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Morphology of Noncardiogenic Pulmonary Edema Induced by Perilla Ketone in Sheep

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A single infusion of Perilla ketone (PK) into sheep causes marked increases in lung fluid and solute exchange in the absence of any alteration in either pulmonary arterial or left atrial pressures. These alterations are most compatible with increased pulmonary microvascular permeability. The present paper describes the morphologic changes that accompany the previously described alterations in lung function. In five anesthetized open-chest sheep, lung biopsy tissue was taken at baseline and at 15, 30, 60, 120, and 180 minutes after the start of a single infusion of PK (15–20 mg/kg given over a 20 minute period). Biopsy tissue was taken from different lobes of the lung in random sequence, fixed, and processed for light and electron microscopic examination. Three control sheep received the vehicle, dimethyl sulfoxide, alone. Just 15 minutes after the start of PK infusion, alveolar capillary congestion, accumulation of peripheral lung neutrophils, and intraalveolar and interstitial edema were apparent. Electron microscopy revealed early evidence of damage to both the microvascular endothelial cells and Type I pneumonocytes. The damage became more severe with time. From 30 minutes, occasional nonciliated cells in the airway epithelium exhibited dilated rough and agranular endoplasmic reticulum. Thus, PK causes rapid onset of pulmonary edema accompanied by structural evidence of damage to the microvascular endothelium and Type I pneumonocytes. Pulmonary inflammation was also evident. These structural changes occur before the described alterations in either pulmonary microvascular permeability or reduction in pulmonary compliance. (Am J Pathol 1988, 133:285–297)

PERILLA KETONE (PK) is a 3-substituted furan present in the purple mint plant, *Perilla frutescens*, which is known to cause respiratory failure in grazing animals.[1-3] The mechanism of lung injury that follows ingestion of PK is not known but its chemical similarity to 4-ipomeanol suggests that the injury involves conversion of PK to a toxic metabolite through the cytochrome P450 system.[4,5] Previous reports with 4-ipomeanol toxicity in rabbits,[6] rats,[4,7] and hamsters[7] suggest that the nonciliated bronchiolar cells are the initial site of lung damage. The light and electron microscopic appearance of the lungs following administration of PK have not been described in detail previously.

An earlier study used PK to develop an animal model of increased pulmonary microvascular permeability in chronically instrumented sheep.[8] PK was found to cause increases in lung lymph flow and lymph to plasma protein concentration without changes in pulmonary artery or left atrial pressures. These changes in lung fluid solute exchange are typical of increased microvascular permeability in the lungs. PK also caused hypoxemia, decreased pulmonary compliance, respiratory failure, and radiographic evidence of pulmonary edema.[8] Thus, PK appears to be an excellent model of increased pulmonary microvascular permeability and a potentially valuable animal model for the adult respiratory distress syndrome in man.[9,10]

The present study describes the light microscopic and ultrastructural changes in sheep lungs induced by a single infusion of PK. Biopsy tissue was taken sequentially and randomly from the lungs of open-chest, anesthetized sheep over a 3-hour period. PK

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Table 1—Semi-Quantitative Data of Light Microscopic Changes in the Lungs of Sheep After Perilla Ketone Infusion (N = 5)

	Time after PK (minutes)					
	0	15	30	60	120	180
Alveolar Congestion	0.3 ± 0.1	1.1 ± 0.5	2.1 ± 0.3*	1.2 ± 0.5	1.9 ± 0.5	2.1 ± 0.4*
Interstitial Edema	0.2 ± 0.2	0.4 ± 0.2	0.6 ± 0.2	0.1 ± 0.1	0.8 ± 0.3	1.4 ± 0.4
Intra-alveolar Edema	0.2 ± 0.2	1.4 ± 0.6	1.7 ± 0.6*	1.4 ± 0.3*	2.3 ± 0.5*	2.2 ± 0.5*
Neutrophil margination in blood vessels	0.4 ± 0.2	1.0 ± 0.4	1.4 ± 0.3*	1.2 ± 0.2*	1.0 ± 0.3	2.3 ± 0.3*
Neutrophil migration†	0.0	0.2 ± 0.2	0.4 ± 0.2	0.5 ± 0.2	1.2 ± 0.3*	1.0 ± 0.6
Perivascular edema	0.2 ± 0.2	0.1 ± 0.1	0.8 ± 0.5	0.6 ± 0.4	1.4 ± 0.6	0.9 ± 0.5
Airway inflammation	0.9 ± 0.3	1.1 ± 0.4	1.4 ± 0.2	1.5 ± 0.3	1.6 ± 0.4	1.8 ± 0.2

Data are expressed as mean ± SE. Data from control animals was similar to time 0.
* $P < 0.05$.
† Evidence of neutrophils passing from capillary lumen into interstitium.

caused early structural alterations to both microvascular endothelial cells and alveolar epithelium that were accompanied by evidence of interstitial and intraalveolar edema. These structural changes doubtless account for the observed changes in pulmonary microvascular and epithelial permeability and lung mechanics after PK infusion.

Materials and Methods

Animal Preparation

Eight yearling, mixed breed sheep, five experimental sheep, and three controls, were used in this study. Anesthesia was induced with sodium thiamylal (Abbott Laboratories, Chicago, IL) and maintained throughout the study with a mixture of halothane (Fluothane, Ayerst Laboratories, Inc., New York, NY), 80% oxygen, nitrous oxide, and air. The sheep were ventilated with a volume ventilator maintaining a 5-cm positive end expiratory pressure and $PaCO_2$ between 35 and 40 torr. A catheter was placed in the superior vena cava through the internal jugular vein for administration of PK. The sheep was placed on its back, the sternum split to expose the lungs, and the experiment was begun.

Preparation of Perilla Ketone

1-(3-furyl)-4-methylpentan-1-one (Perilla ketone) was prepared from 3-furoic acid (Sigma Chemical Co., St Louis, MO) as described by Garst and Wilson.[9] The prepared PK was then mixed 1:1 (vol/vol) with dimethyl sulfoxide.

Experimental Protocol

After anesthesia and opening of the chest, each sheep was allowed to stabilize for 1 hour before a baseline lung biopsy was taken. Five sheep then received a single infusion of PK (15–20 mg/kg) into the internal jugular catheter over a 20-minute period. Three control sheep received an infusion of a similar volume of dimethyl sulfoxide, approximately 1–2 ml. After the beginning of infusion, lung biopsy tissue was taken from random lobes at 15, 30, 60, 120, and 180 min-

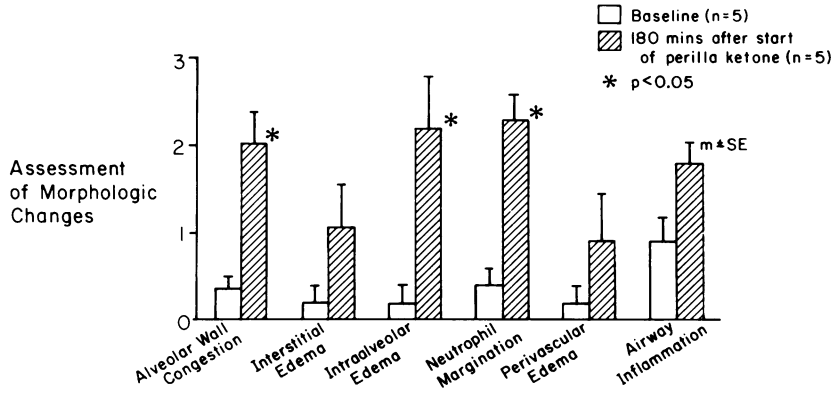

Figure 1—Semi-quantitative assessment of morphologic changes in lung biopsy tissue 3 hours after infusion of Perilla ketone into sheep.

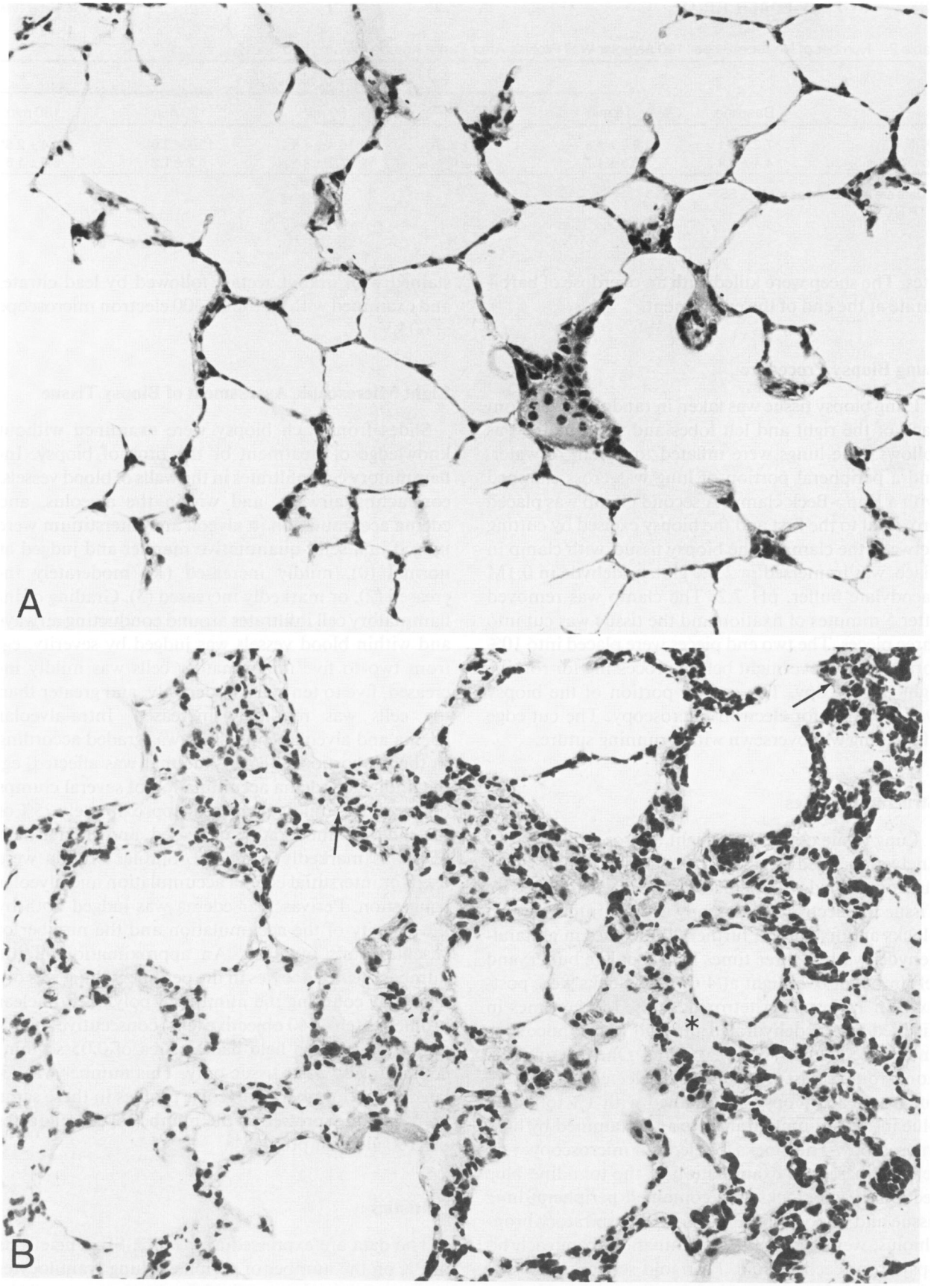

Figure 2—Light micrographs of control lung tissue from sheep (**A**) and 15 minutes after infusion of Perilla ketone (**B**). Congestion of capillaries by red blood cells, increased numbers of granulocytes in the alveolar walls (eg, at arrows) interstitial thickening and intra-alveolar edema (*) is evident (B). ×300

Table 2—Number of Neutrophils per 100 Alveolar Wall Profiles After Perilla Ketone Infusion into Sheep

		Time of biopsy				
	Baseline	15 min	30 min	60 min	120 min	180 min
PK (N = 5)	7.2 ± 1.1	9.8 ± 1.3*	12.6 ± 2.2*	15.4 ± 4.5	13.7 ± 3.6*	14.4 ± 2.8*
Control (N = 3)	4.4 ± 0.8	6.3 ± 1.7	5.1 ± 0.7	7.2 ± 2.1	6.2 ± 1.2	6.9 ± 1.6

Data are expressed as mean ± SE.
* $P < 0.05$.

utes. The sheep were killed with an overdose of barbiturate at the end of the experiment.

Lung Biopsy Procedure

Lung biopsy tissue was taken in random order from each of the right and left lobes and was obtained as follows. The lungs were inflated to 35 cm of water, and a peripheral portion of lung was cross clamped with a Kapp-Beck clamp. A second clamp was placed proximal to the first and the biopsy excised by cutting between the clamps. The biopsy tissue, with clamp in place, was immersed in 2.5% glutaraldehyde in 0.1M cacodylate buffer, pH 7.2. The clamp was removed after 5 minutes of fixation and the tissue was cut into three pieces. The two end pieces were placed into 10% formol-saline overnight before processing for routine light microscopy. The central portion of the biopsy was prepared for electron microscopy. The cut edge of the lung was oversewn with a running suture.

Structural Studies

Lung tissue obtained for light microscopy was routinely embedded in paraffin, sections (5 μ thick) were cut and stained with hematoxylin and eosin (H & E). Tissue for electron microscopy was cut into 2 cu mm blocks and fixed for a further 90 minutes in glutaraldehyde, washed three times in cacodylate buffer, and left in buffer overnight at 4 C. The blocks were postfixed in 1% osmium tetroxide, rinsed three times in distilled water, dehydrated through graded alcohols, and embedded in Epon/Araldite. One-micron sections from at least two randomly selected blocks were cut from each biopsy and stained with 1% toluidine blue in 1% sodium tetraborate and examined by light microscopy. The blocks for electron microscopy were selected based on examination of the toluidine blue sections. Two blocks that contained peripheral lung tissue and, if possible, a terminal or respiratory bronchiolus, were selected for examination from each biopsy from each animal. Pale gold sections were cut with a LKB Ultrotome V and a diamond knife. Sections were mounted on 200 mesh copper grids, stained with uranyl acetate followed by lead citrate, and examined with a Phillips 300 electron microscope at 60 kV.

Light Microscopic Assessment of Biopsy Tissue

Slides from each biopsy were examined without knowledge of treatment or the time of biopsy. Inflammatory cell infiltrates in the walls of blood vessels, conducting airways, and within the alveolus, and edema accumulation in alveoli and interstitium were judged in a semi-quantitative manner and judged as normal (0), mildly increased (1), moderately increased (2), or markedly increased (3). Grading of inflammatory cell infiltrates around conducting airways and within blood vessels was judged by severity, eg, from two to five inflammatory cells was mildly increased, five to ten cells moderately, and greater than ten cells was markedly increased. Intra-alveolar edema and alveolar congestion was graded according to the proportion of each slide that was affected, eg, the finding of edema accumulation of several clumps of alveoli was mildly increased, approximately 25% of the slide was moderately increased, and greater than 25% was markedly increased. Similar criteria were used for interstitial edema accumulation and alveolar congestion. Perivascular edema was judged both by the severity of the accumulation and the number of vascular cuffs involved. An approximation of the number of granulocytes in the peripheral lung was obtained by counting the number of polylobed nuclear profiles in ten (×40 objective lens) consecutive microscopic fields (each field had an area of 0.05 sq mm) containing alveolar tissue only. This number was related to the number of alveolar profiles in these same fields and is expressed as the number of granulocytes per 100 alveolar profiles.[12]

Statistics

The data are expressed as mean ± SE. The effects of PK on the number of peripheral lung granulocytes were assessed using a paired Student's *t*-test and the semi-quantitative light microscopic changes were ex-

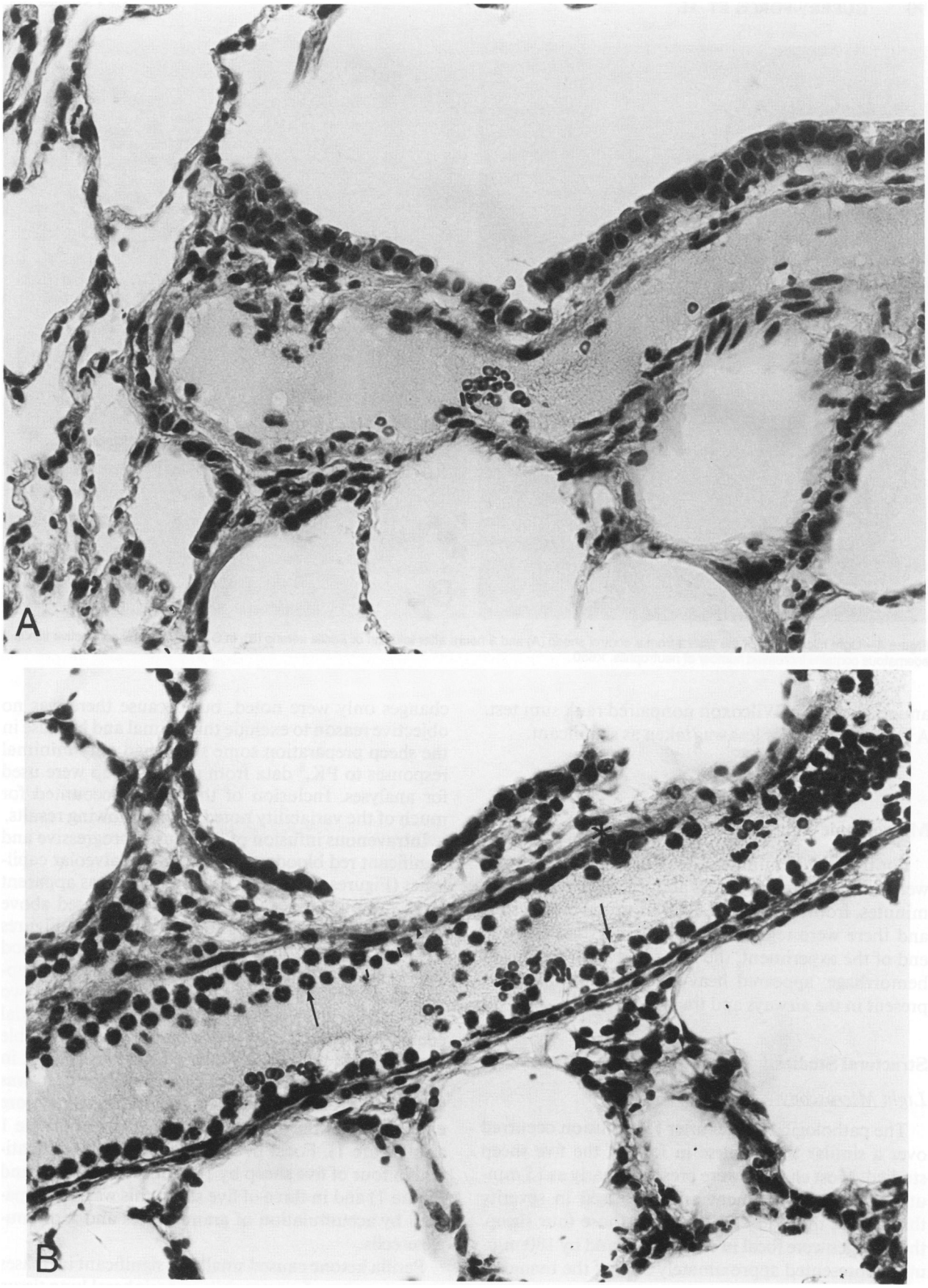

Figure 3—Light micrographs of a pulmonary artery running with a respiratory bronchiolus from a control sheep (**A**) and a sheep 30 minutes after infusion of Perilla ketone (**B**). Margination of neutrophils (eg, at arrows) is evident in B (compare with A) and some neutrophils seem to migrating across the endothelial layer (*). ×700

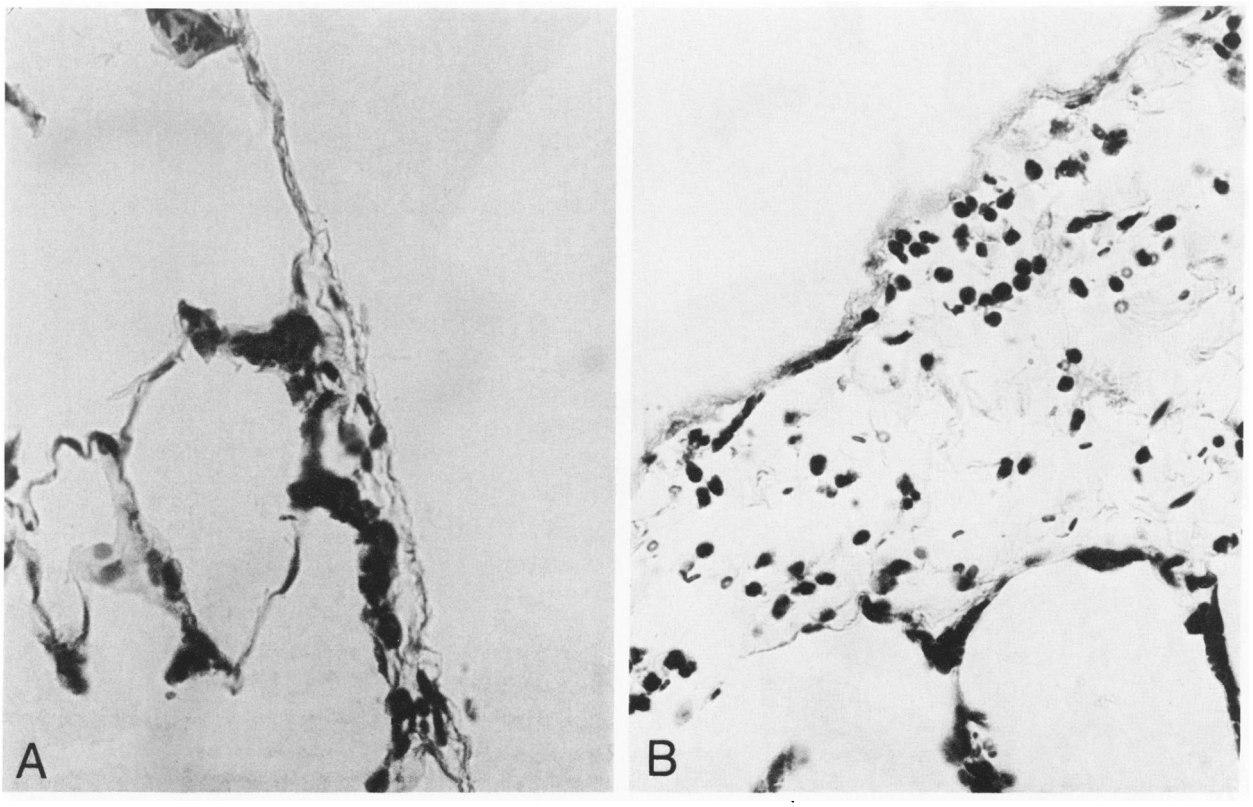

Figure 4—Light micrograph of the pleura from a control sheep (A) and 3 hours after infusion of Perilla ketone (B). In B the subpleural connective tissue is edematous contains increased number of neutrophils. ×650

amined using the Wilcoxon nonpaired rank sum test. A P value of 0.05 or less was taken as significant.

Results

Macroscopic Appearance of Lungs

For the first 60 minutes after PK infusion the lungs were pink without any gross abnormality, but by 120 minutes, frothy material leaked from the biopsy sites and there were regions of focal hemorrhage. At the end of the experiment, the lungs had focal regions of hemorrhage, appeared heavy, and edema fluid was present in the airways and trachea.

Structural Studies

Light Microscopy

The pathologic changes after PK infusion occurred over a similar time course in four of the five sheep studied. Most changes were present as early as 15 minutes into the experiment and increased in severity throughout the study (Table 1). In those four sheep, the changes were focal in each biopsy and by 180 minutes, represented approximately 50% of the tissue examined. In the remaining sheep, minimal structural changes only were noted, but because there was no objective reason to exclude this animal and because in the sheep preparation some sheep had only minimal responses to PK,[8] data from all five sheep were used for analyses. Inclusion of this sheep accounted for much of the variability noted in the following results.

Intravenous infusion of PK caused progressive and significant red blood cell congestion of alveolar capillaries (Figures 1 and 2). The congestion was apparent at 15 minutes and was significantly increased above baseline at 30 and 180 minutes (Table 1 and Figures 1 and 2). By 180 minutes, extravasation of red blood cells into the interstitium and alveolar spaces was apparent. The alveolar walls appeared edematous in two of five sheep at 15 minutes (Figure 2) and interstitial edema was present in all sheep by 180 minutes (Table 1 and Figure 1). Intra-alveolar edema was present in four of five PK sheep at 15 minutes (Figure 2), was seen in all PK sheep by 30 minutes, and became more extensive over the course of the experiment (Table 1 and Figure 1). Focal perivascular edema was identified in four of five sheep by 180 minutes (Table 1 and Figure 1) and in three of five sheep this was accompanied by accumulation of granulocytes and mononuclear cells.

Perilla ketone caused small but significant increases in the number of neutrophils in peripheral lung tissue

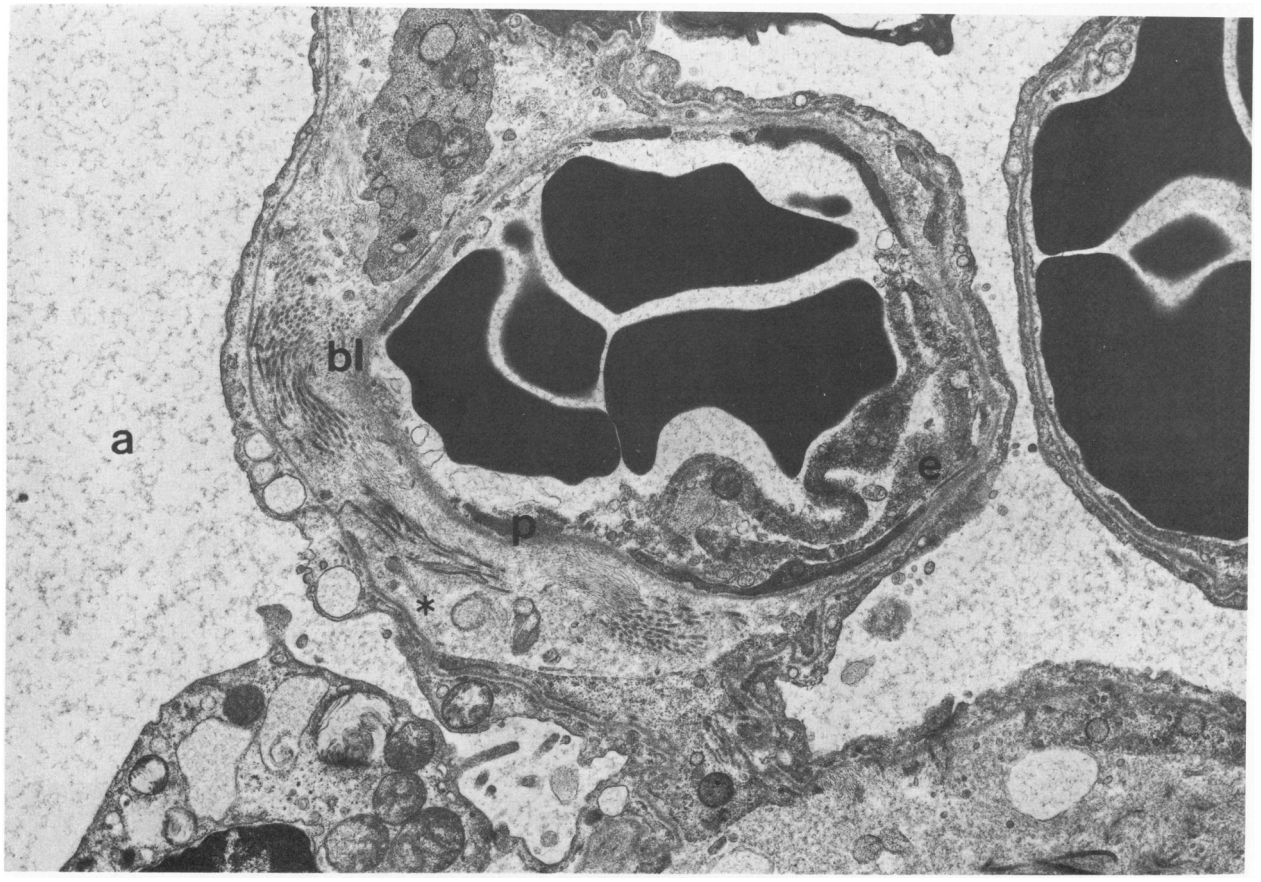

Figure 5—Electron micrograph taken 60 minutes after Perilla ketone infusion showing part of an alveolar wall. The interstitium contains proteinaceous edema fluid (*). The capillary endothelium (e) is electron dense and fragmented. In places, the endothelium is lifted from the basal lamina. Edema fluid is present in the alveolar space (a) and red blood cells are seen between the endothelium and basal lamina. The pericyte (p) is more electron dense than normal and the Type I pneumonocyte is vacuolated (v). ×11,400

including alveolar capillaries and interstitium (Table 2 and Figure 2). The increase was significant by 15 minutes after the start of PK infusion, and from 60 minutes the number of neutrophils per 100 alveolar wall profiles was twice baseline (baseline, 7.2 ± 1.1; mean ± SE; 60 minutes PK, 15.4 ± 4.5; Table 2). Neutrophil counts in peripheral lungs of control animals did not change significantly from baseline (4.4 ± 0.8 to 7.2 ± 2.1).

From 15 minutes after the start of PK infusion, margination and migration of neutrophils also was seen in muscular and nonmuscular vessels (Table 1 and Figures 1 and 3). Margination of neutrophils was significantly increased above baseline at 30, 60, and 180 minutes but neutrophil migration was increased significantly above baseline only at 120 minutes (Table 1 and Figure 1).

Over the course of the experiment, the PK sheep showed a gradual but nonsignificant accumulation of neutrophils and mononuclear cells in the walls of the terminal and respiratory bronchioli (Table 1 and Figure 1). These peribronchiolar sheaths occasionally were edematous, particularly when interstitial and intra-alveolar edema were prominent. In general, the pleura did not appear thickened but, from 30 minutes, three sheep showed focal accumulations of granulocytes in the subpleural connective tissue (Figure 4). No evidence of regional variation among different lobes was found for any of the semi-quantitative light microscopic changes examined in the PK treated sheep.

Electron Microscopy

While interstitial edema occasionally was difficult to assess by light microscopy, especially if mild, it was seen more easily by electron microscopy. Loose, flocculent material representing edema fluid was seen in the interstitial spaces from 15 minutes in all sheep (Figures 5–7). Intra-alveolar edema (Figures 5 and 7) was also present in three of five sheep at 15 minutes, and was present in all sheep from 30 minutes.

By 15 minutes, PK caused focal but striking endothelial damage (Figures 6–8). The damage was seen as an increase in electron density, vesiculation, vacuoli-

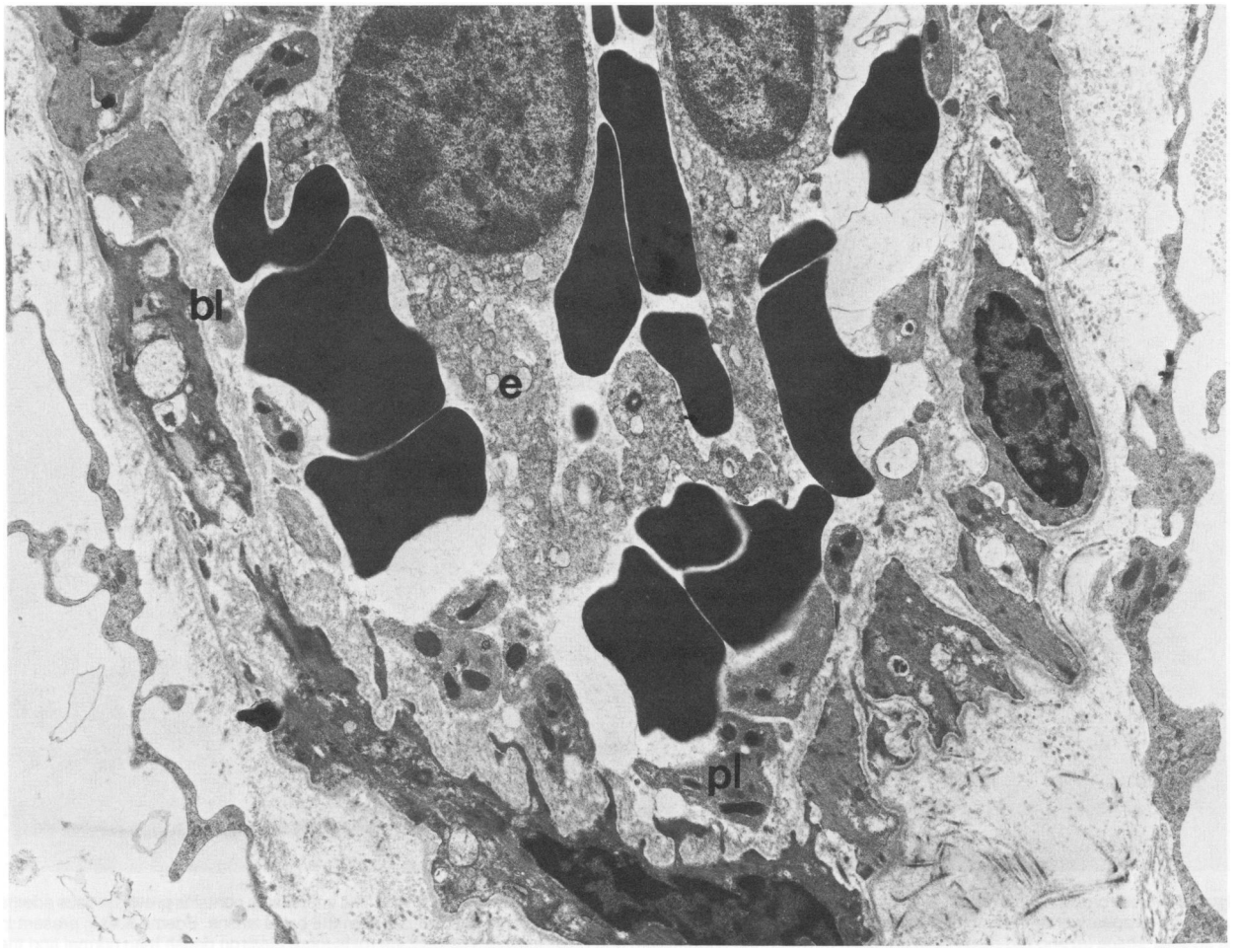

Figure 6—Electron micrograph taken 30 minutes after Perilla ketone infusion showing a small muscular vessel. The endothelial layer (e) is desquamated from the basal lamina (bl). Some platelets (pl) are adherent to the exposed subendothelium. ×8200

zation, and fragmentation of the cytoplasm, clumping of nuclear chromatin, and eventual sloughing of the endothelial cells from their basal lamina; platelet aggregates were often associated with these areas of bare basal lamina (Figures 6 and 8). These changes were seen in the endothelial cells of alveolar capillaries (Figures 5 and 7) and small partially muscular and muscular arteries and veins (Figure 6). At capillary level, the pericytes associated with damaged endothelial cells often were more electron dense than normal, suggesting damage to the entire microvasculature (Figures 5 and 8). By 120 minutes, the damage to endothelial cells was extensive and disruption of the endothelial layer was accompanied by intravascular fibrin clots. Flocculent debris, red blood cells, and fibrin were identified in the interstitium adjacent to the damaged endothelium. While endothelial damage was always associated with the presence of interstitial edema, at early time points there were foci of interstitial edema in areas without obvious endothelial damage. Occasional profiles of neutrophils passing through the endothelial layer of small blood were found (Figure 9).

In four of five sheep, occasional Type I pneumonocytes showed cytoplasmic swelling, vesiculation, and vacuolization, and lifting from the basal lamina from 15 minutes (Figures 5 and 7). From 30 minutes, all sheep showed this change. In later biopsy tissue, focal Type I cells were sloughed from the alveolar basal lamina (Figure 7); the alveolar spaces in these regions contained abundant proteinaceous fluid and fibrin (Figure 7). By 180 minutes, approximately half of the Type I pneumonocytes appeared damaged. The Type II pneumonocytes appeared normal at all times examined.

Perilla ketone caused some alterations to terminal and respiratory bronchioli. From 30 minutes, occasional airways showed edema of the subepithelial region and in focal regions, nonciliated secretory bronchiolar epithelial cells were more electron lucent than normal and displayed dilatation of both rough and

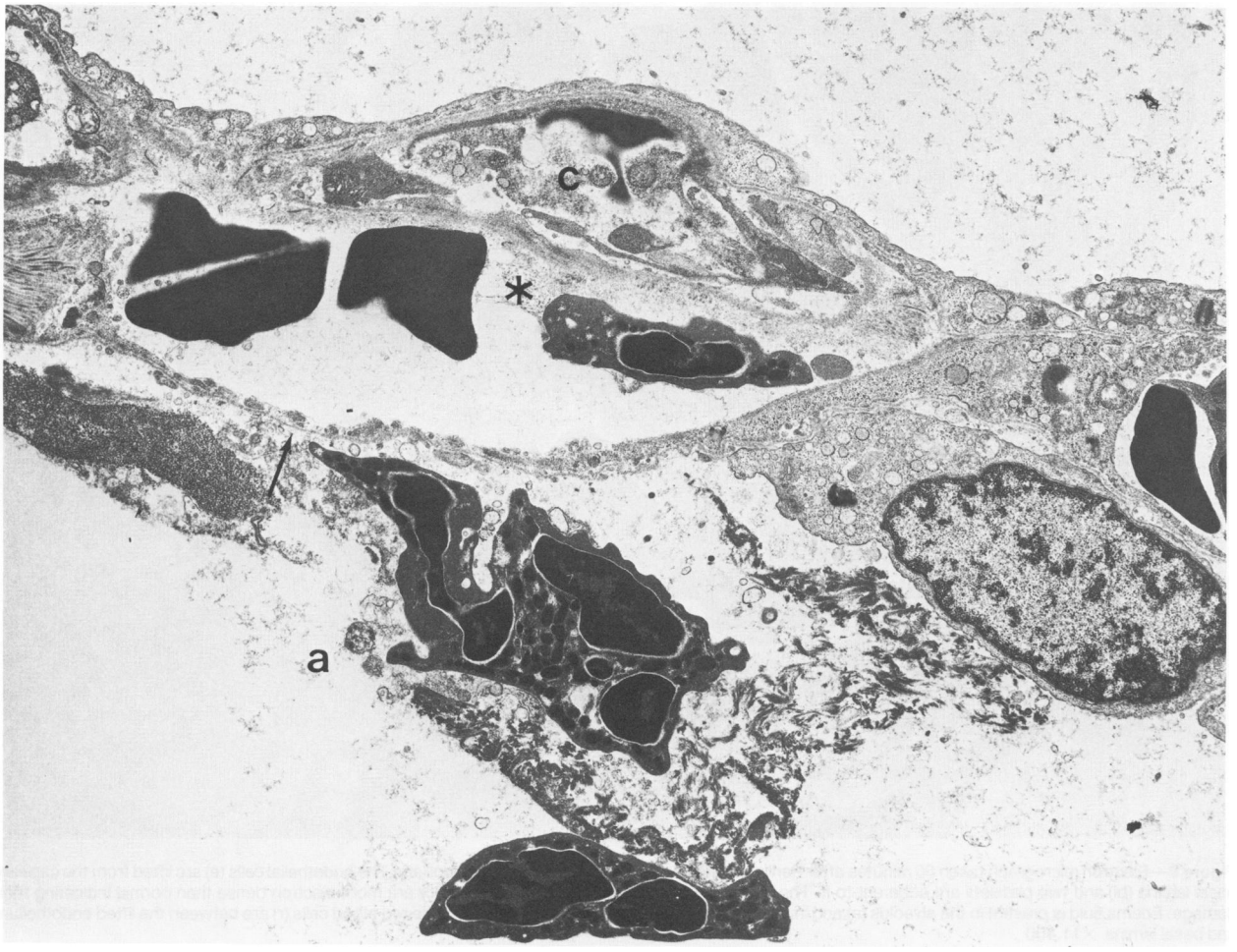

Figure 7—Electron micrograph of part of an alveolar wall 30 minutes after infusion of Perilla ketone. The capillary (c) is disrupted and edema fluid, red blood cells, and a granulocyte are seen in the interstitial space (*). The thin cytoplasmic extension of the Type I pneumonocyte has been sloughed away at arrows and edema fluid, fibrin and two granulocytes are identified in the alveolus (a). ×8000

agranular endoplasmic reticulum (Figure 10); the secretory granules appeared intact. These injured cells were more numerous in later biopsies (120 and 180 minutes), when approximately 25% of the cells were affected by this change. Ciliated epithelial cells showed no apparent structural abnormalities.

Control sheep showed no morphologic alterations over the course of the experiment. These samples were similar to baseline biopsy tissue taken from the experimental sheep.

Discussion

Perilla ketone lung injury in sheep is characterized physiologically by an increase in protein-rich lung lymph flow without any concomitant increase in pulmonary artery or left atrial pressure, or pulmonary vascular resistance.[8] There is radiographic evidence for developing interstitial and alveolar pulmonary edema as well as hypoxemia, decreased dynamic compliance and functional residual capacity, and clinical respiratory failure.[8] These changes are progressive, increasing in severity over a 5-hour period. Measurements of epithelial solute flux using ^{51}Cr-EDTA/^{125}I-antipyrine supply physiologic evidence for damage to the epithelium as well as the endothelium.[13] The present morphologic study demonstrates the pathologic correlates of these physiologic changes. The structural changes after Perilla ketone infusion include damage to both the endothelial and epithelial cells of the lung, accumulation of interstitial and intra-alveolar edema, and peripheral lung neutrophilia. These structural changes are first evident before the onset of significant physiologic alteration.

Interstitial and intra-alveolar edema, as well as endothelial and epithelial damage, were evident as early as 15 minutes after injection of Perilla ketone. The rapid disruption of endothelial and epithelial barriers

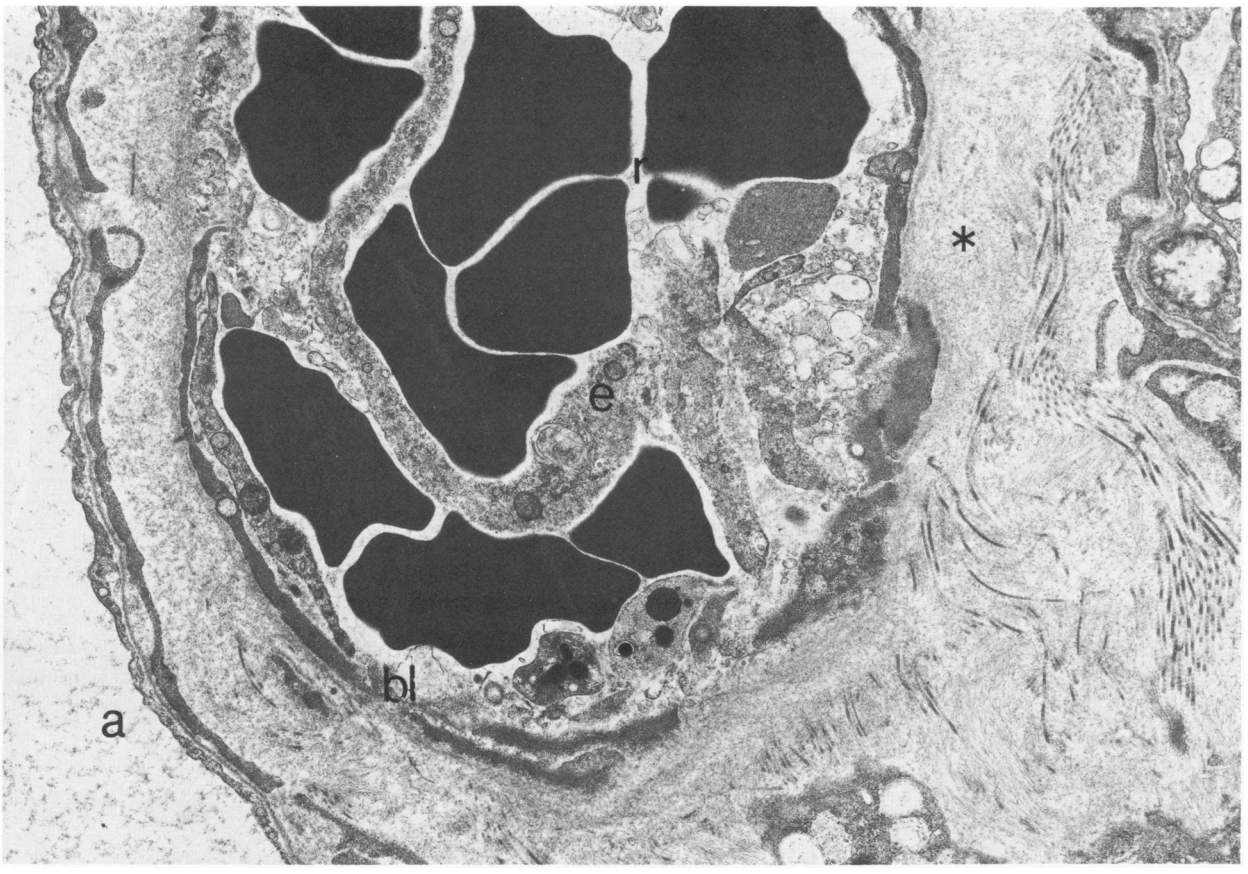

Figure 8—Electron micrograph taken 60 minutes after Perilla ketone showing a damaged alveolar capillary. The endothelial cells (e) are lifted from the capillary basal lamina (bl) and two platelets are adherent to it. The pericyte processes surrounding the capillary are more electron dense than normal indicating their damage. Edema fluid is present in the alveolus (a) and in the alveolar interstitium (*). As in Figure 3, some red blood cells (r) are between the lifted endothelium and basal lamina. ×11,400

to fluid and solute flux may contribute directly to the development of interstitial and intra-alveolar edema as well as the observed abnormalities described previously in lung fluid and solute exchange, lung mechanics, and $^{51}Cr/EDTA^{125}I$-antipyrine flux.[8] It is probable that the morphologic changes account for the progressive alterations observed following Perilla ketone infusion.

The epithelial cells of the peripheral conducting airways (respiratory and terminal bronchioli) of the sheep treated with Perilla ketone showed little early evidence of damage, although some of the secretory cells did show dilatation of both rough and agranular endoplasmic reticulum. In addition, there was modest accumulation of inflammatory cells and edema fluid in the connective tissue sheaths around the airways. This damage to the airways, however, occurred later and was relatively mild when compared with the effects of Perilla ketone on the endothelial and alveolar epithelial cells and as such probably contributes little to the development of intra-alveolar edema. Whether such morphologic changes contribute to the altered lung mechanics and $^{51}Cr/EDTA^{125}I$-antipyrine flux that follows infusion of Perilla ketone[8] is not known.

The mechanism of Perilla ketone toxicity is uncertain. Because of its chemical similarity to 4-ipomeanol (both are 3-substituted furans), it has been postulated that Perilla ketone is converted to its ultimate toxic metabolite via the cytochrome P450 system.[4,5] Cytochrome P450 activity has been demonstrated in the secretory cells of the peripheral lung airways (Clara cells)[4] and it has been suggested that the structural changes induced in these cells by 4-ipomeanol reflect alterations in this system.

The effects of 4-ipomeanol on mouse lung have been described by Durham, Boyd, and Castleman.[16] These authors found mild interstitial edema associated with damaged endothelial cells in the alveolar capillaries from 2 hours. Capillary endothelial cell damage was most severe from 12 to 24 hours and occurred in association with intra-alveolar edema. Damage to the nonciliated bronchiolar cell was apparent by 4 hours. Thus, the present findings in sheep follow-

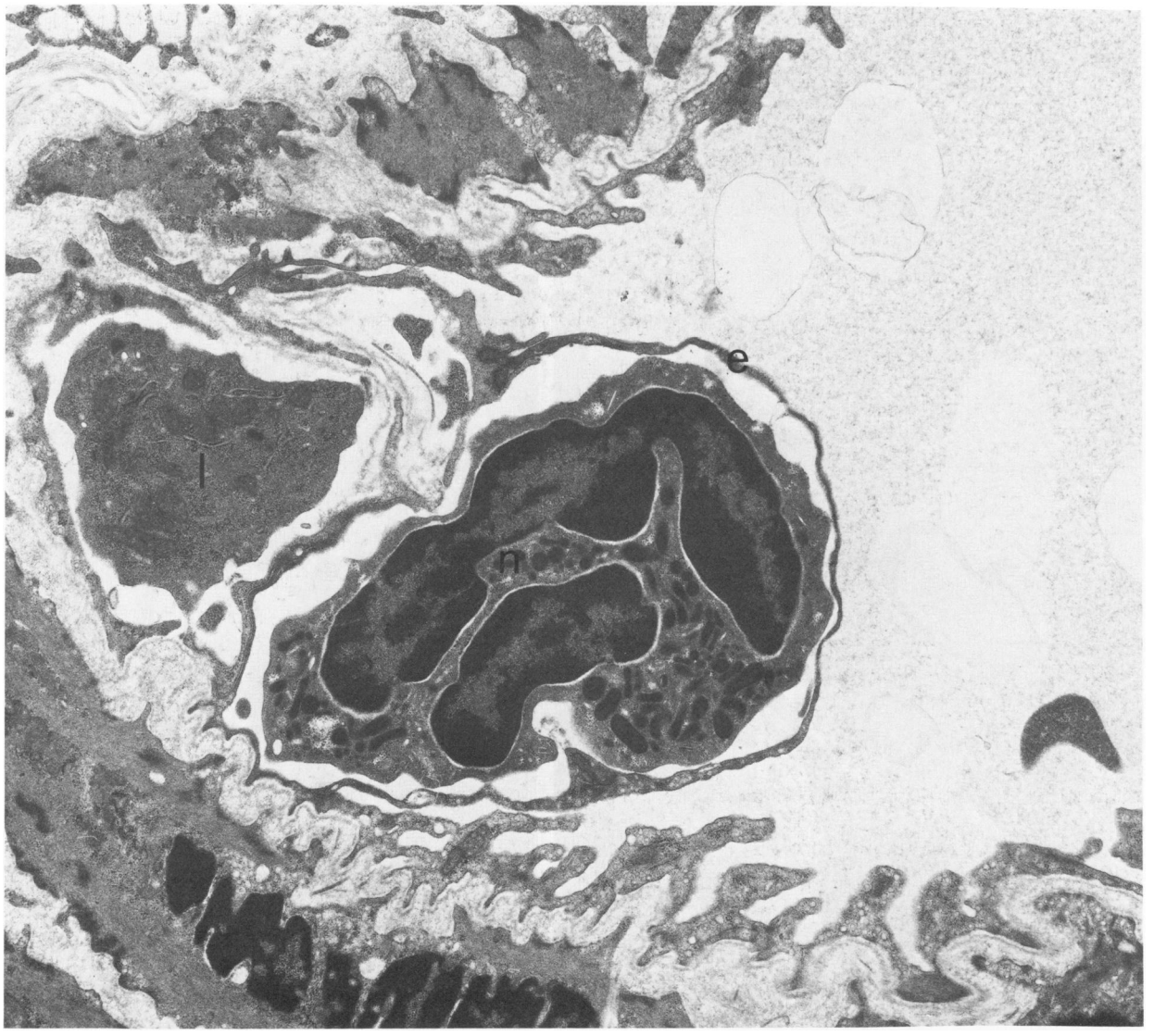

Figure 9—Electron micrograph of a small muscular blood vessel from sheep lung 120 minutes after infusion of Perilla ketone. Part of a nucleated neutrophil (n) and a portion of a leukocyte (l) are surrounded by endothelium (e) suggesting migration of these cells across the endothelial layer and into the vascular wall. ×13,000

ing Perilla ketone infusion—endothelial damage, and interstitial and intraalveolar edema by 15 minutes—while similar to those seen in the mouse injected with 4-ipomeanol, are more rapid and severe in onset. Furthermore, after Perilla ketone infusion, striking damage to the Type I pneumonocytes was found as early as 15 minutes after injection, while Durham and colleagues found only minimal damage to alveolar epithelial cells by 12 hours.

The differences in the response of mouse lung to 4-ipomeanol and sheep lung to Perilla ketone may be due to species variations. Each species has a different capacity of specific cells to metabolize xenobiotic compounds to ultimate toxins. Alternatively, the differences may result from differences between the two compounds administered, differences in the routes of administration of the compound—intraperitoneal in the mouse and into the pulmonary artery in studies with sheep—or both.

Infusion of 3-methylindole, a bacterial metabolite of tryptophan that is chemically similar to Perilla ketone but lacking the 3-substituted furan, into goats also causes structural alterations to the nonciliated bronchiolar cell, the Type I pneumonocyte and capillary endothelium by 30 minutes of administration.[15] Proliferation and dilation of the agranular endoplasmic reticulum in both the Type I pneumonocyte and nonciliated secretory cell of the peripheral airways also were noted after 3-methylindole administration. These authors considered these changes consistent

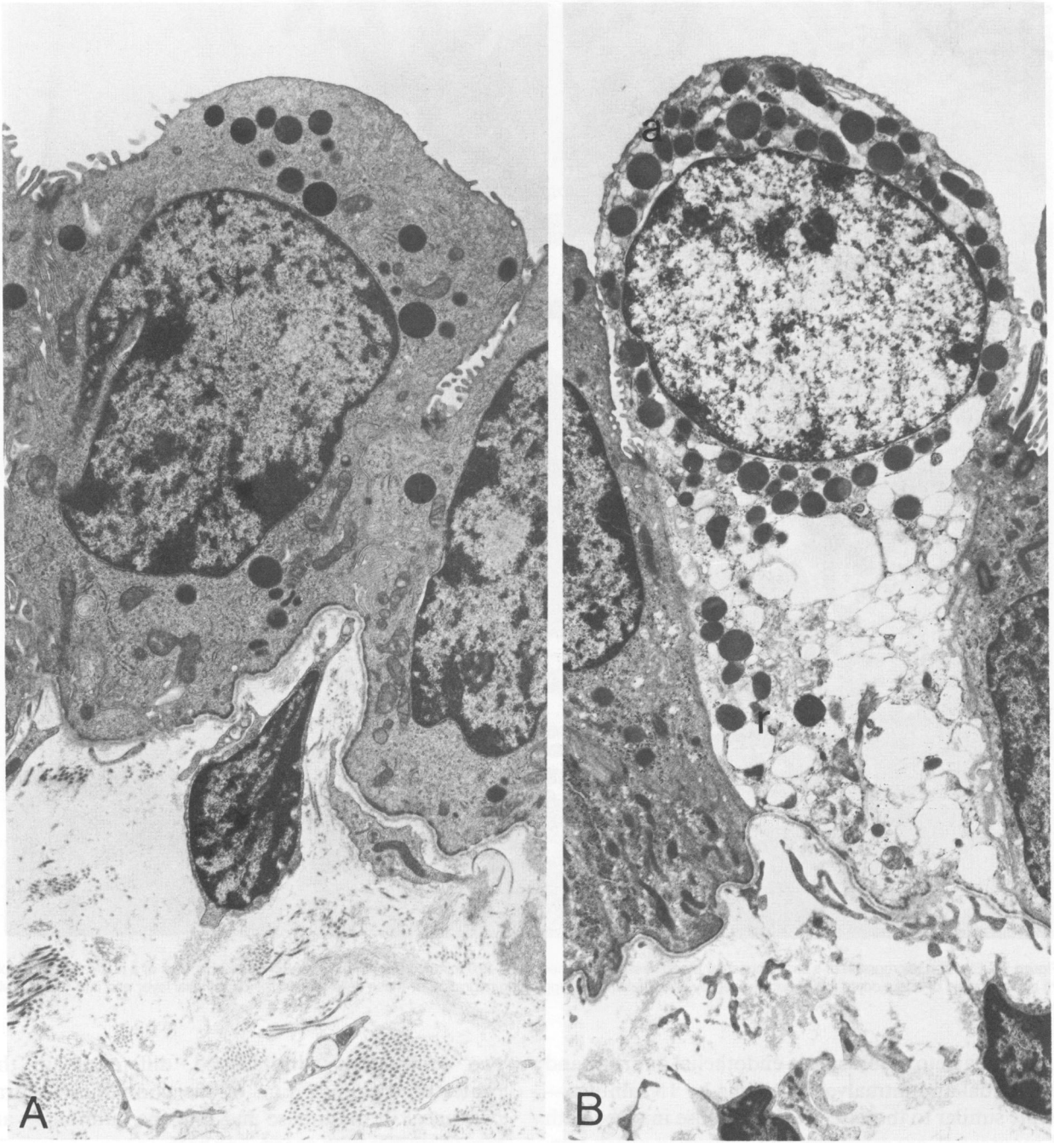

Figure 10—Electron micrographs showing part of a small bronchiolus from a control sheep showing a normal non-ciliated secretory cell (**A**) and from a Perilla ketone treated sheep 120 minutes after infusion (**B**). The nonciliated secretory cell in B is more electron lucent than that in A, and contains dilated cisternae of rough (r) and agranular (a) endoplasmic reticulum (d). ×8000

with involvement of the mixed function oxidase system.[16] The present study showed cytoplasmic vacuolization of the nonciliated secretory cell and the Type I pneumonocyte and dilation of the agranular endoplasmic reticulum in the nonciliated secretory cell, but did not reveal proliferation of the agranular endoplasmic reticulum. Thus, the finding of vesiculation and vacuolization of the Type I pneumonocyte and endothelial cell as well as the secretory cell of the airways are perhaps consistent with induction of cytochrome P450 activity and confirm the idea that the mixed function oxidase system is present in a number of lung cells.

Accumulation of neutrophils in the lungs of animals after administration of Perilla ketone has not been noted previously. In other sheep models of acute

lung injury, such as endotoxemia[17] and air embolization,[18] sequestration and margination of neutrophils in the lung have been linked to the onset of increased pulmonary vascular permeability.[19] *In vitro* studies also suggest that neutrophils may contribute to endothelial injury.[20] The sequestration of neutrophils after Perilla ketone infusion into sheep, however, was modest when compared with other sheep models of acute lung injury and whether such neutrophil accumulation is key to the onset of acute lung injury in the Perilla ketone model requires further study.

Infusion of Perilla ketone also caused platelet accumulation in regions of endothelial injury. Fibrin was often seen in these platelet-rich areas and was also found in alveoli. It is well recognized that platelets accumulate in regions of endothelial injury and that this accumulation is associated both with the intravascular coagulation and release of thromboxane. While none of the indicators of the coagulation were measured, a previous study failed to reveal any increase in thromboxane in lung lymph,[8] but because thromboxane release can occur in the absence of circulating platelets in sheep,[21] this measurement is not a specific indicator of platelet activation and accumulation in the lung. Measurements of the coagulation cascade, humoral mediators, and vasoactive agents, such as leukotrienes and platelet activating factor, require further study.

In summary, the morphologic changes induced in sheep lungs by infusion of Perilla ketone correlate with the previously described alterations in pulmonary physiology. The early appearance of both interstitial and intraalveolar edema, together with changes in the endothelial layer and Type I pneumonocytes, may account for the increased pulmonary microvascular permeability, reduced pulmonary compliance, and increased ^{51}Cr-EDTA/^{125}I-antipyrine uptake noted after Perilla ketone infusion.

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