# Bronchial Venular Leakage During Endotoxin Shock

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**The pulmonary effects of endotoxin shock were investigated in dogs by a combination of anatomic and physiologic technics. Shock was produced in 14 dogs by injecting *Escherichia coli* lipopolysaccharide intravenously. Three dogs in hypovolemic shock and 6 untreated dogs served as controls. Colloidal carbon was injected intravenously to detect sites of pathologic increase in vascular permeability. During the first hour of endotoxin shock, bronchial venules allowed carbon and blood elements to traverse their walls, whereas no leakage of these large particles or ultrastructural changes could be detected in the alveolar walls. Only after the first hour was bronchial venular leakage accompanied by focal degenerative changes in the alveolar endothelium, focal interstitial edema in the alveolar septum and sequestration of damaged leukocytes in the alveolar capillaries. In contrast to these observations in endotoxin shock, control dogs in hypovolemic shock did not show bronchial venous leakage. Our findings suggest that the leakage of bronchial venules may be involved in the pathogenesis of pulmonary interstitial edema caused by endotoxin shock (Am J Pathol 77:387–406, 1974).**

STRIKING AND CHARACTERISTIC CHANGES often designated as "congestive atelectasis" may occur in the lungs of patients who die in septic shock. However, the distinctive histologic appearance of shock lung is not unique for sepsis, but may result from a wide variety of apparently unrelated initiating mechanisms, including hemorrhagic hypotension, fat emboli to the lungs and nonthoracic trauma.[1] Moreover, the final anatomic picture is often complicated by the administration of high concentrations of oxygen in the inspired gas and large transfusions prior to death.

Certain initiating mechanisms, such as endotoxinemia, have raised the possibility that primary alterations in the permeability of pulmonary capillaries may initiate a sequence leading to shock lung. Thus, Chien and Gregersen *et al*[2] demonstrated in the dog that the systemic microcirculation became more permeable to macromolecules after injection of endotoxin. According to Brigham, Woolverton and Staub[3] a similar phenomenon occurs in the pulmonary circulation of conscious sheep after the infusion of suspensions of *Pseudomonas aeruginosa* organisms.

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Their conclusion was based on the demonstration of an increase both in the rate of lymph flow and in the lymph plasma ratios of protein concentration after the organisms were injected intravenously. Finally, Snell and Ramsey,[4] using the double indicator-dilution method to determine the quantity of water in the lungs of the dog, described remarkable increments in the water content in the lungs following injection of endotoxin. But despite this imposing physiologic evidence for heightened vascular permeability after administering endotoxin, the anatomic basis for this increase in pulmonary vascular permeability has not yet been elucidated.

The present study undertook to examine directly the structural basis for changes in pulmonary vascular permeability following injections of *E, coli* endotoxin. For this purpose, we reproduced in dogs the characteristic hemodynamic picture of endotoxin shock as described by others [6,7] and used colloidal carbon as an electron-dense marker to detect pathologic changes in permeability.[5] Despite these attempts to reproduce the experimental preparations of others, we were unable to demonstrate either that the pulmonary microcirculation became highly permeable to the tracer particles or that large quantities of water had accumulated in the lungs. However, we did find leakage of carbon particles through the walls of bronchial venules, in the same pattern that we had previously observed in dogs after the administration of either histamine or bradykinin.[8] In addition, although we did demonstrate that the water content of the lungs increased after endotoxin, in contrast to the results of Snell and Ramsey,[4] the increments were small.

The significance of these observations is considered in terms of the possible role of the bronchial circulation in the dynamics of liquid exchange in lungs and with respect to the relevance of the canine model of endotoxin shock to the pathogenesis of pulmonary effects of septic shock in man.

## Materials and Methods

The experiments were performed on mongrel dogs weighing 20 to 30 kg. They were free of heart worms and respiratory infections. In each dog, the hematocrit was greater than 35%. The experiments were subdivided into four groups according to the following protocols.

### Experimental Groups

#### Group I

In this group of 11 dogs, circulatory and respiratory variables were monitored at regular intervals to determine the physiologic derangements elicited by our

injections of endotoxin and to relate them to the observations of others.[6,7] In each dog, a single injection of *E coli* endotoxin was made intravenously (5 mg/kg of *E coli* lipopolysaccharide, batch 0127 B-8, Difco, Detroit, Mich) following anesthesia with chloralose, (1% solution, 100 mg/kg body weight) administered intravenously. During each experiment, the dog was prone, breathing spontaneously through a glass cannula, 15 mm in diameter, that was inserted through a tracheostomy. Cournand No. 8 catheters were positioned in the pulmonary artery and left atrium under fluoroscopic guidance via the jugular vein and carotid artery, respectively. The aorta was cannulated via the femoral artery using polyethylene tubing. These catheters were connected to Statham P23Db transducers, and the mean values of pressure were obtained by electronic attenuation of the phasic signals.

Transpulmonary pressure was measured by connecting the proximal end of a balloon-tipped catheter positioned in the esophagus and a lateral tap of the endotracheal cannula to the opposite sides of a differential strain gauge (Statham PR 23 2D 300). Air flow was measured using a Fleisch pneumotachograph (Instrumentation Associates, Inc, New York, NY) connected to the tracheal cannula. The dead space of this system was 75 ml. Tidal volumes were obtained by electronic integration of the air flow signals. Dynamic lung compliance loops over the tidal volume range were recorded at regular intervals using the subtraction method of Mead and Whittenberger.[9]

Photographic records of pressure tracings and compliance loops were obtained using a multichannel oscilloscopic recorder (Electronics for Medicine, White Plains, NY). Arterial blood samples were analyzed at regular intervals for pH, $pO_2$ and $pCO_2$ by electronic technics using a commercial electrode assembly (Radiometer, London Co, Westlake, Ohio). Hematocrits were measured using a centrifuge for capillary tubes (Model MB, International Equipment Co, Needham Heights, Mass).

Colloidal carbon, 0.5 ml/kg (Pelikan Biological Ink, Guenther Wagner Pelikan Co, New York, NY) was used as an electron-opaque tracer to detect pathologic vascular permeability. In anticipation of increased pulmonary vascular permeability (which did not materialize), and to define the time of onset and the duration of the changes in permeability, the tracer was given intravenously at different times after the injection of the endotoxin: in 8 dogs the tracer was administered 10 minutes before the injection of endotoxin; in 2 other dogs, the tracer was given 1.5 hours after the administration of endotoxin; in the last dog, the total dose was divided and given intermittently during the course of 3 hours.

At the close of the experiment, each animal was killed and the lungs were fixed as described in the subsequent section "Anatomic Studies". One dog was killed at 15, 2 at 60, 1 at 120, and 7 at 180 minutes.

### Group II

In the second group of experiments, we attempted to learn whether longer survival than in group I would be associated with greater pulmonary damage and more severe pulmonary edema. Since the dose of endotoxin (5 mg/kg) had killed the dogs in group I in less than 4 hours, this second group of 3 dogs received smaller quantities, *ie*, 2.5 mg/kg of *E coli* endotoxin intravenously. They were then returned to their cages. They were not injected with carbon and no physiologic measurements were made. These animals were killed, using the same technic as above, at 6, 8 and 18 hours after the injection of endotoxin. Before terminating the experiment and fixing the lungs, the dogs were anesthetized with chloralose as in the previous group of experiments.

### Group III

These animals were used to gain insight into the increase in bronchial venular permeability found in groups I and II. They were subjected to acute severe hemorrhage to determine if the increase in bronchial venular permeability after endotoxin injection would be reproduced by a mechanism other than endotoxin for inducing systemic hypotension and acidosis.

Three dogs were anesthetized with an intravenous injection of chloralose, as in group I. A large-bore polyethylene catheter was introduced into a femoral artery and connected to a Statham P2SD6 transducer to record systemic blood pressure. The animals were then bled rapidly through a side tap of the same catheter at a rate which reproduced the pattern of all in systemic pressure observed in dogs treated with endotoxin (group I). The blood from the animals was collected in a plastic bag containing 5,000 units of heparin so that it could be reinfused as necessary to maintain the systemic pressure at the desired level. Arterial blood was sampled periodically for determinations of pH and blood gases. One dog died after 2 hours of hemorrhagic hypotension; the other 2 were killed during *in situ* fixation of the lungs as in the previous experiments.

### Group IV

Six additional dogs were used as controls for group I. They were anesthetized and treated in the same way as the dogs in group I except that injections of endotoxin were omitted. Each of these dogs was killed at the end of 3 hours as described in the next section.

## Anatomic Studies

Each experiment was terminated in such a way that one lung was fixed *in situ* by the intrabronchial instillation of fixative. In groups I, III and IV, in which the dogs were anesthetized throughout the period of observation, this simply involved connecting the tracheal cannula to a ventilatory pump (Harvard Apparatus Co), unilateral thoracotomy and clamping of the ipsilateral bronchus, followed by introduction of fixative into the contralateral bronchus. In group II the dogs were anesthetized before killing and lung fixation.

Fixation was accomplished using formaldehyde–glutaraldehyde fixative [10] diluted in 0.1 M sodium cacodylate buffer (pH 7.4) to a final osmolality of 500 mOsmoles. The lung was inflated with the fixative at 10 to 15 cm $H_2O$ for 10 minutes. The fixed lung was then removed and postfixed for 4 hours in fresh formaldehyde–glutaraldehyde fixative. After attempts to detect carbon leakage using a dissecting microscope ($\times$ 40) had proved to be impractical because of uncertainty created by retained carbon particles within the lumen of the blood vessels, samples of lung tissue which included both airways and air spaces were taken randomly and processed for optical and electron microscopy using methods previously described.[8] Segments of the small intestine and diaphragm were also processed for light microscopy to determine whether leakage of carbon particles had occurred in tissues other than the lung.

In groups I and IV, the unfixed lung (with clamped bronchus) was removed and drained of blood for approximately 5 minutes. Aliquots of lung tissue (each approximately 2 g in weight) were removed, homogenized and weighed. The hemoglobin contained in the homogenate was converted into hematin by the addition of acetic acid and extracted with ether-alcohol. The optical density was read in a DU spectrophotometer, and the blood content was calculated by comparison with a graph prepared from standards using each dog's own blood, sampled

shortly before killing.[11] The remainder of this lung was weighed fresh and then dried to constant weight in an oven at 45 C. The water content of the lung was determined as the difference between wet and dry weights per unit of dry weight after subtracting the weight of blood contained in the lungs.

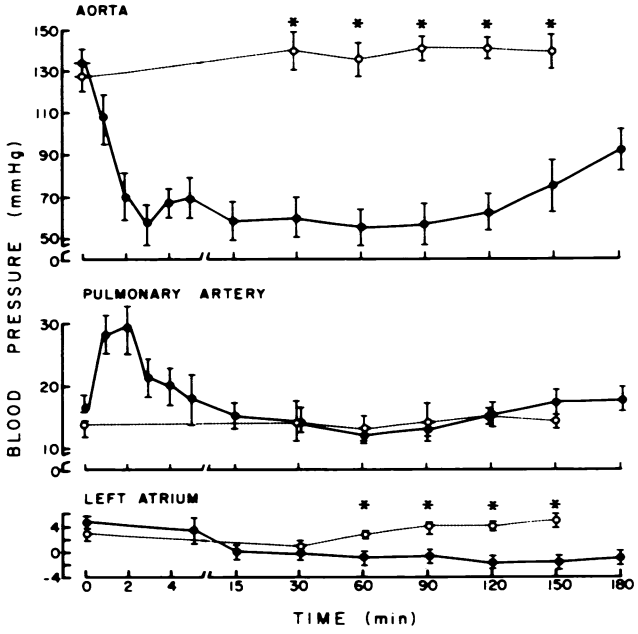
The statistical significance of differences between mean values of control and endotoxin-infused groups was evaluated by Student's *t*-test.

## Results

### Physiologic Observations

#### Comparison of Groups I and IV (Single Large Injection of Endotoxin vs Controls)

Within 3 minutes following the injection of endotoxin, systemic blood pressure fell to about 60 mmHg and remained approximately at this level through the remainder of the experiment. Pulmonary artery pressure increased transiently from 15 to 30 mmHg and returned to normal levels within 15 to 30 minutes. The increment in pulmonary artery pressure was not secondary to increases in left atrial pressure (Text-figure 1). After the endotoxin injection, dynamic compliance decreased




TEXT-FIG 1—Hemodynamic consequences of *E coli* endotoxin injections (5 mg/kg) (*closed circles*) compared to time controls (*open circles*). There is rapid onset of systemic hypotension which is sustained over 3 hours. Pulmonary artery pressure rises abruptly but returns to control levels within 30 minutes without changes in left atrial pressures. The bars represent standard errors and the asterisk indicates significant difference of mean at $P < .02$ level or better.

abruptly to approximately one-half of control values but returned to control levels within 30 minutes (Text-figure 2). Arterial $pO_2$ also decreased abruptly shortly after endotoxin injection and returned toward normal within 30 minutes. Arterial $pCO_2$ and pH decreased progressively, whereas minute ventilation continued to increase during the experiment; the ventilation finally reached a level three times greater than control (Text-figure 3). Also, the hematocrit rose progressively from 38 to 57%.

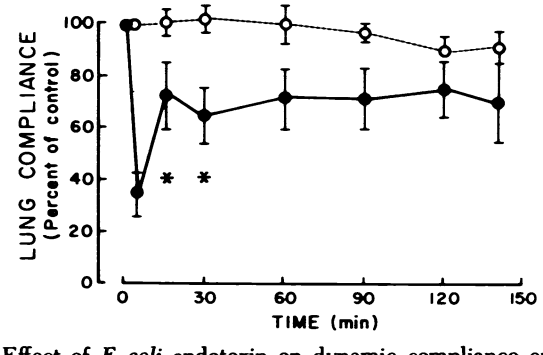
The water content of the lungs of the endotoxin-treated dogs averaged 3.41 ± 42 (SD) g/g dry lung; the water content of the lungs of the control dogs averaged 2.66 ± 55 (SD); the difference between these mean values was statistically significant ($P < .05$).

#### Group II (Low-dose Endotoxin)

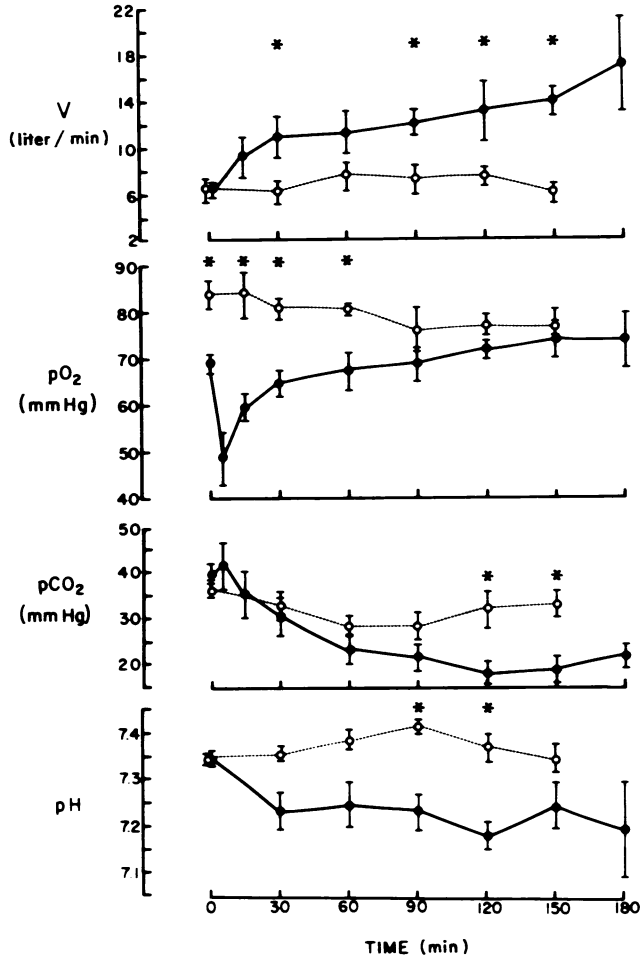
No physiologic observations were made in this group of dogs since interest was confined to anatomic changes in the lungs after more prolonged survival than in the dogs of Group I. These low-dose dogs were not anesthetized and became listless after the endotoxin injection. However, they remained conscious until anesthetized prior to sacrifice for examination and sampling of the lungs.

#### Group III (Hemorrhagic Hypotension)

Physiologic measurements in this group of dogs were confined to a) serial determinations of systemic arterial blood pressure to match the fall in Group I, and b) frequent determination of arterial blood gases and pH. Previous studies in this laboratory have characterized the




TEXT-FIG 2—Effect of *E coli* endotoxin on dynamic compliance expressed as percent of control values, compared to time controls. There is an immediate decrease which returns rapidly towards control levels. By 60 minutes the differences are no longer significant. Symbols as in Text-figure 1.




TEXT-FIG 3—Effect of *E coli* endotoxin on minute ventilation ($V$) and arterial blood gases compared to timed controls. The ventilation increases progressively throughout the 3-hour period accompanied by progressive decrease in arterial $pCO_2$ and pH. Arterial hypoxemia occurs early and is transient; control levels of oxygenation are restored spontaneously within 1 hour. Symbols as in preceding illustrations.

hemodynamic course of dogs bled in a similar pattern to produce hemorrhagic hypotension.[12]

Each of the 3 dogs developed hemorrhagic hypotension and required prompt reinfusion of shed blood to sustain aortic pressure between 5 to 70 mmHg for the 2 to 3 hours of the experiment. Each also developed severe metabolic acidosis (arterial pH = 7.23, 6.80, 7.09, respectively) hypocapnia (arterial $pCO_2$ = 26, 35, 19 mmHg, respectively); in none did the arterial $pO_2$ fall below control levels.

### Anatomic Observations

#### Endotoxic Shock (Group I, Single Large Injection of Endotoxin)

The most dramatic and consistent changes were seen macroscopically and microscopically in the mucosa of the small intestine. This observation is consistent with the fact that in the dog the gut is a primary target for the effects of endotoxin. However, only the pulmonary changes are of concern in the present study, and these will be described in detail.

*Macroscopic and Optical Microscopy Observations.* The severity of the anatomic changes in terms of bronchial leakage of carbon or pulmonary pathology, as detected by gross examination or optical microscopy, did not correlate with the duration of the shock state.

FIFTEEN MINUTES after administering endotoxin, the lungs were black with carbon and focally atelectatic. The mucosa of large bronchi showed patches of black discoloration due to accumulation of carbon in small blood vessels of the bronchial venous plexus. However, the black discoloration did not affect all bronchi equally: some branches appeared entirely normal, other branches of the same parent bronchus were extensively involved.

Microscopically, the capillaries, small arteries and veins were found to contain a large number of carbon particles and polymorphonuclear leukocytes (PMNs) in their lumens. In the areas of atelectasis, the air spaces and respiratory bronchioles appeared collapsed and the vascular bed congested. Extravascular accumulation of carbon was confined to the adventitia of bronchial venules, predominantly those venules in the mucosa of large bronchi, and was associated with moderate interstitial edema. Carbon leakage and interstitial edema were present both in atelectatic and nonatelectatic areas but appeared to be more severe in the areas of atelectasis.

ONE HOUR after administering endotoxin, the lungs were diffusely gray, except for several black patches of atelectasis 3 to 5 cm in diameter.

Microscopically, the pulmonary blood vessels were engorged with erythrocytes, carbon particles and a moderate number of PMNs. The occurrence of carbon outside of blood vessels was still limited to bronchial venules, mainly in the large bronchi. However, the extent of bronchial venular leakage varied in the 2 dogs. In 1 animal, it was marked and extended down the bronchial tree to affect small bronchi of the order of 4 to 5 mm in diameter. As seen in Figure 1, it was associated with an acute inflammatory exudate with marked edema and migration of PMNs into the interstitial space. In some bronchi the acute

inflammatory exudate was associated with focal accumulation of lymphocytes and plasma cells. In the other dog, leakage was only slight and patchy, sparing some bronchi and focally involving others.

TWO AND THREE HOURS after the injection of endotoxin, the lungs of 2 dogs were uniformly black, those of the remaining dogs were grey. Only in 1 dog were areas of atelectasis identified macroscopically.

Microscopically, the difference between black lungs and grey lungs was in the extent of pulmonary intravascular trapping of carbon particles. All lungs showed marked engorgement of pulmonary vessels with erythrocytes and PMNs. In 2 dogs, focal interstitial and alveolar hemorrhage were also present; in 1 of the 2 dogs with black lungs, fibrin thrombi were present in a few capillaries. Leakage of bronchial venules was present in each dog regardless of the schedule of administration of the tracer after the injection of endotoxin, indicating that changes in permeability of the bronchial venules were not limited to the initial phases of endotoxinemia. Although leakage from bronchial venules was widespread, it was moderate in 3 dogs and was limited to only a few venules per bronchus in 5 others. Furthermore, it was not related to the extent of carbon trapping in the pulmonary capillary bed or areas of atelectasis.

AT SIX, EIGHT AND EIGHTEEN HOURS, in the dogs injected with smaller doses of endotoxin and thereafter sacrificed (group II), the lung showed marked congestion, focal atelectasis and microscopically extensive trapping of PMNs. In 2 animals (6 and 18 hours), numerous fat emboli in muscular pulmonary blood vessels ranging from 100 to 300 μ in diameter were found.

Alveolar edema was not observed in any of the dogs treated with endotoxin.

*Electron Microscopy.* Although the extent and severity of the anatomic changes after endotoxin varied somewhat from dog to dog, in general, the ultrastructural changes reached their peak within 1 hour of shock.

FIFTEEN MINUTES after endotoxin administration, the lungs of the dog revealed moderate congestion and accumulation of intact PMNs in the vascular lumens. Carbon particles were seen not only lying free in the lumen of alveolar capillaries but also within phagocytic vacuoles in PMNs and, occasionally, in endothelial cells. All the elements (endothelium, interstitial space and epithelium) of the alveolar septa were intact.

Electron microscopy established that the carbon labeling of bronchial venules was due to the accumulation of carbon particles in the ad-

ventitia of small blood vessels (Figure 2), *ie*, between endothelium and pericytes. This leakage of carbon was associated with separation of collagen fibers of the interstitial space with electron-lucent areas, presumably edema fluid. Margination of leukocytes was common, but migration of PMNs in the interstitium was rare.

AT ONE HOUR after endotoxin administration, the lungs of the 2 dogs killed at that time showed not only leakage of carbon and interstitial edema but also marked-to-moderate migration of PMNs from small blood vessels (Figure 1). In 1 dog the inflammatory exudate was associated with perivascular hemorrhages. The pulmonary blood vessels were congested and filled with numerous PMNs which were either free in the vascular lumen or adherent to the endothelium. Carbon particles, either lying free in the lumen or contained in leukocytic or endothelial phagosomes and platelets aggregates were common. The endothelium, alveolar interstitium and epithelium appeared normal.

TWO TO THREE HOURS after the onset of shock, leakage of carbon from the bronchial venules and interstitial exudate in the bronchial mucosa was qualitatively similar to that described in the previous group. The quantitative differences have been mentioned in the section devoted to light microscopy. The most characteristic lesions at this time were in the pulmonary blood vessels. These consisted of marked accumulation of PMNs in the lumens of capillaries. Leukocytes were commonly adherent to the endothelium. Their cytoplasm was rounded and contained irregular electron-lucent areas (Figure 3). The leukocytic granules were often less electron dense than usual (Figure 3). These changes were seen in all the dogs. In 2 dogs, leukocytes were also found in the interstitial space of alveolar septa and around muscular blood vessels. In the alveolar septa (Figure 3) and in the adventitia of muscular blood vessels at the confluence of three or more alveolar septa, focal separation of the elements of the connective tissue and electron-lucent areas suggested the accumulation of edema fluid. The endothelium lining the alveolar capillaries and muscular blood vessels was usually intact except for rare focal areas of swelling and electron lucency of the cell sap (Figure 3). This swelling was not associated with alterations of mitochondria or of other cell organelles. The membranous pneumocytes showed focal blebbing of the cytoplasm. The granular pneumocytes were generally well preserved, except for rare blunting of microvilli. Platelet clumps were seen occasionally, but only in 1 dog was there evidence of intravascular coagulation as manifested by fibrin strands in the lumen of pulmonary capillaries. Leakage of colloidal carbon was always limited to bronchial venules, irrespective of the

time of administration of the tracer, before or after the administration of endotoxin.

SIX, EIGHT AND EIGHTEEN HOURS after the injections of endotoxin, in dogs of group II, administration of carbon was withheld in an attempt to study the pulmonary lesions uncomplicated by the administration of foreign particles. Also, having established that carbon invariably leaked from the bronchial venules following the administration of endotoxin, we concentrated on other aspects of pulmonary pathology in this group of dogs. To our surprise, the pulmonary changes in these 3 dogs were similar to those described in the dogs killed 2 to 3 hours after endotoxin injection, except for more severe and extensive degenerative changes of the PMNs. These cells showed almost complete replacement of their cytoplasm with irregular nonmembrane-limited electron-lucent spaces and loss of osmiophilic granules. Large osmiophilic bodies (fat emboli) completely filled the lumens of pulmonary arterioles and capillaries of 2 dogs (killed at 6 and 18 hours).

Both by light and electron microscopy in all dogs the mast cells contained intact cytoplasmic granules.

### Hemorrhagic Hypotension (Group III)

On opening the chest cavity, the lungs were undistinguishable from those of the dogs that had received endotoxin. They were diffusely gray-black and focally atelectatic.

Light microscopy revealed large amounts of carbon particles within the lumens of pulmonary small vessels. Congestion was moderate. Peribronchial edema and leakage of tracer in the adventitia of bronchial vessels were consistently absent. The mast cells appeared normal. As seen in Figure 4, the ultrastructural appearance of the lungs of the dogs in hemorrhagic hypotension was similar to that of the lungs of dogs in endotoxic shock. They revealed focal interstitial edema of the alveolar septa, focal electron lucency of endothelial cells and membranous pneumocytes and extensive electron-lucent areas in the cytoplasm of circulating leukocytes. Carbon particles were numerous in the lumens of blood vessels and sometimes within phagocytic vacuoles of the PMNs and endothelium. Indeed, the only difference was the absence of leakage of carbon, edema or exudation around the bronchial small blood vessels.

### Control Dogs (Group IV)

In contrast to the dogs subjected to endotoxin or hemorrhage, the lungs of these dogs appeared pink. On the surface were a few focal

areas of hemorrhage. Light and electron microscopy showed that most of the carbon had been cleared from the circulation. Occasionally leukocytes with electron-lucent areas in the cytoplasm were found in pulmonary capillaries. However, in contrast to the previous groups, endothelial and epithelial cells were well preserved and there was no evidence of carbon leakage.

#### Mechanism of Bronchial Venular Leakage

Most of the leaky bronchial venules showed intact endothelium and offered no clues as to the mechanism of perivascular accumulation of tracer. After prolonged search we found gaps in the walls of small bronchial venules of 2 dogs. We interpreted these gaps as open endothelial junctions. Accordingly, we attribute the increased permeability of bronchial small blood vessels to the tracer, to the temporary opening of endothelial junctions as we saw previously in dogs treated with histamine or bradykinin.[8]

## Discussion

Our study undertook to determine the ultrastructural basis for the changes in permeability of pulmonary capillaries that have been presumed to occur following intravenous injections of *E coli* endotoxin in dogs.[2,4] Although the mechanisms responsible for the physiologic derangements after administering endotoxin remain unsettled,[13,15] the cardiorespiratory changes that occurred in our experiments were comparable in pattern and severity to those observed by others using similar doses of endotoxin in dogs.[6,7]

The ultrastructural changes that we detected in the minute vessels of the pulmonary circulation after endotoxin were minor and their frequency varied from animal to animal. Thus, no structural changes in alveolar capillaries could be detected before 1 hour and focal endothelial swelling, edema of the alveolar septa and trapping of degranulated leukocytes only became manifest 2 to 3 hours after the injection of endotoxin. This meager involvement of pulmonary alveolar capillaries is consistent both with the observations of Finegold, who administered staphylococcal enterotoxin to monkeys,[16] and with those of Coalson, Hinshaw and Guenter, who administered *E coli* endotoxin to dogs.[17] Nor could we attribute the structural changes in pulmonary alveolar capillaries that we observed to the effects of endotoxin, per se, since they also occurred, to the same degree and extent, in the experiments with hemorrhagic hypotension. Similarly, the sparsity of these lesions as well as their lack of specificity detracted from speculation

concerning their possible role as initiating lesions for "shock lung" during septicemia in man.

In contrast to the lack of evidence for extensive increase in *pulmonary* capillary permeability was the unequivocal evidence for *bronchial* venular leakage. Thus, during the first hour following the administration of endotoxin the bronchial small blood vessels became leaky, *ie*, carbon particles, edema fluid and PMNs accumulated in the perivascular interstitial space of the bronchi. The degree of interstitial peribronchial edema varied from dog to dog: in some it was massive and easily detected by low-power light microscopy (Figure 1); in others it was only evident by electron microscopy. An increase in bronchial venular permeability was not manifested during hemorrhagic hypotension, indicating that it resulted from factors other than hypotension and acidosis.

The occurrence of selective leakage of bronchial venules and peribronchial interstitial edema after administering endotoxin is identical with our previous observations in dogs after administering histamine, bradykinin and mast cell degranulators. Although histamine and bradykinin are released from mast cells during the first phases of endotoxin shock,[18-20] it is unlikely that local pulmonary mast cells were the source of these agents in our experiments, since we never observed pulmonary degranulated mast cells. It is also unlikely that local release of serotonin (*eg*, from platelets) was responsible for the changes in permeability of bronchial venules since in our earlier experiments [8] we were unable to elicit changes in permeability of bronchial venules with massive doses of serotonin. Accordingly, the intimate mechanism for increased permeability of the bronchial venules after administering endotoxin remains uncertain.

The bronchial venules are the "systemic venules" of the lungs. The significance of the increase in their permeability with respect to the pathogenesis of "congestive atelectasis" also remains conjectural. However, if they are pictured as having an important reabsorptive function for fluids draining from distal pulmonary interstitial spaces, it is conceivable that damage to their reabsorptive ability will promote interstitial edema.

The evidence that injection of endotoxin increases the permeability of bronchial venules complicates the prevalent notion that increased lymph flow and protein concentration in endotoxin shock simply represent the consequences of an increment in net filtration at the level of the alveolar septae or an increase in the protein content of interstitial fluid surrounding alveolar capillaries.[3] Even if increased entry of water

and protein into the alveolar, pericapillary interstitial space were to occur in septic shock, the demonstration of leaky bronchial venules suggests that the composition of lymph might be seriously modified as it traverses the peribronchial channels en route to collection into the major lymph ducts.

Our experimental results differ quantitatively from those of Snell and Ramsey who reported large increments in extravascular lung water following injections of *E coli* in dogs.[4] Although we too observed increments in lung water that are statistically significant, the increments were small. One explanation for the discrepancy may be the different methods used in the two series of observations: Snell and Ramsey used the double indicator-dilution method to estimate pulmonary extravascular lung water; we measured lung water content directly. The double indicator-dilution method is importantly influenced by the distribution of capillary blood flow in the lung,[21] and the data of Snell and Ramsey were collected at a time when pulmonary arterial pressure after endotoxin injection was still high. This increase in pulmonary artery pressure affords the prospect of recruiting additional capillary vessels. Accordingly, the numerical value for lung water determined by the indicator-dilution technic might be artificially high because of the larger tissue volume being perfused.

The present study failed to identify unique changes in alveolar capillary vessels that could be indicted as the initial event in a pathogenetic sequence leading to lung injury in septic shock. Therefore, unless the susceptibility of the human lung to endotoxin is much greater than that evinced by the dog lung, the initiating mechanism for "congestive atelectasis" after endotoxin shock cannot be attributed to increased pulmonary capillary permeability.

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## Acknowledgments

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[*Illustrations follow*]

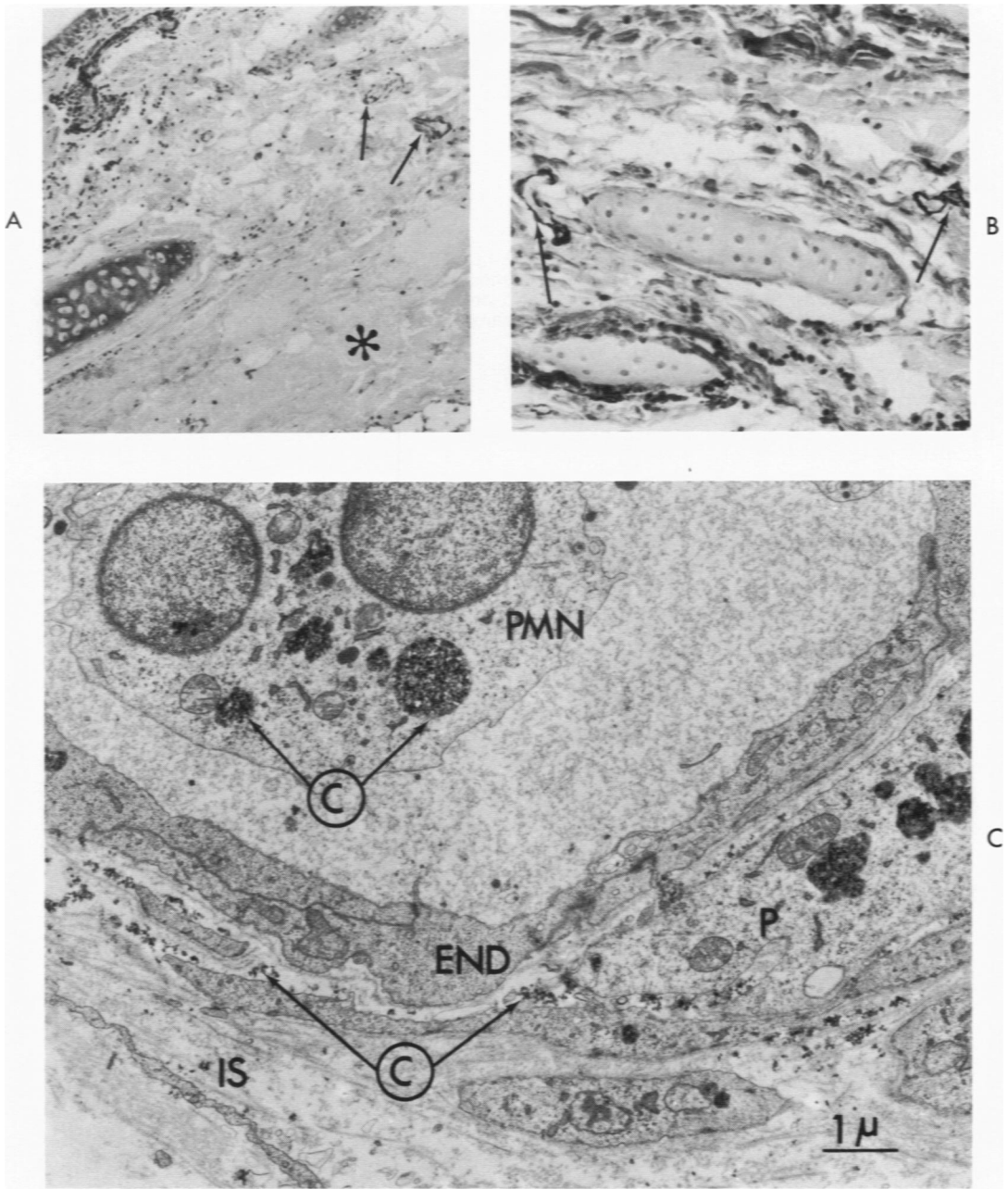
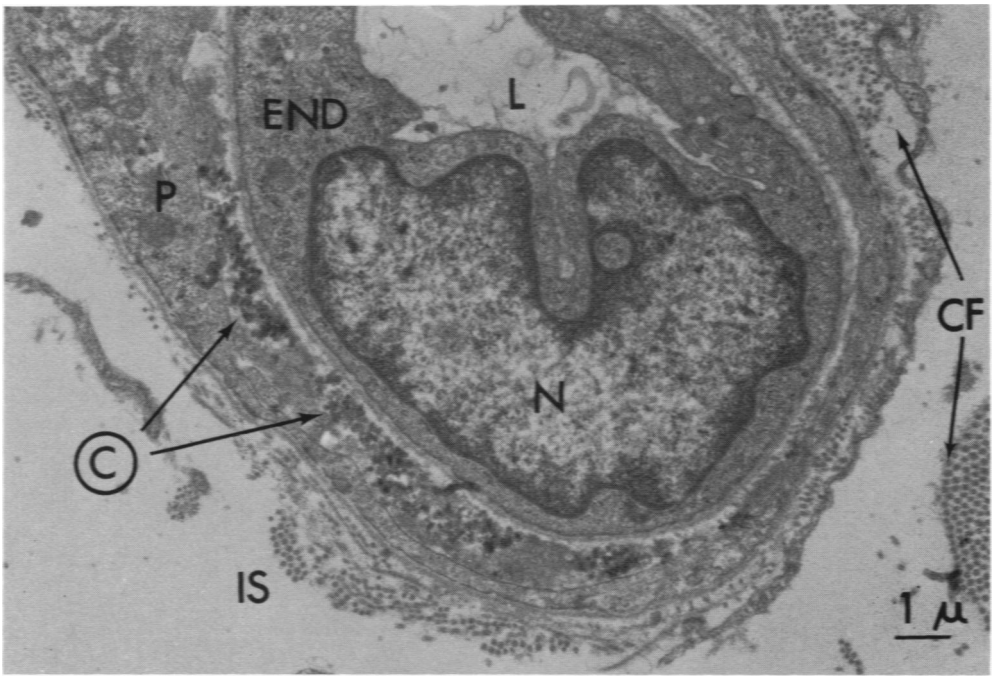



**Fig 1**—Dog killed 1 hour after injection of endotoxin. **A**—Marked widening of the peribronchial interstitium with edema fluid (*asterisk*). Leaky venules are indicated by *arrows*. Some of the submucosal venules are surrounded by inflammatory cells (H&E, × 130).

**B**—Higher power of leaky bronchial venules (*arrows*). At this power it is not possible to decide whether the carbon is sticking to the endothelium or is in the perivascular space (H&E, × 400). **C**—With the electron microscope, carbon particles are seen within the adventitia of a venule, few particles are free in the vascular lumen and others are within phagosomes of a neutrophil granulocyte (*PMN*) and of a pericyte (*P*). *END*=endothelium, *IS*=interstitium (Uranyl acetate and lead citrate, × 20,000).

Fig 2—Leaky bronchial venule of a dog killed 15 minutes after injection of endotoxin. At this early time, carbon particles are seen only between endothelium and pericyte. The electron lucency of the interstitial space among collagen fibers (*CF*) suggests the presence of edema fluid. *N*=nucleus (Uranyl acetate and lead citrate, × 6000).

Fig 3—A neutrophil granulocyte fills the lumen of an alveolar capillary in a dog killed 3 hours after injection of endotoxin. Large electron-lucent areas (*arrowheads*) in the cytoplasm of the leukocyte indicate loss of glycogen. The leukocyte granules also contain electron-lucent spaces (*small arrows*). The endothelium shows focal swelling of cell sap and disruption of the cell membrane at point *X*. The collagen fibers of the interstitial space are separated by electron-lucent areas. *ALV*=alveolar space (Uranyl acetate and lead citrate, × 25,000).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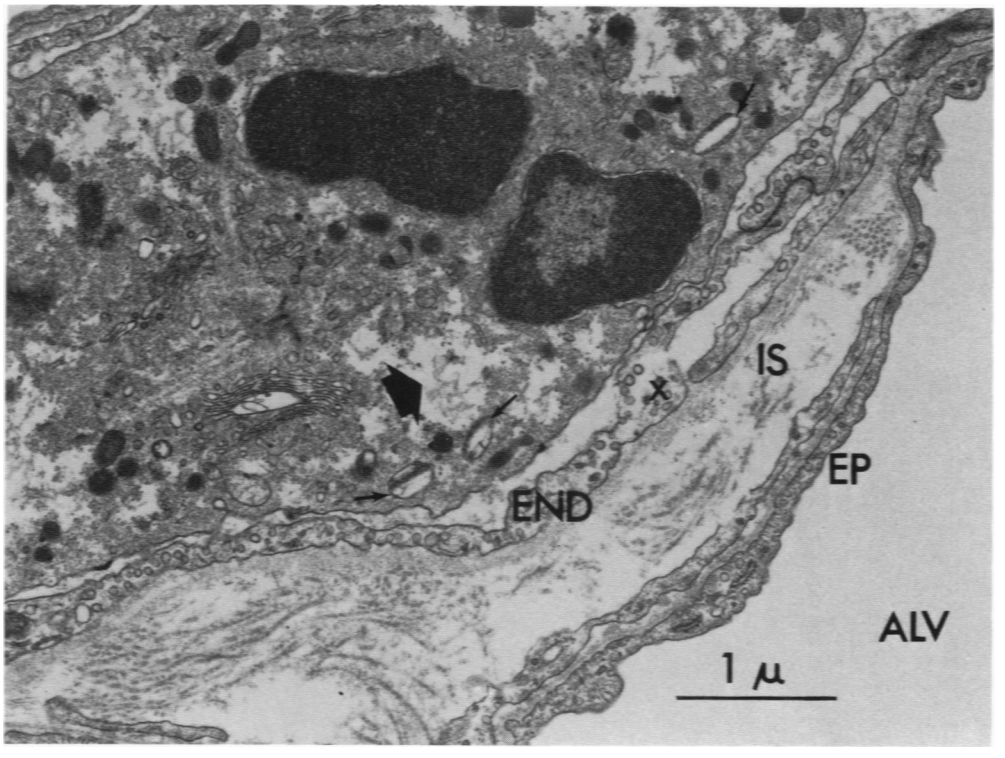
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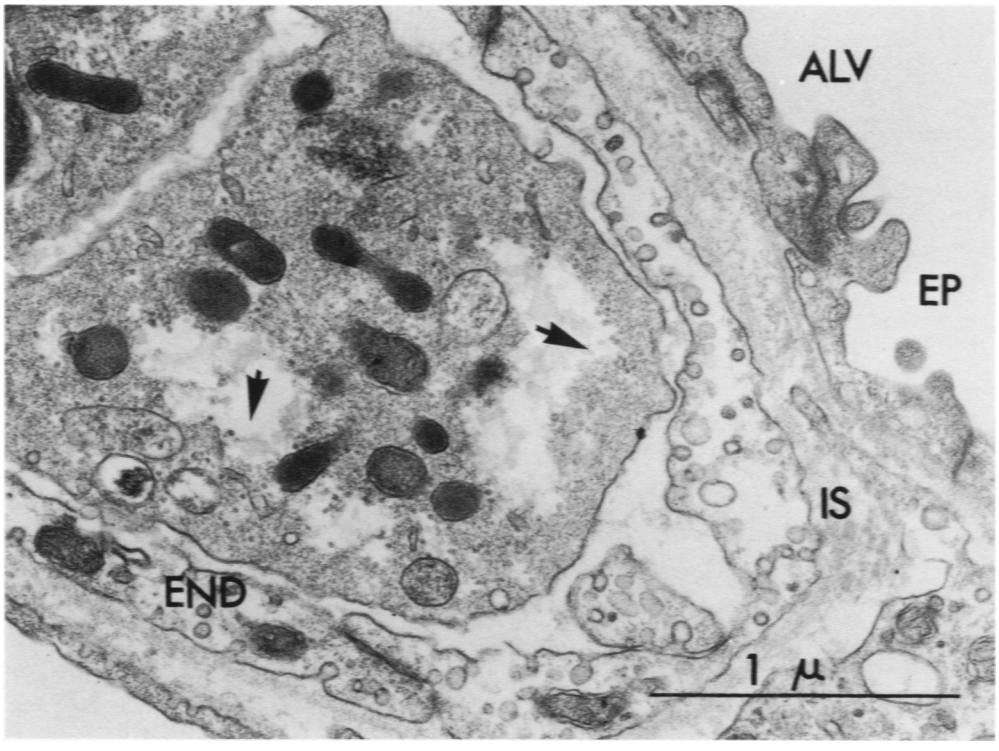


**Fig 4**—Alveolar capillary of a dog killed 3 hours after the onset of hemorrhagic hypotension. The changes in the endothelium and neutrophil granulocytes are indistinguishable from those seen in dogs in endotoxin shock (Uranyl acetate and lead citrate, × 37,000).