POLYMORPHONUCLEAR leukocyte aggregation in the pulmonary vascular bed (pulmonary leukostasis) is known to occur during both hemodialysis and cardiopulmonary bypass (Craddock et al., 1977a; Bolanowski et al., 1977; de Bugh Daly et al., 1954; Neville et al., 1963; Kaplow and Goffinet, 1968; Toren et al., 1970). Such pulmonary leukostasis is associated with a marked but transient peripheral leukopenia, which usually recovers within 1 hour after onset of extracorporeal perfusion.

Craddock and his associates (1977a) have demonstrated that exposure of blood to dialyzer cellophane activates complement, with resulting peripheral leukopenia and pulmonary leukostasis. This mechanical obstruction of pulmonary capillaries with white cells was postulated by these investigators to be the most likely explanation for the pulmonary dysfunction observed following the onset of hemodialysis in patients, and for the rise in pulmonary artery pressure observed in sheep following infusion of autologous complement-activated plasma (Craddock et al., 1977a, 1977b, 1978).

We previously have observed a similar pulmonary response in sheep after the onset of venovenous membrane oxygenator perfusion (Birek et al., 1976). However, our finding that pretreatment of the animal with sulfipyrazone abolished the pulmonary response without altering the leukopenic response cast doubt that leukostasis alone was an adequate explanation for the pulmonary response following complement activation. The present study was designed to investigate further the relationship between complement-mediated leukopenia and alterations in pulmonary vascular resistance and pulmonary gas exchange.
Methods

Experiments in Rabbits

Seven New Zealand white rabbits, weighing 3 kg, were anesthetized with 2% sodium thiopental, iv (0.5 ml/kg), and polyethylene cannulas were inserted surgically into the left carotid artery and through the left jugular vein into the superior vena cava. In addition, a no. 4 French Swan-Ganz catheter was inserted into the right internal jugular vein and advanced into the pulmonary artery using a technique described by J. B. Forrest (1979). The arterial, pulmonary artery, and mean pulmonary artery pressures were recorded continuously on a multichannel recorder (Litton Medical Products Incorporated).

When the rabbits had recovered from the anesthetic, 15 ml of complement-activated plasma were prepared according to the method of Craddock et al. (1977a). Heparinized plasma (1 unit/ml) was prepared, and complement was activated by incubating the plasma with zymosan (I.C.N. Pharmaceuticals) at a ratio of 5 mg zymosan per ml of plasma for 30 minutes at 37°C. The plasma was centrifuged at 20,000 g for 10 minutes at 4°C to remove the zymosan particles. The plasma then was reinfused into the central venous cannula at a rate of 1 ml/min for 15 minutes, using a motor driven syringe pump (Harvard Apparatus Company Incorporated). Arterial blood samples were drawn just prior to plasma infusion and at frequent intervals thereafter for measurement of arterial blood gas and pH determinations (Radiometer Instruments). Arterial blood samples for automated leucocyte determinations were drawn at frequent intervals (Coulter model S, Coulter Electronics). Blood was drawn simultaneously from the central venous catheter and carotid artery at 30 seconds and at 1 minute after the onset of plasma infusion to record the loss of leucocytes across the pulmonary circulation. In three experiments, the total hemolytic complement (CH₅₀) level was measured in the incubated plasma using the technique described by Kabat and Mayer (1971a). These same plasma samples were subject also to immunoelectrophoretic analysis of the C₃ component of complement using antirabbit C₃ antiserum. In three of the rabbits, which were killed by an overdose of sodium thiopental, iv, rapid pneumonectomy was performed at the end of the experiment, and sections of each lung were prepared for histology. Specimens were fixed in a combination of 4% formaldehyde and 1% glutaraldehyde in a buffer of 176 mOsmol/l (McDowell and Trump, 1976) and embedded in epoxy resin. Sections 1-μm thick were cut and stained with 1% toluidine blue and examined by light microscopy.

To ensure that the observed effects of plasma infusion resulted from complement activation of the plasma, and not from some unknown consequence of plasma preparation, experiments were repeated in two rabbits, and the heparinized plasma was prepared, handled, and heparinized in identical fashion, without the addition of zymosan.

Experiments in Sheep

Suffolk sheep, weighing 30-40 kg, were sedated with intramuscular ketamine (22 mg/kg). The right carotid artery and right jugular vein were exposed surgically using local anesthesia to supplement the ketamine. The carotid artery was cannulated for blood sampling and pressure monitoring. A no. 7 French Swan-Ganz thermodilution catheter (specialy modified to have the proximal injection site 12.5 cm from the tip) was inserted via the jugular vein and advanced into the pulmonary artery for measurement of the pulmonary artery and pulmonary wedge pressures and for determination of cardiac output by the thermodilution method (model 9510 Cardiac Output Computer, Edwards Laboratories). A separate central venous cannula was placed for infusion of plasma.

After the sheep had recovered fully from the sedation, heparinized plasma was collected, and 20 ml of complement-activated plasma were prepared as for the experiments on rabbits. The zymosan-activated plasma then was reinfused through the central venous cannula for 4 minutes at a rate of 5 ml/min. This was done in seven sheep (group I). In seven additional experiments (group II), 2 g of intravenous sulfipyrazone (Ciba-Geigy, Dorval, PQ) were given to the sheep after blood had been drawn for preparation of zymosan-incubated plasma, but 30 minutes before the plasma reinfusion. In four additional control experiments, heparinized plasma was prepared, incubated at 37°C for 30 minutes without zymosan, and reinfused. In four other experiments, plasma complement was heat inactivated at 56°C for 30 minutes (Kabat and Mayer, 1971b) before incubation with zymosan and reinfusion. These latter two groups of experiments were performed to ensure that the response to plasma infusion in groups I and II was due to complement activation and not to unknown effects of plasma preparation or to zymosan itself.

For all sheep, arterial blood samples were taken prior to plasma infusion and at frequent intervals thereafter for blood gas determination, automated leucocyte count, and platelet count. Platelet counts were measured automatically (Coulter model ZF, Coulter Electronics), and in three experiments from each group, in vitro platelet aggregation in response to ADP was measured using a Payton aggregometer (Payton Associates Ltd.). For this evaluation, platelet-rich plasma (PRP) was obtained by centrifugation at 80 g for 10 minutes. A concentration of 1 mg of ADP/ml of PRP was used to induce maximal aggregation.

Blood samples were drawn simultaneously from the Swan-Ganz catheter and the carotid artery.
A rapid leukopenia developed after the onset of plasma infusion (Fig. 1). This was associated with a selective loss of polymorphonuclear leukocytes (PMN). From the blood samples taken simultaneously from the right atrium and carotid artery shortly after the start of infusion, the loss of PMN across the lungs could be calculated. The number of leukocytes per cubic mm in the carotid arterial sample was expressed as a percentage of the number recorded simultaneously in the vena cava, and it was found that 99.7 ± 0.33% were lost into the pulmonary vascular bed. No significant loss of other leukocyte fractions occurred.

The arterial oxygen tensions (Pao₂) are shown in Table 1. The slight fall in Pao₂ recorded at 10 minutes did not reach the level of statistical significance when compared with the preinfusion value (P = 0.2). The Paco₂ values for these experiments showed no change after plasma infusion.

In the three rabbits with Swan-Ganz catheters, no change in pulmonary artery pressure occurred. In the two control experiments (reinfusion of non-activated plasma) no change in the leukocyte count or the arterial blood gases resulted.

Measurement of CH₅₀ showed complete loss of activity in the zymosan-activated plasma. Immunoelectrophoretic analysis of C₃ showed increased anodal mobility, indicative of C₃ activation. Histology of the autopsied lungs showed consistent evidence of pulmonary capillary leukocyte aggregation.

### Experiments in Sheep

(Groups I—infusion of activated plasma, n = 7; group II—sulfinpyrazone pretreatment followed by infusion of complement-activated plasma, n = 7.) As in the experiments on rabbits, an abrupt leukopenia followed the onset of plasma infusion in both groups (Fig. 2). Calculation of PMN loss across the lungs was carried out as described. At 30 seconds after the onset of plasma infusion in group I experiments, 92.4 ± 3.9% of PMN were trapped in the lungs, compared with 82.3 ± 17.8% for group II. No significant loss of other leukocyte fractions occurred.

The mean arterial oxygen tension for groups I and II are shown in Figure 3. The pulmonary artery pressures are shown in Figure 4. In group I sheep,

### Table 1: Arterial Pao₂ (mm Hg) in Seven Rabbits before, during, and after Infusion of Complement-Activated Plasma

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>Preinfusion</th>
<th>1 min</th>
<th>3 min</th>
<th>5 min</th>
<th>10 min</th>
<th>15 min</th>
<th>20 min</th>
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<tbody>
<tr>
<td>1</td>
<td>89</td>
<td>84</td>
<td>83</td>
<td>79</td>
<td>80</td>
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<td>82</td>
<td>74</td>
<td>77</td>
<td>74</td>
<td>86</td>
<td>82</td>
</tr>
<tr>
<td>Mean ± SEM</td>
<td>83.9 ± 2.8</td>
<td>85 ± 2.7</td>
<td>82.9 ± 2.4</td>
<td>82.4 ± 3.8</td>
<td>78.6 ± 2.8</td>
<td>84.3 ± 3.0</td>
<td>88.1 ± 2.0</td>
<td>84.1 ± 2.7</td>
</tr>
</tbody>
</table>
plasma infusion resulted in a marked fall in PaO₂ and a marked rise in the mean pulmonary artery pressure. In group II sheep, no such response was seen. The difference in the PaO₂ response between group I and group II was statistically significant (P < 0.01 at 5 and at 10 minutes). Similarly, the pulmonary vascular response in group I sheep was significantly different from that seen in group II (P < 0.001 at 1 minute and 3 minutes, P < 0.02 at 5 and 10 minutes).

In group I sheep, the mean cardiac output fell from a preinfusion value of 6.6 liters/min to 5.3 liters/min at the height of the pulmonary vascular response, indicating that the rise in mean pulmonary artery pressure resulted entirely from increased pulmonary vascular resistance.

No change in Paco₂ or pH occurred in any sheep after plasma infusion. No change in cardiac output occurred in group II sheep.

FIGURE 2 Leukocyte counts (mean ± SEM) in sheep during and after infusion of complement-activated plasma. Group I (n = 7) received no sulfinpyrazone. Group II (n = 7) received sulfinpyrazone prior to plasma infusion. All counts are expressed as a percentage of preinfusion value, which was 11.0 ± 1.4 x 10⁳ for group I and 11.9 ± 1.4 x 10⁴ for group II.

Heat inactivation of plasma, prior to zymosan incubation, completely abolished the leukocyte, blood gas, and pulmonary vascular responses. Similarly, reinfusion of nonactivated plasma samples produced no effect. We conclude that the demonstrable effects of the plasma infusion were due specifically to activation of the complement system.

Serial platelet counts were performed in three sheep from group I and 3 sheep from group II. In neither group were there significant changes in platelet count (group I, —8% decline; group II, 6% decline) or in alteration in platelet aggregation in response to ADP after plasma infusion. Platelet aggregation in response to ADP was diminished slightly in the sulfinpyrazone-treated sheep, but thereafter no further change occurred in association with the plasma infusion.

Discussion

The experimental model used in these experiments has been demonstrated previously to produce pulmonary capillary engorgement with PMN (Craddock et al., 1977a). Similar pathological observations have been observed in humans after cardiopulmonary bypass (Wilson, 1974). Furthermore, in a variety of pathological conditions associated with pulmonary dysfunction, pulmonary leukostasis has been observed (Hechtman et al., 1978; Turino et al., 1974), but the exact relationship between pulmonary leukostasis and pulmonary dysfunction remains unclear.

The present experiments indicate that leukopenia and pulmonary leukostasis result from complement activation produced by plasma incubation with zymosan. In the experiments on rabbits, no pulmonary dysfunction was demonstrated in spite of the concomitant leukopenia and pulmonary leukostasis. On the other hand, infusion of comple-
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ment-activated plasma in sheep resulted in a significant elevation of pulmonary vascular resistance and a significant decline in PaO₂. The differences in these experiments may relate to a species difference, either in the nature of the complement fragments produced or the responsiveness of the pulmonary vasculature. The experiments on rabbits, however, suggest that pulmonary leukostasis alone cannot be the explanation for significant changes in pulmonary vascular resistance and gas exchange.

The ability of sulfinpyrazone to block the pulmonary response in sheep, without altering the leukocyte response, further supports our conclusion that the acute pulmonary dysfunction is not due to pulmonary leukostasis alone. It is likely, however, that the pulmonary leukostasis is in some way related to the pulmonary dysfunction. It has been demonstrated that the C₅ fraction of complement probably is responsible for leukocyte aggregation (Craddock et al., 1977c) and that anaphylatoxins produced by C₃ and C₅ components cause the release of histamine from mast cells, contraction of smooth muscle, and increased capillary permeability (Vallota and Muller-Eberhard, 1973). Complement activation can cause release of histamine (Grant et al., 1975) and lysozomal enzymes (Goldstein et al., 1973) from human leukocytes. The finding of Craddock and his co-workers (1977b) that complement-activated plasma produces no pulmonary response in sheep rendered acutely neutropenic further supports a role for leukocytes in the pathogenesis of the pulmonary response to complement activation.

The pulmonary vascular response observed in sheep after the onset of extracorporeal veno-venous membrane oxygenator perfusion (ECMO), (Birek et al., 1976) is quite similar to the response seen after the infusion of zymosan-activated plasma. Presumably the pulmonary response in the ECMO sheep also is due to complement activation, since such activation has been demonstrated following exposure of plasma to silicone rubber membranes (Lindsay et al., 1976). In the ECMO experiments on sheep, as in the current experiments, sulfinpyrazone pretreatment eliminated the pulmonary response without altering the leukopenic response.

The role of sulfinpyrazone in blocking the pulmonary response has not been identified yet. We have found that sulfinpyrazone does not act by preventing the complement activation, since addition of sulfinpyrazone to heparinized plasma, prior to incubation with zymosan, does not prevent the pulmonary response, when this plasma is reinfused into the non-pretreated sheep.

It is also apparent that sulfinpyrazone is not acting as a pulmonary vasodilator, since there was no change in the pulmonary artery pressure or in pulmonary vascular resistance after the administration of sulfinpyrazone to these sheep. This finding is supported by the work of Mlczoch and co-workers (1978) who found that the administration of sulfinpyrazone to dogs did not alter any hemodynamic parameters. Furthermore, they demonstrated that the increase in pulmonary vascular resistance produced in response to hypoxia (8.5% oxygen in nitrogen) in dogs was the same before and after sulfinpyrazone administration, indicating that sulfinpyrazone does not act as a direct pulmonary vasodilator.

Sulfinpyrazone could be acting as an inhibitor of the release of vasoactive substances by platelets. However, since there was no measurable change in platelet count or in platelet response to ADP after plasma infusion, platelet aggregation at least is not occurring. This does not exclude absolutely the possibility that the infusion of activated plasma is causing a platelet release reaction without concomitant aggregation, but we believe this mechanism is unlikely.

The most probable mechanism for sulfinpyrazone's action is inhibition of prostaglandin synthesis. It has been demonstrated (Ali and McDonald, 1977) that platelet prostaglandin synthesis is inhibited strongly by sulfinpyrazone. Presumably such inhibition may occur in white cells and in pulmonary vessel walls as well.

In several of the experiments on sheep, the platelets showed demonstrable aggregation on exposure to collagen prior to sulfinpyrazone but no aggregation after sulfinpyrazone. This suggests that prostaglandin synthesis (usually involved in collagen-induced aggregation) was inhibited. Further evidence in this regard is suggested by preliminary data from our laboratory, which indicates that pretreatment of sheep with the cyclo-oxygenase inhibitor, indomethacin (10 mg/kg per day), for 3 days, prevents the pulmonary vascular response and pulmonary dysfunction otherwise caused by infusion of complement-activated plasma (Cooper, Menkes, Masterson, and Klement, unpublished observations).

It is apparent that pulmonary leukostasis, per se, cannot be invoked as the cause of pulmonary dysfunction after infusion of complement-activated plasma. The role of leukocytes in the production of such dysfunction requires further elucidation.

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