As for the lung water measurements after embolization, we had difficulty in accounting for the weight of the glass beads infused. Even in control experiments, we could recover only about 85% of beads added to the lung. Subtraction of the weight of glass beads is a large correction in the dry weight of the lung. Even small error may lead to a large difference in apparent lung water. This, we believe, accounts for the variability we found.

Leukocytes must play a central role in this type of increased permeability edema, since all of our leukopenic animals, both acute and chronic, showed less response to microemboli. Our prior experiments in sheep with fibrinogen or platelet depletions did not reveal any effect on lung lymph and protein flow after emboli (Binder et al., 1979, 1980). Actually, platelets do mediate an increase in permeability. After administration of antiplatelet serum, there is an immediate increase in pulmonary artery pressure and fluid flow out of the lung (Binder et al., 1980), at a time when platelets are probably aggregating in the lung. However, this increase is transient (about 1 hour), and whatever platelets do to increase permeability is not sustained. The sustained injury to the microvascular endothelium does not occur immediately. In both septicemia (Brigham et al., 1974) and with microembolization (Ohkuda et al., 1978), the full effect of injury is not reached for 2-5 hours.

A role for leukocytes in other forms of permea-
bility edema in sheep has been shown. Depletion of circulating leukocytes prevents permeability edema after infusion of sheep plasma which has been in-cubated with hemodialyzer cellophane or zymosan (Craddock et al., 1977), and after endotoxemia (Heflin and Brigham, 1979).

However, a central role for leukocytes in the mechanism of permeability edema has not been shown in all animal models. Leukopenia does not prevent the hemodynamic and blood gas changes after *Escherichia coli* endotoxemia in baboons (Guenter, 1971), ethchlorvynol administration in dogs (Millen et al., 1978), or intravenous injection of thrombin and a fibrinolysis inhibitor in dogs (Busch et al., 1974). It is possible that the mechanism of permeability edema is different in dogs and baboons than in sheep. With respect to humans, we think it is likely that leukocytes do play a role in many types of permeability edema that are not mediated directly by chemical toxins, and that the clotting cascade and platelets serve an augmenting function by trapping leukocytes in the pulmonary microvasculature. However, comparative studies across species and with uniform protocols, including lung lymph collections, must be done.

We do not know how leukocytes damage the endothelium. It has been suggested that alterations in membranes cause leukocytes to adhere to the endothelium (Cross and Hyde, 1978), and that adherence and activation may be caused by complement (Craddock et al., 1977). The leukocyte may damage the endothelial physically, providing a channel for fluid and protein flow, as it migrates through the endothelium into the lung’s interstitium (Henson et al., 1979). The injury may be mediated by the release of superoxides, lysosomal enzymes, prostaglandins, leukotrienes, or other metabolites of the leukocyte closely approximated to the endothelial cell in the microvasculature. Although the exact sequence of events in the mechanism of permeability edema is still not known, our experiments show that the increased microvascular permeability which follows pulmonary microemboli in sheep requires circulating leukocytes.

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