Role of Intravascular Coagulation and Granulocytes in Lung Vascular Injury after Bone Marrow Embolism

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SUMMARY. We examined the role of intravascular coagulation and granulocytes in mediating the alterations in pulmonary transvascular fluid and protein exchange after an intravenous injection of bone marrow. The injection of 0.2 ml/kg of bone marrow suspension (BMS) increased pulmonary arterial pressure (Pp)a and pulmonary vascular resistance (PVR) indicating the embolization of pulmonary vessels. BMS also produced steady state increases in pulmonary lymph flow (Qlym), lymph-to-plasma concentration (L/P) ratio, and lymph protein clearance (Qlym X L/P ratio). An increase in pulmonary microvascular pressure (Pmv) produced by inflating a left atrial balloon catheter after injection of BMS produced further increases in Qlym and protein clearance without a decrease in L/P ratio, indicating that the small quantities of marrow increased lung endothelial permeability to proteins. Defibrinogenation induced by treating sheep with Ancrod prevented the increases in Qlym, L/P ratio, protein clearance, Pmv, and PVR after the injection of BMS. To test whether defibrinogenation prevented the increase in endothelial permeability, Pmv was increased after injection of BMS in Ancrod-treated sheep. In contrast to the control animals, in the defibrinogenated sheep, Qlym increased but L/P ratio decreased. In another group, the role of granulocytes in mediating the marrow-induced increase in lung vascular permeability was examined by granulocyte depletion produced with hydroxyurea. Injection of BMS resulted in an increase in Qlym and decreased in L/P ratio, changes which could be explained by increased Pmv. Therefore, granulocyte depletion, like fibrinogen depletion, also inhibited the increase in permeability. However, in contrast to defibrinogenation, granulocytopenia did not inhibit the increases in Pmv and PVR after the marrow injection. The results indicate that fibrinogen depletion prevents pulmonary vascular obstruction after injection of BMS and the associated increase in pulmonary endothelial permeability, whereas granulocyte depletion prevents only the increase in permeability. Therefore, both intravascular coagulation and granulocytes are required for the development of lung vascular injury after bone marrow pulmonary embolism. (Circ Res 50: 830-838, 1982)

PULMONARY edema and pulmonary insufficiency have been observed following long bone fractures and other non-thoracic injuries in which pulmonary vessels are embolized with bone marrow (Collins et al., 1968; Blaisdell and Lewis, 1977; Saldeen, 1979; Shier and Wilson, 1980). The edema is often associated with intravascular coagulation (McKay, 1975; Bone et al., 1976; Saldeen, 1979). Saldeen (1979) observed microemboli consisting of fibrin and formed elements in small pulmonary arteries and in capillaries of patients dying with post-traumatic pulmonary insufficiency. Studies in dogs (Busch et al., 1974; Malik and van der Zee, 1977; Malik et al., 1979) indicated a relationship between the degree of intravascular coagulation in the lungs, which was accentuated by inhibition of fibrinolysis. Thus, the activation of intravascular coagulation may play a role in mediating post-traumatic pulmonary edema.

In a previous study (Barie et al., 1981), we demonstrated in sheep that an intravenous injection of a suspension of homologous bone marrow, which simulated the release of bone marrow into the circulation after injury (Sevitt, 1962; Feltier et al., 1974; Saldeen, 1979), produced pulmonary edema that was due to increased lung endothelial permeability to proteins. Furthermore, the increase in permeability was accompanied by decreased plasma fibrinogen concentration and increased levels of fibrin degradation products, indicating that both coagulation and fibrinolysis cascades were activated (Barie et al., 1981). Also, the leukocyte count was transiently decreased after the bone marrow injection (Barie et al., 1981). In the present study, we examined the roles of intravascular coagulation and leukocytes in the development of lung vascular injury after bone marrow pulmonary embolism.

Methods

Studies were made in 29 sheep ranging in weight from 19 to 28 kg. All animals were fasted except for unlimited access to water for 48 hours prior to study. Anesthesia was induced with sodium thiopental (25 mg/kg, iv). After endotracheal intubation, pancuronium bromide, 0.04 mg/kg, iv, was given to abolish spontaneous respiration while anesthesia was maintained with a mixture of 1% halothane in oxygen and nitrous oxide delivered by a Harvard respirator. The inspired oxygen concentration (Fio2) was adjusted during the experiment in the sheep developing hypoxemia so as to maintain normal blood gases and pH in the normal range. The Fio2 never exceeded 40% O2.
Circulating fibrinogen was depleted in 12 of these animals with Ancrod (Prentice et al., 1974), an enzyme derived from Malayan pit viper venom (Abbott Laboratories). An intravenous injection of 0.35 NIH U/kg of Ancrod was given daily for 3 days to deplete fibrinogen (Johnson and Malik, 1980a). Plasma fibrinogen and fibrin degradation products were measured by the radial immunodiffusion technique (Kaplan, 1979). Granulocytes were depleted in nine sheep with hydroxyurea using 200 mg/kg per day for 4 days (Johnson and Malik, 1980b).

The caudal mediastinal node and its efferent duct were identified through a right thoracotomy; the duct was isolated for collection of pulmonary lymph (Erdmann et al., 1975; Malik and van der Zee, 1978). The node was resected just caudal to the distal margin of the pulmonary ligament to eliminate most systemic contamination (Erdmann et al., 1975; Malik and van der Zee, 1978). All identifiable diaphragmatic and chest wall afferents were ligated. The efferent duct was then ligated and cannulated with a Silastic catheter (C.R. Bard, Inc.) and secured in position. Blood-free pulmonary lymph was obtained in all 20 sheep.

Polyethylene catheters (10-F) were placed in the aorta and jugular vein and a thermistor-tip Swan-Ganz (7-F) catheter (Edwards Laboratories) was positioned in a pulmonary artery. Aortic pulmonary arterial, and either pulmonary arterial wedge or left atrial pressures were obtained using Statham P23Db pressure transducers referred to the level of the left atrial appendage. Pressures were recorded on a polygraph (Grass Instruments, model 7B). Pulmonary perfusion pressure was calculated as the difference between mean pulmonary arterial and mean pulmonary artery wedge or left atrial pressures. Pulmonary blood flow was determined in triplicate at 30-minute intervals by the thermal dilution technique (Edwards Laboratories, model 9250).

The simultaneous collection of lymph and plasma samples was begun at 15-minute intervals with the sheep in the supine position. Steady state lymph flow was defined by at least three consecutive samples which differed by less than 0.23 ml/hr. Lymph and plasma protein concentrations were determined with the Biuret technique.

Studies were made in six groups of sheep.

**Group I (n = 8)**

These animals received 0.2 ml/kg of bone marrow suspension (BMS) via the jugular venous catheter. Tibias were excised 1 day before each experiment from other sheep immediately postmortem and stored intact overnight at 4°C. The marrow was cut and frozen approximately 30 minutes before the injection to avoid the possibility of microbial or endotoxin contamination of the injectate. Just before injection, a suspension of 50% by volume of bone marrow in sterile normal saline was prepared in a laboratory blender (Waring) and maintained at normal sheep body temperature (38°C). It was necessary to suspend the marrow to produce particles (50-150 μm) that were injectable and would emulsify only small pulmonary vessels. The tibia content of the BMS is 5.9 ± 1.2 μmol/ml (mean ± SEM for n = 6). The BMS was injected over a 5- to 10-minute period to ensure adequate mixing in the right heart. We chose a fixed dose of BMS in this study since, previously, we demonstrated that the BMS produced a dose-dependent increase in lymph protein clearance after injection (Barie, 1981); the dosage of 0.2 ml/kg was chosen to double the pulmonary transvascular protein clearance (Barie, 1981). Pulmonary hemodynamics and lymph flow were monitored for at least 2 hours after the BMS injection or until a new steady state was attained.

In four of the eight controls of group I, a 12-F Foley balloon catheter, which had been placed in the left atrium via the atrial appendage, was inflated during the steady state obtained after the BMS injection. The purpose of the inflation was to increase the left atrial pressure, which increased pulmonary microvascular pressure (Pmv), so as to examine the effects of BMS on pulmonary vascular permeability to proteins (Brigham, 1977; Taylor, et al., 1981).

**Group II (n = 4)**

The sheep were defibrinogenated with Ancrod (0.35 NIH U/kg day for 3 days) prior to the study (Prentice et al., 1974; Johnson and Malik, 1980a). After a steady state baseline, the left atrial pressure was increased by inflation of the Foley left atrial balloon catheter. The purpose of this study was to determine the effects of an increase in Pmv on pulmonary transvascular fluid and protein exchange after Ancrod treatment.

**Group III (n = 3)**

These sheep were also defibrinogenated with Ancrod before the study, as in group II. After the baseline period, 0.2 ml/kg of BMS was injected as in group I, and the animals were studied until a new steady state was attained.

**Group IV (n = 5)**

Fibrinogen was depleted with Ancrod before the study, as in groups II and III. After the baseline period, 0.2 ml/kg of BMS was injected, and the left atrial balloon catheter was inflated immediately after the injection. The purpose of the increase in Pmv was to determine whether fibrinogen depletion prevented the marrow-induced increase in vascular permeability.

**Group V (n = 3)**

Granulocytes were depleted with hydroxyurea (200 mg/kg per day for 4 days) prior to the study (Johnson and Malik, 1980b). After a steady state baseline, Pmv was increased by inflation of the left atrial balloon catheter. The purpose of this study was to determine the effects of an increase in Pmv on pulmonary transvascular fluid and protein exchange after hydroxyurea treatment.

**Group VI (n = 6)**

Granulocytes were depleted using hydroxyurea as in group V; 0.2 ml/kg of BMS was injected as in group I, and the sheep were studied until a new steady state. The purpose of this study was to examine the effect of granulocyte depletion on the marrow-induced alterations in pulmonary fluid and protein exchange.

The significance of steady state changes from baseline was tested using the paired t-test. Analysis of variance was done between groups to determine whether there was intergroup variability between the baseline values. The significance of difference between slopes of lines was tested using the analysis of covariance.

**Results**

**Effect of Bone Marrow Injection**

The effects of injection of 0.2 ml/kg of bone marrow suspension (BMS) on pulmonary fluid balance and hemodynamics in group I (n = 8) are shown in Table 1. Pulmonary lymph flow (Qlym), the lymph-
TABLE 1  
Alterations in Steady State Pulmonary Fluid Balance and Hemodynamics after Bone Marrow Injection (Group I) (n = 8)  

<table>
<thead>
<tr>
<th>Baseline</th>
<th>Bone marrow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean pulmonary artery pressure (torr)</td>
<td>14.1 ± 1.2</td>
</tr>
<tr>
<td>Mean left atrial pressure (torr)</td>
<td>4.6 ± 0.8</td>
</tr>
<tr>
<td>Pulmonary blood flow (liters/min)</td>
<td>2.15 ± 0.17</td>
</tr>
<tr>
<td>Pulmonary vascular resistance, (torr/liter per min)</td>
<td>4.1 ± 0.6</td>
</tr>
<tr>
<td>Pulmonary lymph flow (ml/hr)</td>
<td>4.1 ± 0.7</td>
</tr>
<tr>
<td>Lymph/plasma protein concentration ratio</td>
<td>0.78 ± 0.04</td>
</tr>
<tr>
<td>Lymph protein clearance (ml/hr)</td>
<td>3.3 ± 0.6</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM.  
* Significantly different from baseline (P < 0.05).

to-plasma protein concentration (L/P) ratio, and transvascular protein clearance (C_L), i.e., the product of Qlym and C_L, increased after the marrow injection. The injection of BMS produced significant increases in mean pulmonary artery pressure (P_{PA}) from 14.1 ± 1.2 torr to 19.0 ± 2.4 torr and in pulmonary vascular resistance (PVR) from 4.1 ± 0.6 torr/liter per min to 7.4 ± 1.3 torr/liter per min (Table 1), indicating obstruction of pulmonary vessels.

The effects of injection of BMS on pulmonary fluid balance and hemodynamics in one sheep are shown in Figure 1. In this animal, the BMS caused a doubling of Qlym and C_L even though PVR was not increased by the marrow injection and P_{PA} increased by only 3 torr. The inflation of the left atrial balloon catheter to increase the left atrial pressure (P_{L}) by 10 torr after a new steady state produced relatively large increases in Qlym and C_L without a concomitant large decrease in L/P ratio, in contrast to the response of left atrial hypertension in normal sheep (Minnear et al., 1981).

The steady state response to left atrial hypertension after bone marrow is summarized in Table 2. The increase in Pmv after left atrial hypertension produced increases in Qlym and C_L without a significant decrease in L/P ratio (Table 2). An increase in Pmv after left atrial hypertension in normal sheep, however, characteristically produces an increase in Qlym and a resultant decrease in L/P ratio due to increased movement of protein-poor fluid (Erdmann et al., 1975; Minnear et al., 1981).

**Effect of Fibrinogen Depletion**

Plasma fibrinogen was successfully depleted in all 12 sheep treated with Ancrod; fibrinogen concentra-

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**FIGURE 1.** Effects of injection of 0.2 ml/kg of bone marrow suspension in a control sheep. The left atrial pressure (P_{L}) was increased at 120 minutes after the marrow injection by inflation of a left atrial balloon catheter. L/P ratio = lymph-to-plasma protein concentration ratio; P_{PA} = mean pulmonary arterial pressure; PVR = pulmonary vascular resistance.
TABLE 2  
Effects of Bone Marrow Suspension and Increased Left Atrial Pressure on Pulmonary Fluid Balance and Hemodynamics  
(n = 4)  

<table>
<thead>
<tr>
<th></th>
<th>Baseline*</th>
<th>Bone marrow† and left atrial hypertension</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean pulmonary artery pressure (torr)</td>
<td>17.9 ± 1.8</td>
<td>21.0 ± 3.1</td>
</tr>
<tr>
<td>Mean left atrial pressure (torr)</td>
<td>3.9 ± 1.4</td>
<td>4.8 ± 1.2</td>
</tr>
<tr>
<td>Pulmonary blood flow (torr/liter per min)</td>
<td>2.17 ± 0.24</td>
<td>1.63 ± 0.20‡</td>
</tr>
<tr>
<td>Pulmonary vascular resistance (torr/liter per min)</td>
<td>6.6 ± 1.7</td>
<td>9.4 ± 1.9</td>
</tr>
<tr>
<td>Pulmonary lymph flow (ml/hr)</td>
<td>3.1 ± 0.9</td>
<td>6.3 ± 1.3‡</td>
</tr>
<tr>
<td>Lymph/plasma protein concentration ratio</td>
<td>0.78 ± 0.08</td>
<td>0.83 ± 0.04</td>
</tr>
<tr>
<td>Lymph protein clearance (ml/hr)</td>
<td>2.6 ± 1.0</td>
<td>5.6 ± 1.4‡</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM.
* † Data from these four sheep are also included in the data for bone marrow injection into eight sheep shown in Table 1.
‡§ Different from baseline (P < 0.05).
§ Different from bone marrow (P < 0.05).

The fibrinogen concentration decreased from 377 ± 29 mg/dl before treatment to 34 ± 4 mg/dl determined during the steady state baseline period. Fibrinogen depletion with Ancrod also resulted in generation of fibrin degradation products (FDP). Circulating levels of FDP were markedly elevated from pretreatment value of 1.8 ± 0.3 to 154 ± 27 mg/dl after Ancrod treatment.

The effects of increased Pmv on lung fluid balance after Ancrod treatment in a sheep are shown in Figure 2. Qlym increased, whereas CL did not increase to the same extent because of the decrease in the L/P ratio. The pulmonary lymph and hemodynamic data for the four Ancrod-treated sheep in which Pia was increased (group II), are summarized in Table 3. Despite the increase in Qlym produced by raising Pia from 3.5 ± 1.1 to 15.3 ± 1.5 torr, the increase in CL was not significant because of the significant decrease in the L/P ratio. The fibrinogen and FDP concentrations were 29 ± 5 and 170 ± 38 mg/dl, respectively. The changes in pulmonary fluid and protein exchange after increased left atrial pressure in Ancrod-treated sheep (Table 3) were the same as those in untreated sheep after left atrial hypertension (Erdmann et al., 1975; Minnear et al., 1981).

Figure 3 compares the relationships between Qlym and CL (upper panel) and Qlym and L/P ratio (lower panel) for sheep in which Pia was increased (group II). Injection of BMS produced an increase in CL and L/P ratio, and increased Pia in these animals produced a further increase in CL and no change in the L/P ratio as Qlym increased. These changes are in marked contrast to the smaller increase in CL and the decrease in L/P ratio following increased Pia in Ancrod-treated sheep (Fig. 3) and in untreated sheep (Erdmann et al., 1975; Minnear et al., 1981).

The results of bone marrow injection in the fibrinogen-depleted sheep (group III) are shown in Figure 4. The bone marrow injection did not produce increases in Pia or PVR that were observed in the control group after the marrow injection (Table 1). The marrow injection also did not produce significant changes in Qlym, L/P ratio, and CL as it did in the control group (Tables 1 and 3).

Since the absence of increases in Qlym and CL in Group III may be due to the fact that Pia did not increase after BMS injection, in group IV (n = 5) we inflated the left atrial balloon catheter in additional fibrinogen-depleted sheep immediately after bone marrow was injected. The Pia was increased to 20.3 ± 1.8 torr after the BMS injection (Table 3), a level that was comparable to the pressure of 19.0 ± 2.4 torr observed in group I after BMS (Table 1). In group IV, Qlym increased from baseline of 5.2 ± 0.8 ml/hr to 11.4 ± 6.9 ml/hr.

FIGURE 2. Effects of left atrial hypertension in an Ancrod-treated sheep. The fibrinogen concentration was decreased to 15.0 mg/dl.
TABLE 3
Steady State Pulmonary Fluid Balance and Hemodynamics in Ancrod-Treated Groups II, III, and IV

<table>
<thead>
<tr>
<th></th>
<th>Group II (n = 4)</th>
<th>Group III (n = 3)</th>
<th>Group IV (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Left atrial hypertension</td>
<td>Bone marrow + left atrial hypertension</td>
</tr>
<tr>
<td>Mean pulmonary artery pressure (torr)</td>
<td>14.1 ± 1.9</td>
<td>23.6 ± 1.6*</td>
<td>9.5 ± 1.3</td>
</tr>
<tr>
<td>Mean left atrial pressure (torr)</td>
<td>3.5 ± 1.1</td>
<td>15.3 ± 1.5*</td>
<td>1.3 ± 0.9</td>
</tr>
<tr>
<td>Pulmonary blood flow liter/min</td>
<td>2.35 ± 0.16</td>
<td>1.45 ± 0.11*</td>
<td>2.01 ± 0.31</td>
</tr>
<tr>
<td>Pulmonary vascular resistance (torr/liter per min)</td>
<td>4.5 ± 0.3</td>
<td>5.9 ± 0.6*</td>
<td>4.5 ± 1.4</td>
</tr>
<tr>
<td>Pulmonary lymph flow (ml/hr)</td>
<td>4.5 ± 1.0</td>
<td>13.0 ± 2.7*</td>
<td>8.6 ± 2.3</td>
</tr>
<tr>
<td>Lymph/plasma protein concentration ratio</td>
<td>0.72 ± 0.08</td>
<td>0.41 ± 0.06*</td>
<td>0.76 ± 0.11</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± 1 SEM.
* Different from baseline (P < 0.05).

1.9 ml/hr after BMS, but the L/P ratio decreased from 0.61 ± 0.05 to 0.46 ± 0.05 (Table 3).

The relationships between Qlym vs. C(,i) (upper panel) and between Qlym vs. L/P ratio (lower panel) for groups I, II, and IV are summarized in Figure 5. The slope of Qlym vs. C(,i) line was greater (P < 0.05) after BMS in control sheep (group I) than in either of the fibrinogen-depleted groups (groups II and IV). Also, the L/P ratio decreased in the defibrinogenated sheep after the BMS injection (group IV), in contrast to the increase in L/P ratio in control sheep (group I) (Fig. 5). The changes in C(,i) and L/P ratio in both Ancrod-treated groups in which Pmv was increased (groups II and IV), were the same in spite of the injection of bone marrow in group IV.

Effect of Granulocyte Depletion

Granulocyte count decreased after hydroxyurea treatment from 2465 ± 784 cells/μl before treatment to 21 ± 9 cells/μl after treatment, and the total leukocyte count decreased from 8299 ± 1702 to 1039 ± 134 cells/μl. The platelet count before treatment of 319,375 ± 47,602 cells/μl was not significantly different from the value of 272,917 ± 20,518 cells/μl after treatment.

The results of injection of BMS in the granulocyte-depleted sheep (group VI) are summarized in Table 4. The marrow produced increases in Ppi and PVR, but in contrast to the response in control animals after
FIGURE 4. The effect of bone marrow injection in an Ancrod-treated sheep. Fibrinogen concentration was decreased to 18.2 mg/dl prior to injection of the marrow.

BMS (Table 1), Qlym increased and L/P ratio decreased after marrow injection in the granulocytopenic sheep. The increased pulmonary fluid filtration after BMS in the granulocytopenic sheep was therefore due to increased Pmv and not to increased vascular permeability. The increase in Qlym and decrease in L/P ratio in the granulocytopenic sheep after BMS were in contrast to the greater increase in Qlym and an increase in L/P ratio in the untreated sheep after embolization (Fig. 6).

Discussion

Pulmonary edema, and the resultant pulmonary insufficiency, has been identified as a major complication of long bone fracture and other types of non-thoracic injuries (Blaisdell and Lewis, 1977; Collins et al., 1968; Shier and Wilson, 1980; Saldeen, 1979; Sevitt, 1962). Fat microemboli were observed in the pulmonary vascular bed (Armin and Grant, 1951; Saldeen, 1970; Schinella, 1973; Peltier et al., 1974) suggesting that bone marrow is released into the circulation at the fracture site (Schinella, 1973). The other source of fat microemboli may be mobilization of fatty acids by release of catecholamines after injury (Whitaker and McKay, 1969; Baker et al., 1971). The mechanism of the pulmonary edema is unclear, although there is an association between intravascular coagulation, pulmonary leukostasis, and pulmonary insufficiency (Saldeen, 1979; Bone et al., 1976).

In a previous study, we characterized the alterations in lung transvascular fluid and protein exchange after iv injection of homologous bone marrow (Barie et al., 1981). Injection of small amounts of bone marrow (range 0.07 to 0.41 ml/kg body weight) produced dose-dependent increases in pulmonary arterial pressure and pulmonary vascular resistance that indicated obstruction of pulmonary vessels. The pulmonary microembolization was likely due to secondary thrombosis, since the small amount of marrow injected could not explain the 2- to 3-fold increases in pulmonary vascular resistance (Barie et al., 1981). Secondary thrombosis may result from the activation of the extrinsic coagulation pathway since the bone marrow contains thromboplastin (Barie et al., 1981). The coagulation cascade was undoubtedly activated in our study after the marrow injection, since plasma fibrinogen concentration decreased and concentration of fibrin degradation products increased. Moreover, there was evidence of a margination of leukocytes as the leukocyte count decreased rapidly after the marrow injection (Barie et al., 1981), however, the platelet count did not change from the baseline levels (Barie...
et al., 1981). In the present study, we examined (1) the effects of the bone marrow injection on lung vascular permeability to proteins and (2) the contribution of intravascular coagulation and leukocytes to the increase in lung vascular permeability.

We used 0.2 ml/kg of the bone marrow suspension to produce the increase in pulmonary transvascular protein clearance, since this dosage was shown to double the protein clearance with minimal increase in pulmonary vascular resistance (Barie et al., 1981). The intravenous injection of the bone marrow produced increases in pulmonary lymph flow (Qlym), lymph-to-plasma protein concentration (L/P) ratio, and protein clearance, as has been shown previously (Barie et al., 1981). We tested the hypothesis that the increase in protein clearance was due to increased lung endothelial permeability to proteins by raising the pulmonary microvascular pressure in some of the animals that had received the bone marrow suspension. If the interstitial oncotic pressure is increased relative to the plasma oncotic pressure due to an increase in vascular permeability, then an increase in microvascular pressure causes a greater increase in transvascular protein clearance in the lung with injured vessels than in the normal lung (Taylor et al., 1981); moreover, the L/P ratio does not decrease to the same extent after an increase in Qlym in the injured lung (Brigham, 1977; Taylor et al., 1981). These relationships hold because of the greater flow of protein-rich fluid into the interstitium after the increase in endothelial permeability (Brigham, 1977; Taylor et al., 1981). The increase in transvascular protein clearance after injection of marrow probably is due to increased vascular permeability since the elevation in microvascular pressure in sheep that received bone marrow produced a large Qlym increase without a change in L/P ratio; thus, the transvascular protein clearance increased to a greater extent in this group than in normal sheep subjected to the same left atrial hypertension. Left atrial hypertension in normal sheep produces an increase in Qlym and a decrease in L/P ratio (Erdmann et al., 1975; Minnear et al., 1981). In light of these differences in response to increased microvascular pressure, the results of the present study indicate that the bone marrow injection increases pulmonary vascular permeability to proteins.

### Table 4

| Alterations in Pulmonary Fluid Balance and Hemodynamics in Hydroxyurea-Treated Sheep during Increased Left Atrial Pressure (n = 3) and after Bone Marrow Injection (n = 6) |
|-----------------------------------------------|-----------------------------------------------|
| Mean pulmonary artery pressure (torr) | Mean left atrial pressure (torr) |
| Mean pulmonary artery pressure (torr) | Mean left atrial pressure (torr) | 2.7 ± 1.7 |
| Pulmonary blood flow (liter/min) | Pulmonary vascular resistance, (torr/liter per min) | 3.2 ± 0.6 |
| Pulmonary lymph flow (ml/hr) | Pulmonary lymph flow (ml/hr) |
| Lymph/plasma protein concentration ratio | Lymph protein clearance (ml/hr) |
| Lymph protein clearance (ml/hr) | Lymph protein clearance (ml/hr) |

| Baseline | Left atrial hypertension | Baseline | Bone marrow |
|-----------------------------------------------|-----------------------------------------------|
| Mean pulmonary artery pressure (torr) | 9.7 ± 1.5 | 24.3 ± 2.4† | 12.3 ± 2.2 | 17.5 ± 2.5* |
| Mean left atrial pressure (torr) | 2.7 ± 1.7 | 17.0 ± 2.7† | 2.3 ± 0.4 | 3.4 ± 0.4 |
| Pulmonary blood flow (liter/min) | 2.32 ± 0.38 | 1.17 ± 0.28† | 1.60 ± 0.13 | 1.48 ± 0.09 |
| Pulmonary vascular resistance, (torr/liter per min) | 3.2 ± 0.6 | 11.9 ± 5.0 | 6.9 ± 2.0 | 9.4 ± 1.5* |
| Pulmonary lymph flow (ml/hr) | 4.3 ± 1.2 | 10.4 ± 3.8† | 6.9 ± 0.9 | 10.3 ± 1.8* |
| Lymph/plasma protein concentration ratio | 0.90 ± 0.03 | 0.65 ± 0.08† | 0.79 ± 0.04 | 0.70 ± 0.03* |
| Lymph protein clearance (ml/hr) | 3.8 ± 1.0 | 6.3 ± 1.5† | 5.5 ± 0.9 | 7.4 ± 1.6 |
| Lymph protein clearance (ml/hr) | 3.8 ± 1.0 | 6.3 ± 1.5† | 5.5 ± 0.9 | 7.4 ± 1.6 |

Data are expressed as mean ± SEM.

* Different from baseline with paired t-test (P < 0.05).
† Different from baseline with sign test (P < 0.05).

**Figure 6.** Relationship between pulmonary lymph flow and L/P ratio in untreated sheep after marrow injection and in hydroxyurea-treated sheep after marrow injection. Bars indicate ± 1 SEM.
Since a small amount of the bone marrow suspension (0.2 ml/kg) was required to increase permeability, the model approximates the changes occurring after release of minute amounts of marrow into the circulation subsequent to a bone fracture or tissue injury (Bone et al., 1976; Saldeen, 1979; Shier and Wilson, 1980).

In the present study, we also examined whether the intravascular coagulation and leukocyte entrapment are factors in mediating the increase in lung vascular permeability (Sevitt, 1962; Bone et al., 1976; Blaisdell and Lewis, 1977; Saldeen, 1979). The role of intravascular coagulation was examined by studying the effects of bone marrow injection after fibrinogen depletion induced with Ancrod (Prentice et al., 1974; Johnson and Malik, 1980a). Injection of 0.2 ml/kg of bone marrow suspension in defibrinogenated sheep failed to produce increases in either Qlym or transvascular protein clearance. Also, pulmonary arterial pressure and pulmonary vascular resistance did not increase in these animals, indicating that the bone marrow did not produce pulmonary vascular obstruction in the defibrinogenated animals; this contrasted with the significant increases in pulmonary arterial pressure and vascular resistance in untreated sheep after the marrow injection.

Since the failure of the bone marrow suspension to increase Qlym and transvascular protein clearance in fibrinogen-depleted sheep may have been due either to prevention of increased permeability or to smaller increases in microvascular pressures in this group, we differentiated between these possibilities by raising pulmonary microvascular pressure by inflation of the left atrial balloon catheter. In this group, Qlym increased but the L/P ratio decreased in spite of injection of bone marrow, indicating that the increased fluid filtration in this group of defibrinogenated sheep was solely due to increased microvascular pressure. The results indicate that fibrinogen depletion prevented the increased permeability caused by intravascular bone marrow. Therefore, the activation of intravascular coagulation was required for the increase in permeability to occur after the bone marrow embolization.

The immediate consumption of granulocytes after the marrow injection (Barie et al., 1981) also involves granulocytes in the response. Recent evidence indicates granulocytes are required for the mediation of the increase in lung vascular permeability occurring after endotoxemia induced by E. coli (Heflin and Brigham, 1979), pulmonary microembolization (Johnson and Malik, 1980b; Flick et al., 1981; Tahamont et al., 1981). Injection of complement-activated plasma (Craddock et al., 1977), and acute pancreatitis (Malik et al., unpublished observation). Granulocytes may be the final effectors mediating the increase in permeability observed after the bone marrow injection. The generation of the serine protease, plasmin, responsible for fibrinolysis directly activates the complement system (Woolridge et al., 1974; Kaplan et al., 1978), which may then cause the production of chemotactic complement fragments (Craddock et al., 1977). The fibrin degradation products generated in the pulmonary microcirculation as a result of fibrinolysis may also cause chemotaxis of leukocytes (Sueishi et al., 1980).

Injection of bone marrow in sheep made granulocytopenic with hydroxyurea (Johnson and Malik, 1980b) produced increases in pulmonary artery pressure and PVR, presumably because obstruction of the pulmonary microvessels still occurred due to activation of the coagulation cascade and resulting intravascular thrombosis. However, the increase in Qlym in the granulocytopenic sheep was associated with a decrease in the L/P ratio, indicating that the increased filtration was due to increased microvascular pressure rather than to increased permeability. Therefore, granulocyte depletion and fibrinogen depletion independently prevented the increase in pulmonary vascular permeability after the marrow injection, but unlike fibrinogen depletion, granulocyte depletion did not prevent the pulmonary vascular obstruction. The protective effect of fibrinogen depletion may, thus, be due to inhibition of secondary thrombosis and of the subsequent leukocyte margination.

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