The intrapulmonic accumulation of neutrophils is a relatively common finding in certain animal models of increased permeability pulmonary edema and in humans with the adult respiratory distress syndrome. The release of toxic oxygen radicals from these cells can result in acute lung injury. Whether these cells mediate the increased permeability in all models of increased permeability pulmonary edema remains controversial. This review will examine the role of the neutrophils in various models of increased permeability pulmonary edema.

Neutrophils and their toxic oxygen metabolites have been implicated as the central mediators of the increased alveolar capillary membrane (ACM) permeability associated with the adult respiratory distress syndrome. Are they "guilty" as charged? There is evidence which makes the neutrophil a potential culprit:

1. Neutrophil numbers are increased in the lung lavages of animal models and patients with ARDS.¹⁶
2. Neutrophil function is abnormal in the blood of patients with ARDS.⁷⁻¹⁰
3. Neutrophils accumulate in pulmonary capillaries in animal models and human disease.¹¹⁻¹⁷
4. Neutrophil-generated unstable oxygen radicals can produce endothelial cell damage. Scavengers or blockers of unstable oxygen radicals ameliorate the increased ACM permeability in some animal models.¹⁸⁻²²
5. Neutrophil depletion blunts (but does not completely eradicate) the increased ACM permeability and associated cardiopulmonary dysfunction in certain animal models.²³⁻²⁶

These observations have been reported in a plethora of original and review articles.²⁷⁻⁴³ One might conclude that the neutrophil is "guilty" and is indeed the most important mediator of the ACM injury. We believe that this conclusion may be somewhat premature. In this review, we present evidence to support alternative conclusions.

As has been pointed out,⁴⁴⁻⁴⁵ the intrapulmonic accumulation of neutrophils does not necessarily mean that these cells are responsible for ACM damage. As outlined in Table 1, the relationship of neutrophils to increased permeability pulmonary edema could take a number of forms. We will discuss each of these:

1. Intrapulmonic neutrophil trapping and increased permeability pulmonary edema occur together; the former is responsible for the latter. Phorbol myristate acetate (PMA) is an ester derivative of croton oil and a potent stimulant of polymorphonuclear leukocyte oxidative metabolism, aggregation, release of secondary granules, and production of toxic oxygen metabolites.⁴⁶⁻⁴⁷ The intravenous injection of PMA causes an increased permeability pulmonary edema mediated by neutrophils as evidenced by the following:

(A) In rabbits, lung-to-body weight ratios increased from 4.2 ± 0.5 to 6.4 ± 1 x 10⁻³ (p<0.05) with concomitant increases in lung lavage albumin concentrations. In animals, made neutropenic there was no significant increase in lung weight or lavage fluid albumin concentrations following PMA administration.⁹ (B) Four to six hours after PMA injection in sheep with chronic lung lymph fistulas, there was a 2.5-fold increase in protein-rich lymph flow compared to baseline values; this translates into an increase in lung lymph protein clearance from .59 to 1.34 g/hr.⁶ (C) Intrapulmonic granulocytes increased sevenfold within an hour and a half after PMA infusion in sheep. There was also some interstitial thickening and alveolar edema.⁸ In rabbits, 1.5 hours following PMA injection, neutrophils invade the interstitium and by five hours these cells plus erythrocytes and edema fluid appear in the alveoli.⁸ (D) In isolated saline-perfused rabbit lungs, the addition of PMA and granulocytes to the perfusate caused an increase in lung weight of 42 ± 9.2 g compared to only 2 ± 4 and 6 ± 6 g in lungs perfused with granulocytes or PMA alone.⁹ (E) Mepacrine, a neutrophil degranulation blocker and oxygen radical scavenger, blunted the increase in lung lavage albumin concentra-
tion, neutrophil accumulation, and lung/body weight ratios induced by PMA. Additional studies employing cultured endothelial monolayers and isolated perfused lungs demonstrated that PMA stimulated neutrophils, when closely apposed to endothelial cells, increased permeability through an oxygen radical dependent mechanism.

The preponderance of evidence indicates that PMA causes intrapulmonic neutrophil trapping, release of unstable oxygen radicals, damage to the pulmonary endothelium, and increased permeability pulmonary edema. However, neutrophils may not be entirely responsible for the increased permeability: in rats, intratracheal installation of PMA leads to acute hemorrhagic pneumonitis that is independent of neutrophils. No studies have been performed in which PMA was infused into neutropenic sheep with chronic lung lymph fistulas. In neutropenic rabbits, PMA infusion is not associated with the expected increase in lung weight and lung lavage protein and neutrophils; however, there was some increased thickness of the interstitium. Taking all the above into consideration, we conclude that PMA models of increased permeability pulmonary edema are almost entirely neutrophil dependent, but probably have no corollary in human disease.

(2) Intrapulmonic neutrophil trapping and increased permeability pulmonary edema occur together; the former is partially responsible for the latter. With the introduction of the sheep lung lymph fistula model in the early 1970s, the way was open to study ACM permeability in a chronic, awake animal model. Following the infusion of Escherichia coli endotoxin or Pseudomonas bacteria, there is a stereotypical response: (1) the early response (phase 1) is characterized by an increased flow of protein poor lymph and marked pulmonary hypertension, and (2) the later stage (phase 2) is characterized by an increase in protein rich lymph with a decline in pulmonary artery pressures towards normal. An early neutropenia (phase 1) is associated with neutrophil sequestration within the pulmonary capillary bed. That these intrapulmonic neutrophils may play a role in the increased vascular permeability phase is evident in neutropenic sheep. There is a 30 to 50 percent decrease in lymph flow and lymph protein clearance (Table 2) in these animals compared to those with normal neutrophil counts. However, it should be noted that lymph flow and protein flux are still elevated two to three times baseline measurements. Although neutropenia blunts the increased vascular permeability, it does not totally abolish it. This is also true in various models of pulmonary microemboli (Table 2). This contrasts with methylprednisolone pretreatment (Table 2) in which both the increased lymph flow and protein clearance are completely prevented after E. coli infusion.

Table 1—Possible Relationship of Neutrophils to Increased Permeability Pulmonary Edema

<table>
<thead>
<tr>
<th>Model</th>
<th>Q₀, ml/hr</th>
<th>Lymp Protein Clearance, g/hr</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pseudomonas</em> bacteria</td>
<td>.58</td>
<td>3.2</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>.495</td>
<td>2.73</td>
</tr>
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<td><em>E. coli + WBC</em></td>
<td>.536</td>
<td>2.17</td>
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<td>.42</td>
<td>1.06</td>
</tr>
<tr>
<td><em>E. coli + WBC</em></td>
<td>.607</td>
<td>1.44</td>
</tr>
<tr>
<td><em>E. coli + WBC</em></td>
<td>.16</td>
<td>1.12</td>
</tr>
<tr>
<td><em>E. coli + MP</em></td>
<td>.47</td>
<td>1.83</td>
</tr>
<tr>
<td><em>Microemboli + WBC</em></td>
<td>.40</td>
<td>.73</td>
</tr>
<tr>
<td><em>Microemboli + WBC</em></td>
<td>.33</td>
<td>.48</td>
</tr>
<tr>
<td><em>Air emboli + WBC</em></td>
<td>.20</td>
<td>.82</td>
</tr>
<tr>
<td><em>Microemboli + WBC</em></td>
<td>.14</td>
<td>.24</td>
</tr>
<tr>
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<td>1</td>
<td>2.42</td>
</tr>
<tr>
<td><em>Microemboli + WBC</em></td>
<td>1</td>
<td>171</td>
</tr>
</tbody>
</table>

*Parentheses indicate percent change from baseline (BL).
†Q₀, lymph flow; Perm, maximum increase in pulmonary microvascular permeability as reflected by highest Q₀; lymph protein clearance, Q₀ × lymph/plasma protein; + WBC, normal neutrophil count; − WBC, neutropenia; MP, methylprednisolone.
‡BL normalized to 1.
We conclude in sheep infused with bacteria, E coli endotoxin, or microemboli intrapulmonary neutrophil trapping accounts for part, but not all of the increased vascular permeability. In other models, the administration of endotoxin causes pulmonary edema in animals depleted of neutrophils. This is in contrast to E coli infusion in dogs in which intrapulmonary neutrophil trapping occurs, but pulmonary edema is either not present or minimal.

(3) Intrapulmonic neutrophil trapping and pulmonary edema occur with no relationship between the two. A multitude of drugs have been used to induce pulmonary edema including alpha naphthathiourea (ANTU), pyrrolizidine pyrroles, streptococcal endotoxin, ammonium sulfate, and oleic acid. Oleic acid administered intravenously in various animals produces rapid onset, high permeability pulmonary edema. During this early stage, there is interstitial/alveolar hemorrhage and edema, and vascular thrombi composed of platelets, cell debris, and neutrophils. In most, but not all studies, the increased permeability edema is not altered by neutropenia, pretreatment with indomethacin and steroids or by complement depletion.

When isolated dog lung lobes are perfused with a balanced salt solution containing no formed blood elements and oleic acid is added to the perfusate, there is a rapid weight gain compared to control lobes. Thus, in the near absence of blood elements, pulmonary edema develops following oleic acid. Although intrapulmonic neutrophil trapping occurs in vivo, it does not appear to play a role as a mediator of the oleic acid-induced increased permeability edema.

(4) Intrapulmonic neutrophil trapping occurs but increased permeability pulmonary edema is not present. Clinical interest in neutrophil mediated pulmonary dysfunction was stimulated in 1977 when Craddock et al reported transient neutropenia in patients undergoing hemodialysis with a cellophane membrane. In 34 patients, severe neutropenia (24 percent of initial values) was found. Fifteen of these patients had impaired pulmonary function including hypoxemia, increased P(A-a)O gradients and decreased diffusing capacity within 30 minutes of starting hemodialysis. When rabbits were infused with autologous cellophane incubated plasma, a similar neutropenia was noted. Histologic examination of these animals revealed intravascular leukostasis and perivascular lymphatic dilatation. Autologous cellophane incubated plasma infusion in sheep with chronic lung lymph fistulas led to a striking neutropenia with a doubling of the pulmonary artery pressure and a threefold increase in lung lymph flow. Animals made neutropenic showed no alteration in any parameters with the infusion of the autologous cellophane incubated plasma. The authors implied that complement activation by cellophane membranes resulted in intrapulmonic neutrophil accumulation with increases in pulmonary vascular permeability leading to hypoxemia.

Further in vivo and in vitro studies confirmed this series of events and extended the observation that complement stimulated granulocytes released oxygen radicals which damaged endothelial cells. Since complement is activated during cardiopulmonary bypass, it was also postulated that the above series of events could be responsible for the "postpump syndromes." Other studies followed which confirmed that a variety of diverse disorders could activate complement. However, although almost all investigators were able to show pulmonary leukostasis following complement activation, more recent reports found no physiologic or histologic evidence for increased microvascular permeability or acute lung injury.

A careful review of the original article by Craddock et al reveals that intravascular leukocyte trapping and hypoxemia were definitely present. However, the contention that a threefold increase in the lung lymph flow was due to increased microvascular permeability remains unconfirmed. It would seem more probable that the doubling of the pulmonary artery pressure led to the increase in the lymph flow since they were temporally related. The investigators did not report the protein concentration in the lung lymph in these experiments. An alternative conclusion for the increased lymph flow is that it was a "hydrostatic" form of microvascular permeability. The hypoxemia may have been due to the release of vaso and bronchial active arachidonic acid products leading to V/Q mismatch.

More recent studies indicate that the duration of intrapulmonic neutrophil trapping may be critical to lung injury. Repeated or continued infusion of activated plasma leads to pulmonary dysfunction with increases in high protein lymph flow. However, short-term activation of complement does not cause acute lung injury even though arachidonic acid products are released.

In summary, we conclude that a multitude of insults lead to intravascular activation of complement with intrapulmonic or intravascular neutrophil entrapment, though injury rarely occurs. Possibly, the presence of other factors such as hypoxia, surgery, or the release of prostaglandins may be needed to "activate" the neutrophil.

(5) Intrapulmonic neutrophil trapping does not occur and there is increased permeability pulmonary edema. We became interested in the pulmonary edemagenic effects of ethchlorvynol (Placidyl) when we cared for two patients who experienced severe, rapidly reversible ARDS following the intravenous injection of 25 to 40 mg/kg of this agent. Ethchlorvynol is a unique hypnotic sedative drug in that it is a
liquid suspended in a polyethylene glycol diluent, and packaged within a gelatin capsule. The solution is easily removed by puncturing the capsule with a needle. To delineate the effects of ethchlorvynol on cardiopulmonary parameters, we injected from 12 to 80 mg/kg body weight into dogs. Lung weights increased, and there was microscopic evidence of alveolar edema without an increase in pulmonary wedge pressure. Early hypoxemia was universal. In later studies, employing the in vivo saline-filled dog lung model, we confirmed dramatic increases in the ACM flux of endogenous proteins and exogenous dextrans up to 500,000 mw. We concluded that ECV induced a severe, but transient, increased permeability pulmonary edema.

In further studies, we became interested in the possible mediator(s) of this increased permeability. Although we found a slight increase in alveolar epithelial permeability mediated by histamine, this did not explain our previous findings of a marked increase in permeability. To evaluate the role of neutrophils we performed the following: (1) morphologic analysis revealed no intrapulmonic neutrophil trapping; (2) we measured pulmonary artery and systemic artery leukocyte counts before and after ethchlorvynol injection; there was a slight drop in the arterial counts (approximately 1,500 ml/cu mm); (3) in normal dogs injected with ethchlorvynol, there was an increase in lung water from 75 ± 4 to 84 ± 3 percent (p<0.01). In dogs made neutropenic with cyclophosphamide there was a similar increase in lung water; (4) we established an isolated dog lung lobe model and perfused it with plasma free of formed elements. Addition of ethchlorvynol to the perfusate led to a significant increase in lung weights. In lobes of dogs previously made neutropenic, ethchlorvynol also caused a significant increase in lung lobe weight; (5) indomethacin, methylprednisolone, or induced leukopenia didn't protect dogs from an increased right lung lymph flow following ethchlorvynol injection; (6) employing the acute sheep lung lymph fistula model pretreatment of animals with indomethacin, diphenhydramine, and phenotamine had no effect on the increase in high protein lymph flow but there was blunting of the pulmonary hypertension with indomethacin; (7) further unpublished studies in the chronic sheep lung lymph fistula model revealed no increase in intra-alveolar neutrophil counts during any of the four or five days following ethchlorvynol injection, although increased permeability was present as reflected by increases in lung lymph flow with high protein concentrations; (8) in further unpublished studies using the isolated perfused rat lung, the addition of ethchlorvynol to the perfusate led to a significant and rapid increase in lung weight. We therefore conclude that ethchlorvynol can produce a severe, but transient, increased permeability pulmonary edema not mediated by neutrophils.

In summary, intrapulmonic neutrophil trapping is a relatively common occurrence in various forms of increased permeability pulmonary edema. However, the presence of neutrophils in the pulmonary vasculature does not necessarily implicate this cell as the culprit in causing the increased microvascular permeability.

If the neutrophil is not the primary mediator or is just one of many mediators, what are the other possibilities? These include, but are not limited to some combinations of the following: 1) other formed elements such as platelets, fibrin, fibrin degradation products, etc may be important; 2) leukotrienes, cyclo-oxygenase products, bradykinin, histamine, etc may be released and cause increased permeability; 3) alveolar macrophages may be of central importance in modulating acute lung injury; 4) certain substances such as oleic acid and ethchlorvynol could directly injure the endothelial cells; 5) the pulmonary microvasculature and alveolar epithelium possess fixed negative electrical charges which may help modulate normal transACM protein and fluid flux. A decrease or loss of these charges could lead to increased transACM protein/fluid movement. This would be similar to the proteinuria that occurs when glomerular fixed negative charges are either bound or destroyed. When protamine sulfate, a polycation which can bind negative charges, is placed into the perfusate in the isolated, perfused rat lung model there is a rapid lung weight gain with minimal changes in vascular pressures.

At this point in time, a multitude of possible pathways still exist which need extensive exploration before investigators can specify state the cause or causes of the increased permeability edema associated with various animal models and in humans. It is also possible that a single final common pathway to increased permeability does not exist.

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Neutrophil in increased Permeability Pulmonary Edema (Glauser, Fairman)