# Experimental Type 25 Pneumococcal Pneumonia in Rats

## *An Electron-Microscopic Study*

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**A self-healing lobar pneumonia was produced in rats by intratracheal instillation of Type 25 pneumococci. Sequential changes in the lung were examined by electron microscopy from the onset of acute inflammation through resolution. Manifestations of the infection included vascular endothelial changes of acute inflammation and leukocytic mobilization. The pulmonary alveolar epithelium showed only minimal changes throughout the infection. The significance of the findings is discussed in relation to the pathogenesis of pneumococcal lobar pneumonia. (Am J Pathol 1980, 99:231-242)**

PRIOR TO THE DISCOVERY of antibiotics, there was a high mortality with pneumococcal pneumonia, and indeed the overall mortality is still approximately 10%. Despite the severity of the acute illness usually associated with pneumococcal pneumonia, pulmonary necrosis and residual damage to the lung is unusual, in contrast to the parenchymal damage which is often observed in pneumonias caused by staphylococcal or *Enterobacteriaceae.* The reason for the unique preservation of the pulmonary architecture in pneumococcal disease is not known. Pneumococci do not produce any readily demonstrable toxins. They appear to multiply in tissues by virtue of their polysaccharide capsules, which protect them from phagocytosis. This multiplication triggers an extensive inflammatory response without, however, permanent parenchymal changes visible with the light microscope.

In the present study, we have examined the ultrastructure of the lung in rats with Type 25 pneumococcal pneumonia to further characterize the pulmonary response in pneumoccocal pneumonia. Electron microscopy has been employed in the investigation of various pulmonary diseases, but there have been only a few reports of ultrastructural changes in the bacterial pneumonias.[1-5] Loosli and Baker [1] examined Type 1 pneumococcal pneumonia by electron microscopy, but their observations were limited to the vascular changes. In the present study, the alveolar epithelial cells as

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well as the vascular changes have been evaluated by use of sequential observations throughout the course of the infection.

## Materials and Methods

### Pneumococci

Type 25 pneumococci were obtained from the American Type Cultures Collection (ATCC 6325) (Rockville, Md). The organisms were passed in rats and stored at 4 C in rabbit serum. An overnight culture was inoculated into rabbit brain–heart infusion broth that contained 7% heat-inactivated rabbit serum. After 5 to 6 hours at 37 C (logarithmic phase of growth), the concentration of the organisms was determined with a spectrophotometer and adjusted to $2 \times 10^8$ pneumococci/ml with broth. An aliquot was diluted in 12% bacteriologic mucin (Difco Laboratories, Detroit, Mich) for inoculation into the rats.

### Animals

Adult male Sprague–Dawley rats (Charles River Breeding Laboratories, Wilmington, Mass) were used throughout the experiment. Serums of the rats employed for infection with Type 25 pneumococci did not contain any type-specific antibody as measured by indirect immunofluorescence with a modification of the method of Sloyer et al.[6] Experimental and control groups and the schedule of killing after inoculation are shown in Table 1.

### Production of Pneumonia

The rats were anesthesized with methoxyflurane (Pitman-Moore, Inc., Washington Crossing, N.J.). A small incision was made in the skin, and the trachea was exposed by blunt dissection. A tracheostomy was made with a 22 gauge needle, and a plastic catheter (attached to a tuberculin syringe holder) was threaded down the trachea to the left lower lobe. An inoculum of 0.1 ml containing $10^7$ pneumococci in 6% mucin was injected.

### Examination of the Lung

Animals were killed by exsanguination under deep anesthesia. The thorax was opened with a midline incision, and the trachea and lungs were excised *en bloc*. Glutaraldehyde (3%) in 0.2 M cacodylate buffer was instilled through the tracheal cannula at the pressure of 10 cm of water. After the full expansion of the lungs, the trachea was clamped and the lungs were immersed in the same fixative for 2 hours at 4 C. After 2 hours of fixation, the lobe containing consolidation (the left lower lobe in all animals that had visible consolidation) was bisected sagittally. One half was processed for light microscopy. The other half was divided into three parts, consisting of the center and the periphery of the consoli-

Table 1—Schedule of Killing After Inoculation

| | Time after inoculation | | | | | | |
|---|---|---|---|---|---|---|---|
| | 6 hours | 12 hours | 24 hours | 48 hours | 3 days | 5 days | 7 days |
| Experimental pneumonia (type 25 with mucin) | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| Control (mucin alone) | 1 | 1 | 1 | 1 | 1 | 1 | 1 |

dated area and the uninvolved area. Each part was diced into small pieces of tissue and processed for electron microscopy. In brief, the tissue blocks were washed several times in cacodylate buffer and postfixed in 2% osmium tetraoxide in cacodylate buffer. The tissue blocks were stained with uranyl acetate in veronal buffer, pH 5.0, dehydrated through graded ethanols, and embedded in epoxy resin. The sections were cut with a Porter-Blum ultramicrotome, stained with lead citrate, and examined with a Philips 300 electron microscope.

## Results

Five out of 30 experimental animals failed to show any sign of inflammation, grossly or microscopically, at the time of death. They comprised one each from the groups killed at 6 hours, 12 hours, 24 hours, 3 days, and 5 days. The rest of the experimental animals (83%) showed various stages of consolidation, limited to the left lower lobe. No animals died before sacrifice.

At autopsy, the involved lobe was obvious and showed color changes ranging from pink or red to gray, according to the time of sacrifice. At 6–12 hours the pleural surface was dull and at 24–48 hours showed some fibrinous exudate. Beginning at 3 days and in all cases by 7 days, the left lower lobe became grossly indistinguishable from the rest of the lung.

The control animals that received only mucin did not show any evidence of pneumonia grossly.

### Light Microscopy

The animals inoculated with mucin alone showed a focal collection of alveolar macrophages (AMs) in the alveoli. No acute inflammatory response was recognizable in any animals of this group.

The experimental animals showed essentially the changes of evolution of pneumonia as described by Wood.[7,8] At 6 hours after inoculation, the lesion consisted mainly of an "outer edema zone," in which alveoli were filled with fluid and numerous organisms. The perivascular space was markedly widened, indicating interstitial edema (Figure 1). A few polymorphonuclear leukocytes (PMNs) were present in the alveoli and in the interstitial space.

At 12 hours, the zone of "early consolidation and phagocytosis" could be clearly recognized from the edema zone. In early consolidation, alveoli contained numerous PMNs, and free organisms were few (Figure 2). At higher magnification (not shown), PMNs at this stage could be seen to contain many intracytoplasmic organisms. The alveolar septums appeared slightly thickened.

At 24 hours, the three zones described by Wood became distinct. In addition to the outer two zones (ie, the edema and early consolidation

zones), the central part of the lesion became a zone of "advanced consolidation" (Figure 3). In advanced consolidation, alveoli were packed with PMNs, and no free organisms were seen. Fibrin was present in the alveoli as well as in the interstitium.

At 48 hours, the area of advanced consolidation expanded, and the edema zone became less conspicuous. In the center of the area of advanced consolidation, there were focal areas of clearing, with many macrophages in the resolving alveolar exudate. These changes appeared to spread centripetally, and at 7 days the only residuum of the pneumonia was apparent thickening of alveolar septums and focal intraalveolar collection of AMs (Figure 4). Throughout the experiment, the alveolar epithelial cells appeared intact, and there were no foci of necrosis.

#### Electron Microscopy

The fine structure of *Streptococcus pneumoniae* has been described previously by Tomasz et al[9] with a noncapsulated variant. In the present study, the Type 25 pneumococci showed capsular fibrils measuring 40 nm with a rather narrow capsular space (Figure 5).

Control animals inoculated with mucin alone showed no remarkable changes except an accumulation of AMs in the alveolar spaces.

Because in our experimental model the light-microscopic changes followed the events described by Wood,[7] our observations with the electron microscope are presented with reference to the three zones of inflammation described by him.

##### Outer Edema Zone

The pulmonary alveoli were filled with amorphous floccular material, indicating a high protein content in the edema fluid. Embedded in this material were numerous organisms, some of which were in the process of binary division, indicative of active multiplication (Figure 5). Alveoli also contained erythrocytes and myelin figures (pulmonary surfactant material). At the level of peripheral alveoli, epithlial and endothelial cells were firmly attached to the fused, thin basement membrane. The cellular matrix remained compact, and the interstitium showed minimal edema. In contrast, in the proximal portion of respiratory unit (the area near the bronchiole and the bronchiole itself), endothelial cells appeared swollen, with less electron-dense cytoplasmic matrix, suggesting intracellular edema. There were gaps between the endothelial cells with focal fibrin deposition. The interstitium showed focal electron-lucency, suggesting interstitial edema. Epithelial cells (Types I and II) appeared normal (Figure 6).

### Zone of Early Consolidation and Phagocytosis

Alveoli were filled with PMNs containing numerous organisms. The organisms were seen in membrane-bound vacuoles (phagosomes), and some of the phagosome showed attached osmiophilic dense bodies (lysosomes) (Figure 7). The intracellular organisms showed various stages of digestion by PMNs. The blood vessels, especially venules, showed PMNs on the surface of the endothelial cells, at the gap between the endothelial cells, and in the subendothelial space. These changes indicated mobilization of PMNs from the vascular space into alveoli (Figure 8). The interstitial space was widened and electron-lucent, suggesting interstitial edema. The alveolar epithelial cells were firmly attached to the basement membrane, and there was no sign of intracellular edema or organellar degeneration.

### Advanced Zone of Consolidation

Alveoli were packed with PMNs that contained numerous phagocytized materials. There were few intact organisms in the phagosomes. At this stage, AMs appeared increasingly in the alveolar space, engulfing cell debris, fibrin, and erythrocytes. In the early zone of resolution, the intraalveolar cell population consisted exclusively of AMs.

The Type I pneumocyte appeared slightly swollen with electron-lucent cytoplasmic matrix. The basement membrane of the alveolar septa were partially separated by a clear zone (Figure 9). Dense deposits of fibrin were recognized intraalveolarly as well as interstitially. The Type II pneumocyte retained its structure intact throughout all stages of the infection.

## Discussion

The temporal evolution of inflammation in experimental bacterial pneumonia is obviously dependent on the bacterium, the inoculum size, and the choice of experimental animal. In the present study intratracheal inoculation of Type 25 pneumococci in rats led to self-healing lobar pneumonia. The changes detected by light microscopy followed the pattern described by Wood,[7] who employed Type 1 pneumococci in a rat model. In both studies, inflammation developed rapidly and could be readily detected within 6 hours. The early changes observed with electron microscopy were basically the vascular changes of acute inflammation. There were no concomitant changes in the alveolar epithelial cells at the time when these early vascular changes were prominent.

Apparently pneumococci produced the vascular changes of inflammation simply by their presence within the alveoli, because there was no evi-

dence of invasion of host tissue, and pneumococci are not known to produce soluble toxins. The mechanism by which pneumococci excite these early inflammatory changes is not known. Many pneumococci, including Type 25, can activate the alternative complement pathway and do not require specific antibody for complement activation.[10-12] We have demonstrated elsewhere [13] that in rat serum, Type 25 pneumococci activate the alternate pathway exclusively. Moreover, we recently detected C3 and a functionally active alternative complement pathway in the concentrated bronchial lavage fluid of rats (J. D. Coonrod, unpublished data). Others [14] have shown that there is an intact alternative complement pathway in human bronchial lavage fluid. Desai et al [15] have demonstrated that the intratracheal instillation of a preformed chemotactic factor derived from C5 can cause an active inflammatory reaction in hamster lungs. On the basis of these observations, it is tempting to speculate that complement activation is the impetus for the early inflammatory changes in the present model of infection, where there is no direct tissue invasion by the organisms and no apparent production of soluble toxins.

There was no evidence of alveolar cell necrosis at any point in the evolution of the inflammatory response in the present model. There are several studies that show that in the event of Type I cell necrosis the recovery phase is slow in onset[3,4] and the Type I cell is replaced by the Type II pneumocytes or by bronchiolar Clara cells.[16,17] This change, then, is frequently associated with interstitial fibrosis. In the present model, where recovery was rapid, the interaction of host and organism was such that the organisms were eliminated without tissue necrosis or residual fibrosis. It seems likely that this sequence of events in rats may be indicative of events in most patients with pneumococcal pneumonia, where rapid recovery is the rule.

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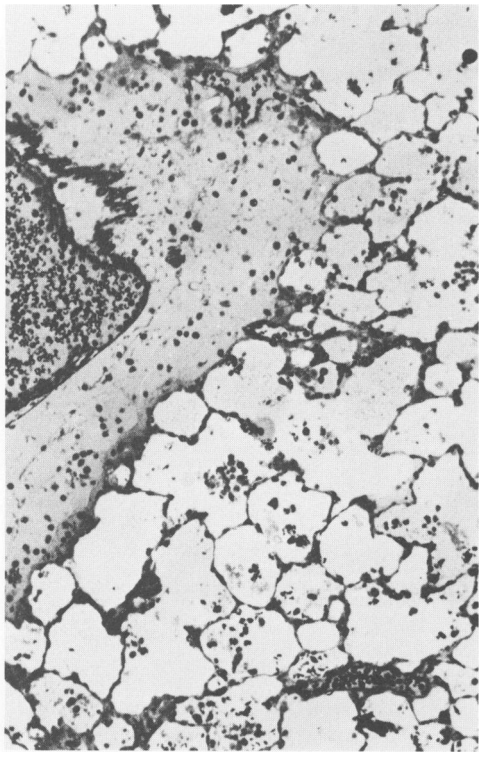
[*Illustrations follow*]



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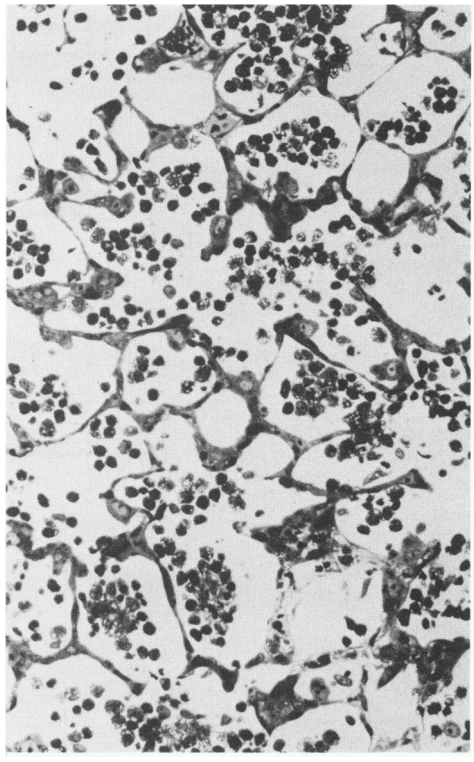

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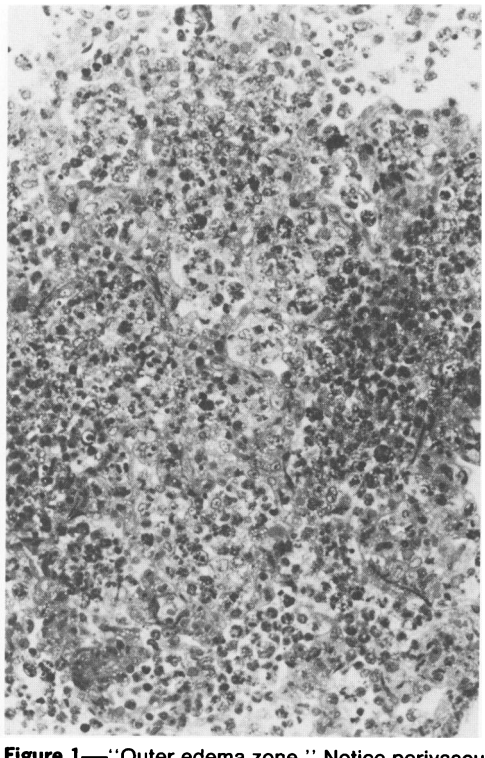

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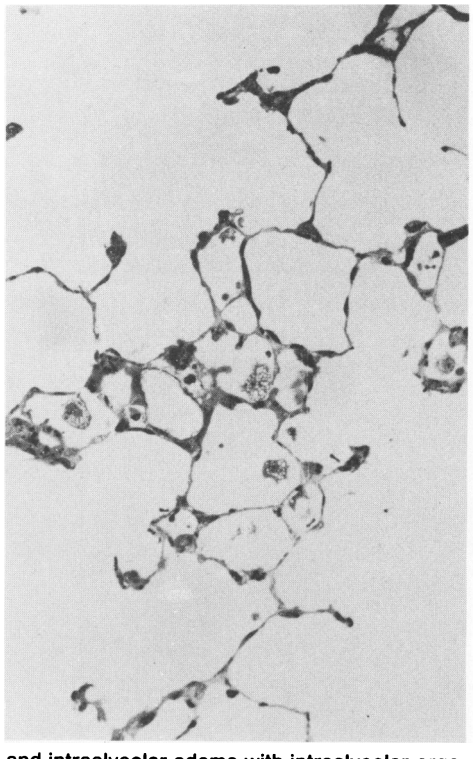

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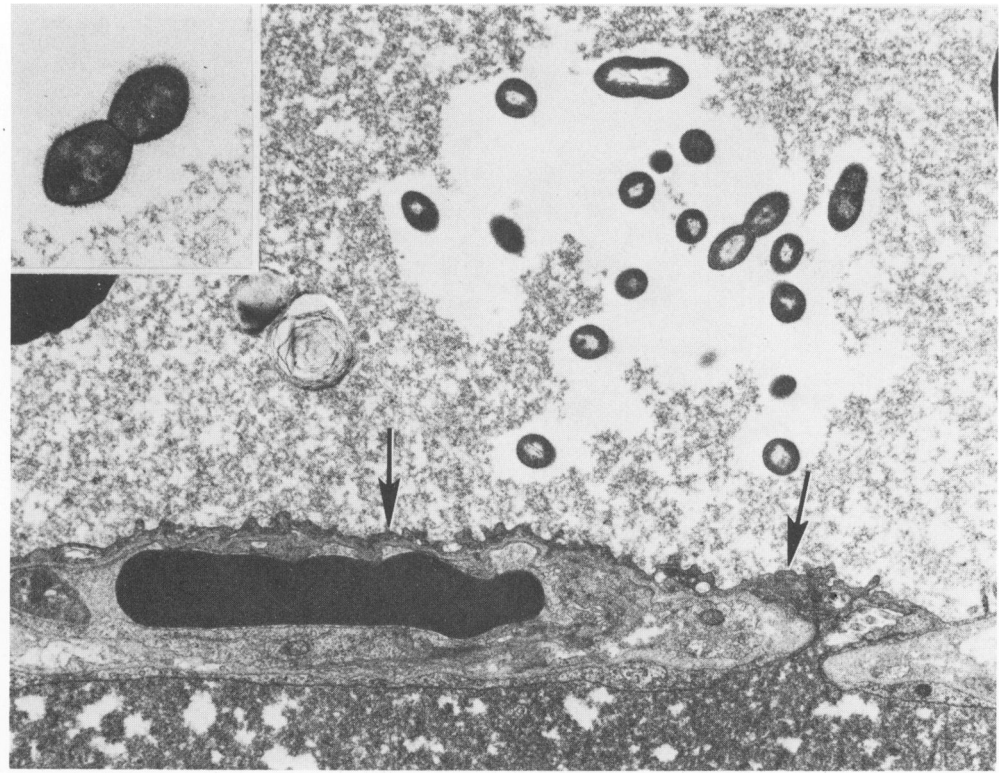
**Figure 1**—"Outer edema zone." Notice perivascular and intraalveolar edema with intraalveolar organisms. (Toluidine blue, ×80) **Figure 2**—"Early consolidation." Notice the intraalveolar accumulation of PMNs. (Toluidine blue, ×100) **Figure 3**—"Advanced consolidation." The alveoli are filled with PMNs. (Toluidine blue, ×100) **Figure 4**—Alveoli 7 days after inoculation. The only abnormalities are AMs and focal thickening of septums. (Toluidine blue, ×100)



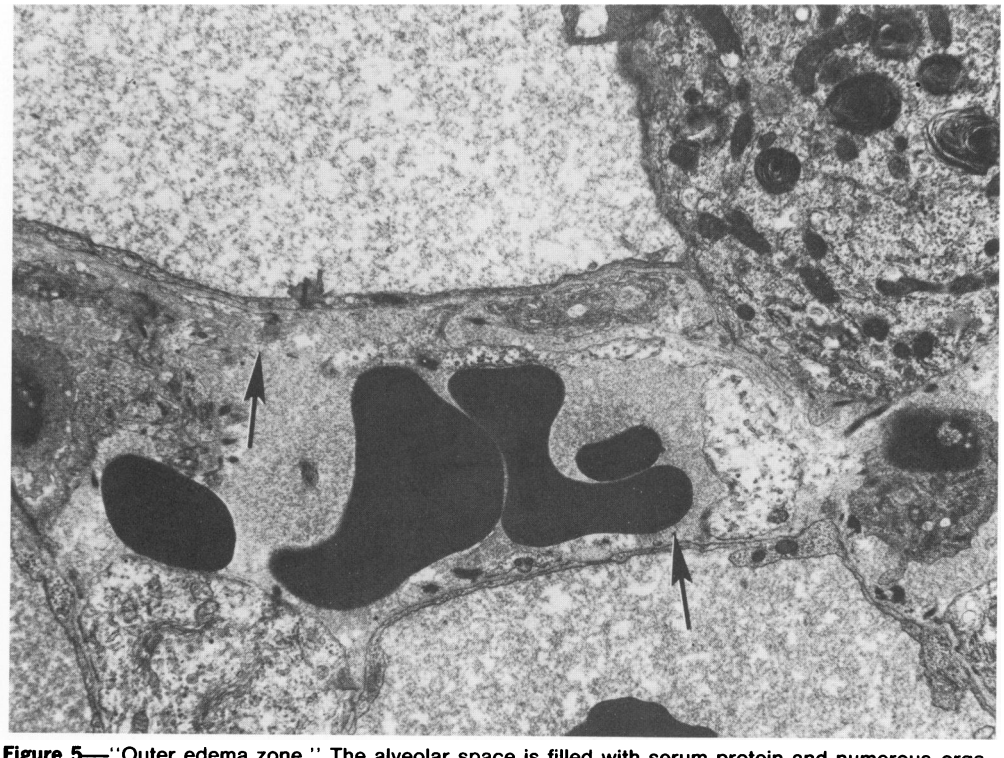


**Figure 5**—"Outer edema zone." The alveolar space is filled with serum protein and numerous organisms. Notice the intact Type I pneumocyte (*arrow*). (Lead citrate and uranyl acetate, ×9000) **Insert**—Higher magnification of pneumococci. Notice the capsular fibrils. (×20,000) **Figure 6**—"Outer edema zone," proximal respiratory unit. Notice the swelling of endothelial cell with gaps (*arrow*). (Lead citrate and uranyl acetate, ×7800)

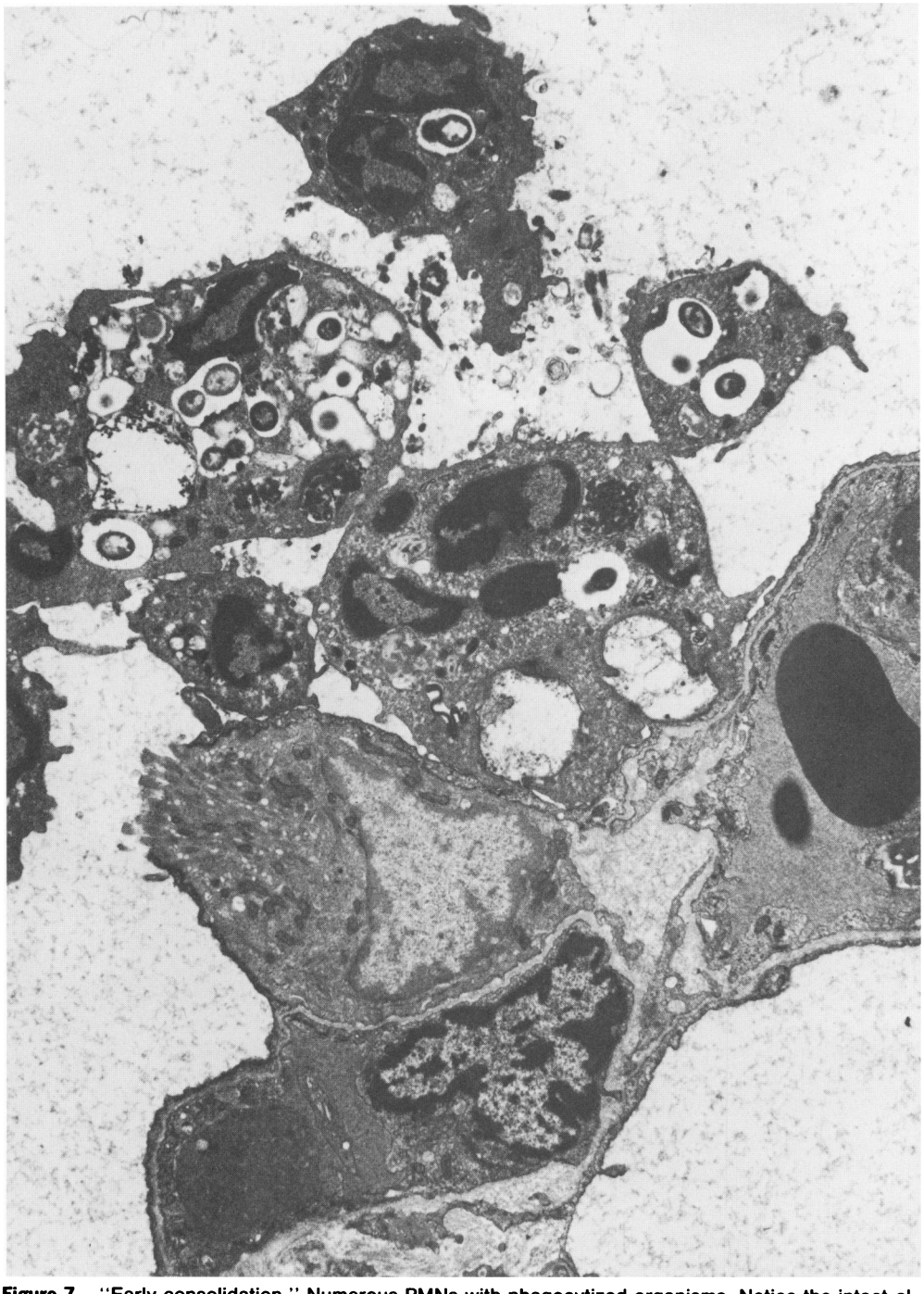
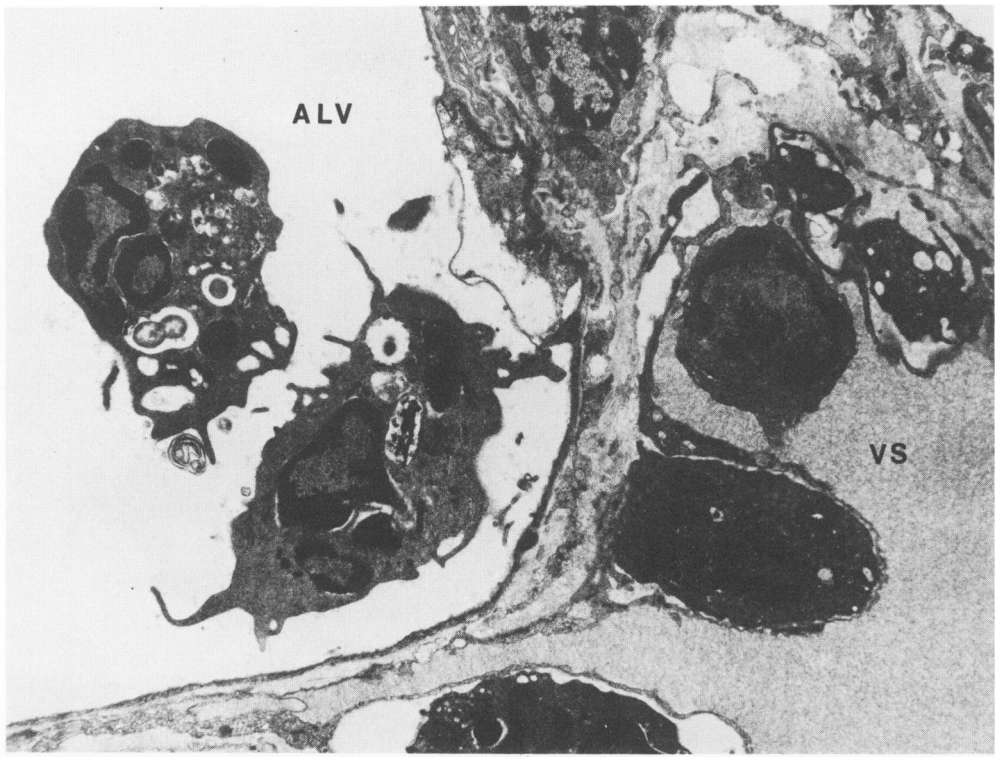


**Figure 7**—"Early consolidation." Numerous PMNs with phagocytized organisms. Notice the intact alveolar epithelial cells and interstitial edema. (Lead citrate and uranyl acetate, ×8300)

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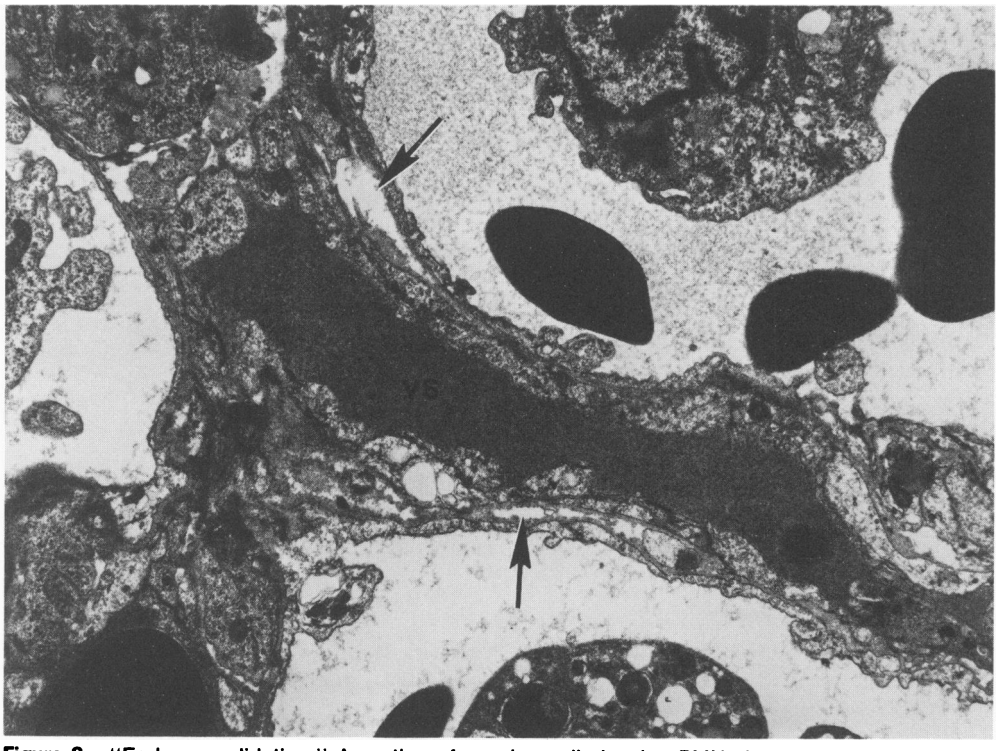


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**Figure 8**—"Early consolidation." A portion of venular wall showing PMNs in stages of mobilization. *ALV*, alveolar space; *VS*, vascular space. (Lead citrate and uranyl acetate, ×6200) **Figure 9**—"Advanced consolidation." No intact organisms are seen in the PMNs. Type I pneumocytes show loose cytoplasmic matrix. The basement membrane is focally separated (*arrow*). *VS*, vascular space (Lead citrate and uranyl acetate, ×9000)