Ultrastructural Pulmonary Changes Induced by Intravenously Administered 3-Methylindole in Goats

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Transmission electron microscopy was used to characterize early pulmonary lesions in goats after a 2-hour intravenous infusion of 0.04 g 3-methylindole (3MI) per kilogram body weight. Groups of 2 or 3 goats were euthanized at 0.5, 2, 4, 8, and 24 hours after the beginning of the infusion. Changes in lung ultrastructure were compared to noninfused and carrier-infused (propylene glycol) controls. By 0.5 hour, mitochondria and intracellular vesicles were swollen in capillary endothelial, alveolar, and nonciliated bronchiolar epithelial cells. Morphologic changes were most severe in the alveolar Type 1 and nonciliated bronchiolar epithelial cells. Interalveolar septums were swollen at 0.5 hour, and interstitial edema was severe at 2 hours. Denuded alveolar epithelial basement membranes were also observed at 2 hours, and some endothelial cells appeared dark and necrotic. Endothelial cells appeared normal after 2 hours. By 4 hours, the remaining intact alveolar Type 1 cells contained larger and more prominent clusters of smooth endoplasmic reticulum, compared with controls. Morphologic changes in alveolar Type 1 and nonciliated bronchiolar epithelial cells became progressively more severe during the 24-hour experiment. These findings demonstrate that 3MI induces a rapid cytotoxic effect primarily on alveolar Type 1 and nonciliated bronchiolar epithelial cells. Proliferation of smooth endoplasmic reticulum in these cells suggests involvement of the mixed function oxidase system in 3MI-induced pneumotoxicity. (Am J Pathol 1980, 99:551-560)

3-METHYLINDOLE (3MI) is a bacterial metabolite of tryptophan that has been shown to cause acute pulmonary edema and interstitial emphysema in ruminants within 1–4 days following oral or intravenous 3MI administration.[1,2] Clinical signs of respiratory distress, such as tachypnea and dyspnea, are evident within several hours after an oral dose. Changes seen by light microscopy appear to be limited to the lung and include thickened alveolar septums, foci of hemorrhage, alveolar edema, interstitial emphysema, and hyaline membrane formation. Hypertrophy and hyperplasia of alveolar epithelial cells occur from 2 to 4 days after dosing.[1–5] Ultrastructural pulmonary lesions have been observed 4 hours after an oral dose of 3MI and involve primarily alveolar and nonciliated bronchiolar epithelial cells.[6] The time sequence of events is shorter and more reproducible following intravenous administration of 3MI, compared with oral dosing.[7,8]

There is ample evidence that 3MI can alter the structure and cause dis-

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ruption of biologic membranes and that these effects are related to its lipophilic properties.[9-11] Even though these direct effects could conceivably cause lung damage, the primary mechanism by which 3MI exerts its pneumotoxic effect is through metabolism by the mixed function oxidase (MFO) system to reactive intermediates.[12,13] A similar mechanism has been suggested for a variety of pneumotoxic chemicals such as 3-substituted furans,[14-17] and bromobenzene.[18]

Many of the pathologic changes associated with 3MI-induced lung damage are related to secondary effects and progressive deterioration of lung tissue. Early morphologic changes that may be associated with biochemical effects of 3MI on lung cells have not been reported. The objective of this study was to characterize the early ultrastructural changes in lung cells associated with intravenous infusion of 3MI. These results are an essential part of our long-term goal to elucidate the sequence of biochemical and morphologic events associated with 3MI-induced pulmonary disease.

Materials and Methods

Fourteen yearling wether goats were infused with 3MI (Sigma Chemical Co., St. Louis, Mo) dissolved in propylene glycol (PG) (0.1 g 3MI/ml PG) through an indwelling jugular catheter. Eleven goats received a total dose of 0.04 g 3MI/kg body weight over a 2-hour period. All time designations are reported as the time after the beginning of the infusion. Groups of 3 goats were euthanized with sodium pentobarbital at 2 and 4 hours, and groups of 2 goats were euthanized at 8 and 24 hours. An additional group of 3 goats euthanized at 0.5 hour had received only a fourth of the total dose (0.01 g 3MI/kg body weight). One goat, scheduled to be euthanized at 8 hours, died of acute pulmonary disease at 6 hours. One control goat was euthanized at each interval of 0.5, 2, 8, and 24 hours after PG infusion (0.36 ml/kg body weight), and 2 control goats were euthanized without any infusion. Lungs were removed from the thoracic cavity within 3–5 minutes after euthanasia. Lung samples for transmission electron microscopy (TEM) were cut from the mid-dorsal area, mid-lateral edge, and caudal tip of the right caudal lobe. Other lobes were not examined. Cubes (1 cu mm) were fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer (pH = 7.3) for 4 hours. Samples were rinsed in buffer, postfixed for 1.5 hour in 1% osmium tetroxide, dehydrated in ethanol, and embedded in Epon (Miller–Stephenson Chemical Co., Inc. Danbury, Conn). Thin sections were stained with uranyl acetate and lead citrate, and examined with a Hitachi Model 125-E transmission electron microscope.

Results

Control Goats

Infusion of the PG carrier did not result in significant ultrastructural changes in the lungs of the goat. At the gross level the lungs appeared morphologically normal in all control goats. Type 1 cells in control goats formed a smooth attenuated layer resting on an intact basement membrane. The Type 1 cell cytoplasm was electron-lucent and contained

small vesicles, occasional mitochondria, and small clusters of smooth endoplasmic reticulum (SER). An interalveolar septum from a PG-infused control goat at 24 hours is shown in Figure 1. Capillary endothelial cell junctions and nonciliated bronchiolar epithelial cells were similar in the PG-infused and noninfused control goats.

Capillary and Interstitial Lesions

By 0.5 hour after beginning the 3MI infusion, capillary endothelial damage was indicated by the presence of swollen mitochondria and enlarged cytoplasmic vesicles (Figure 2). Some endothelial cell junctions appeared loose or slightly widened (10–15 nm), indicating possible damage to these areas and less restricted communication between the capillary lumen and septal interstitial spaces (Figure 3).

At 2 hours, interalveolar septums were dilated, and electron-dense endothelial cells lined part or all of many capillaries (Figure 4). Intracellular vesicles appeared more numerous in these electron-dense cells than in controls, and some damaged cells were separated by gaps of approximately 25 nm (Figure 4, inset). Endothelial cytoplasmic blebs or areas of separation from the basal lamina were evident at 2 hours (Figure 5). Only an occasional swollen organelle or vacuole was observed in endothelial cells at 4 hours, and by 8 hours the cells appeared normal.

Alveolar Type 1 and Type 2 Cell Lesions

Obvious ultrastructural lesions observed at 0.5 hour were swollen vesicles (presumably enlarged profiles of SER) and mitochondria within the alveolar Type 1 epithelial cells. These changes were similar to those seen at 2 hours, shown in Figure 5. By 2 hours, increased swelling of interalveolar septums was noted, and some alveolar spaces were edematous, as indicated by the flocculent electron-dense debris in the alveoli, similar in density to that seen in capillaries (Figure 4). Severe edematous dilation of interalveolar septums at 2 hours apparently caused separation of some alveolar epithelial cells from the basement membrane. By 4 hours, areas of denuded alveolar basal lamina were more numerous (Figure 6), and clusters of SER were seen more frequently in Type 1 cells of 3MI-infused goats than in controls (Figure 7). By 8 and 24 hours, Type 1 cells appeared extremely swollen. Large clusters of SER were often the only distinguishable intercellular organelles observed (Figure 8). Alveolar spaces appeared to contain increased concentrations of proteinaceous debris, and numerous alveolar macrophages were observed.

Some Type 2 cells contained slightly swollen mitochondria (Figure 6),

and only mild cytoplasmic swelling was evident in these cells at 2 and 4 hours. At later periods, the Type 2 cells appeared normal.

Bronchiolar Lesions

The most severe changes in the bronchiolar epithelium occurred in the nonciliated cells. Compared with controls (Figure 9), the nonciliated epithelial cells of goats infused with 3MI for only 0.5 hour contained many cytoplasmic vacuoles, swollen mitochondria, perinuclear space, and electron-dense bodies associated with cytotoxicity (Figure 10). These cellular changes were present before there was evidence of peribronchiolar or alveolar edema. These lesions were progressive, and sloughing of bronchiolar epithelial cells was apparent after 2–4 hours. Less severe ultrastructural changes were present in the ciliated epithelial cells, and later these cells appeared normal.

Discussion

The most striking ultrastructural change observed within 0.5 hour after beginning the 3MI infusion was progressive degeneration of alveolar Type 1 and nonciliated bronchiolar epithelial cells. The early endothelial cell changes induced by 3MI are apparently reversible, since they were not seen after 2 hours in this study or after oral administration of 3MI in a previous experiment.[6] The remission of ultrastructural changes in endothelial cells corresponded to the end of 3MI infusion at 2 hours, and it is possible that these changes may result from direct effects of 3MI on endothelial cells. Less severe changes in other lung cell types emphasize the selective effects of 3MI.

The severity of interstitial and alveolar edema increased with time throughout the experiment. Interstitial edema observed at 0.5 hour preceded the onset of significant alveolar edema, suggesting an increase in capillary permeability and/or changes in pulmonary lymphatic drainage.[19] Alveolar edema developed as a prominent feature after 2 hours and occurred in conjunction with severe interstitial edema and alveolar Type 1 cell damage.

Cytotoxicity in alveolar Type 1 and nonciliated bronchiolar epithelial cells is most likely related to the metabolism of 3MI by the MFO system in these cells. We have previously shown that induction of the MFO system with phenobarbital increases the severity of 3MI-induced lung injury and that lung injury is prevented by piperonyl butoxide, an inhibitor of the MFO system.[12] In addition, the formation of highly reactive intermediates that covalently bind to cellular macromolecules during the course of 3MI metabolism by lung microsomes is probably responsible for

pneumotoxicity.[13] The SER is known to contain the MFO enzymes, and a variety of substrates lead to proliferation of SER and induction of the MFO system.[20] The known involvement of the MFO system in pneumotoxicity, coupled with the proliferation of SER in alveolar Type 1 and nonciliated bronchiolar epithelial cells observed in this and a previous study [6] suggest a relationship between MFO metabolism in these cells and the cytotoxicity of 3MI.

The observations from this study are consistent with the following interpretation of the sequence of major events associated with 3MI-induced lung injury. Exposure of the lung to 3MI in the blood may have direct effects on capillary endothelia and alter the characteristics of the permeability of these cells, which may contribute to the interstitial edema observed within 0.5 hour after exposure to 3MI. Since 3MI is highly lipophilic, it can penetrate cellular membranes and enter lung cells. Metabolism of 3MI by the MFO system in alveolar Type 1 and nonciliated bronchiolar cells results in the formation of reactive metabolite(s), which initiate cytotoxic effects within 0.5 hour, and these effects become progressively more severe, resulting in acute injury to these cells. As the alveolar epithelial barrier is thus weakened, the alveoli are flooded with edema fluid. It is clear from previous studies that hyperplasia of alveolar Type 2 pneumocytes and bronchiolar epithelial cells occurs within 2–3 days to replenish these epithelial cell populations.[4,21,22]

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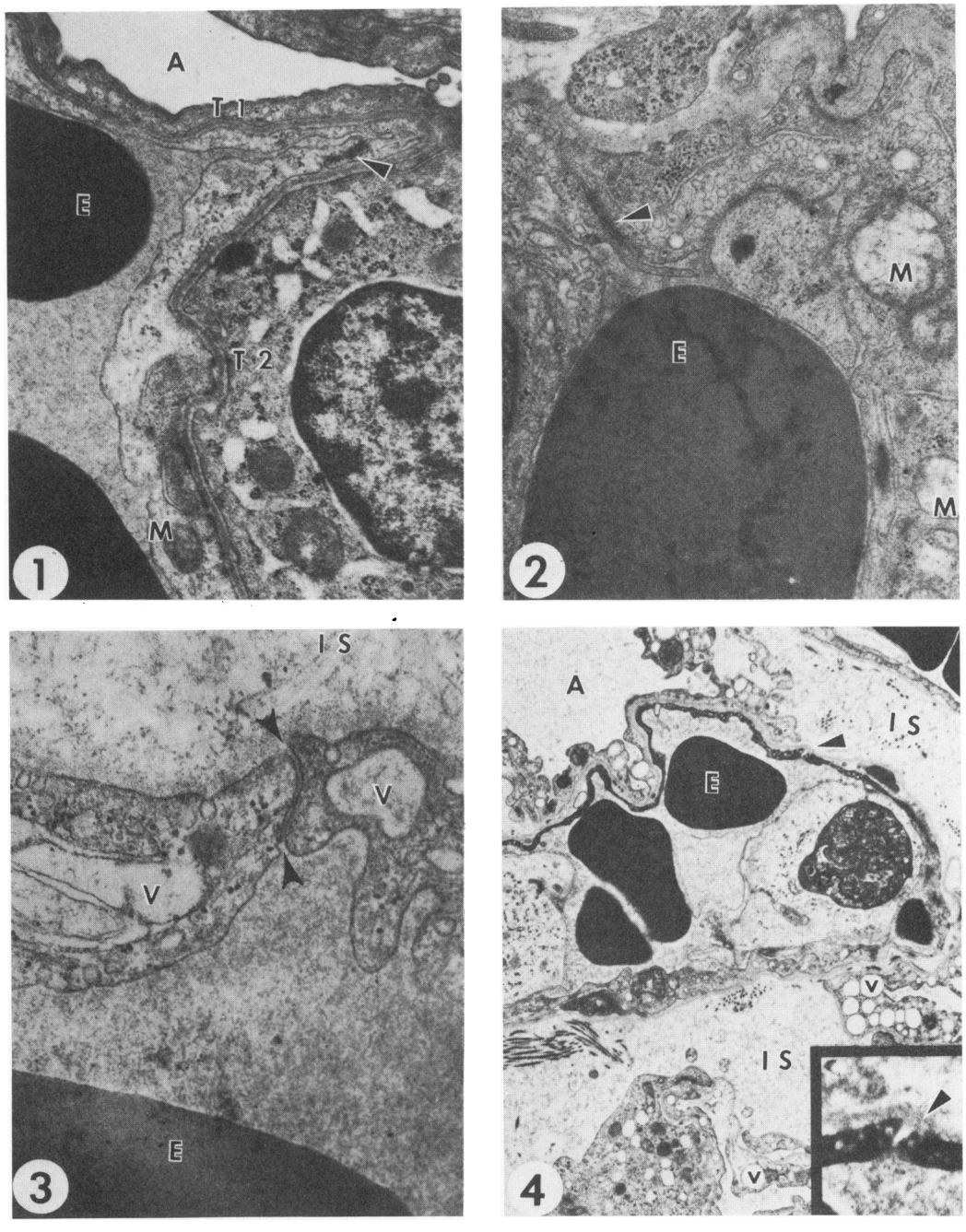


Figure 1—Lung parenchma of a propylene-glycol–infused control goat. Erythrocytes (*E*) are shown within a capillary. A normal endothelial mitochondrion (*M*) and cell junction (*arrow*) are evident. Both Type 1 (*T1*) and Type 2 (*T2*) cells line the alveolar spaces (*A*). (×19,500) **Figure 2**—Swollen mitochondria (*M*) in the capillary endothelia at 0.5 hour after the beginning of the 3MI-infusion. The endothelial cell junction (*arrow*) appears intact. (×24,000) **Figure 3**—By 0.5 hour, some endothelial cell junctions (*arrow*) lack the electron density normally present. Swollen intracellular vesicles (*v*) are present in both adjacent endothelial cells. Edematous interstitial area (*IS*) surrounds the capillary. (×37,500) **Figure 4**—Some endothelial cells appear severely damaged by 2 hours, as evidenced by their electron density and occasional gaps (*arrow*). **Inset** is an enlargement of an electron-dense endothelial cell gap. (×23,100) Interstitial spaces (*IS*) are edematous. Type 1 cells lining edematous alveolar spaces (*A*) contain numerous swollen vesicles (*v*). (×6,300)

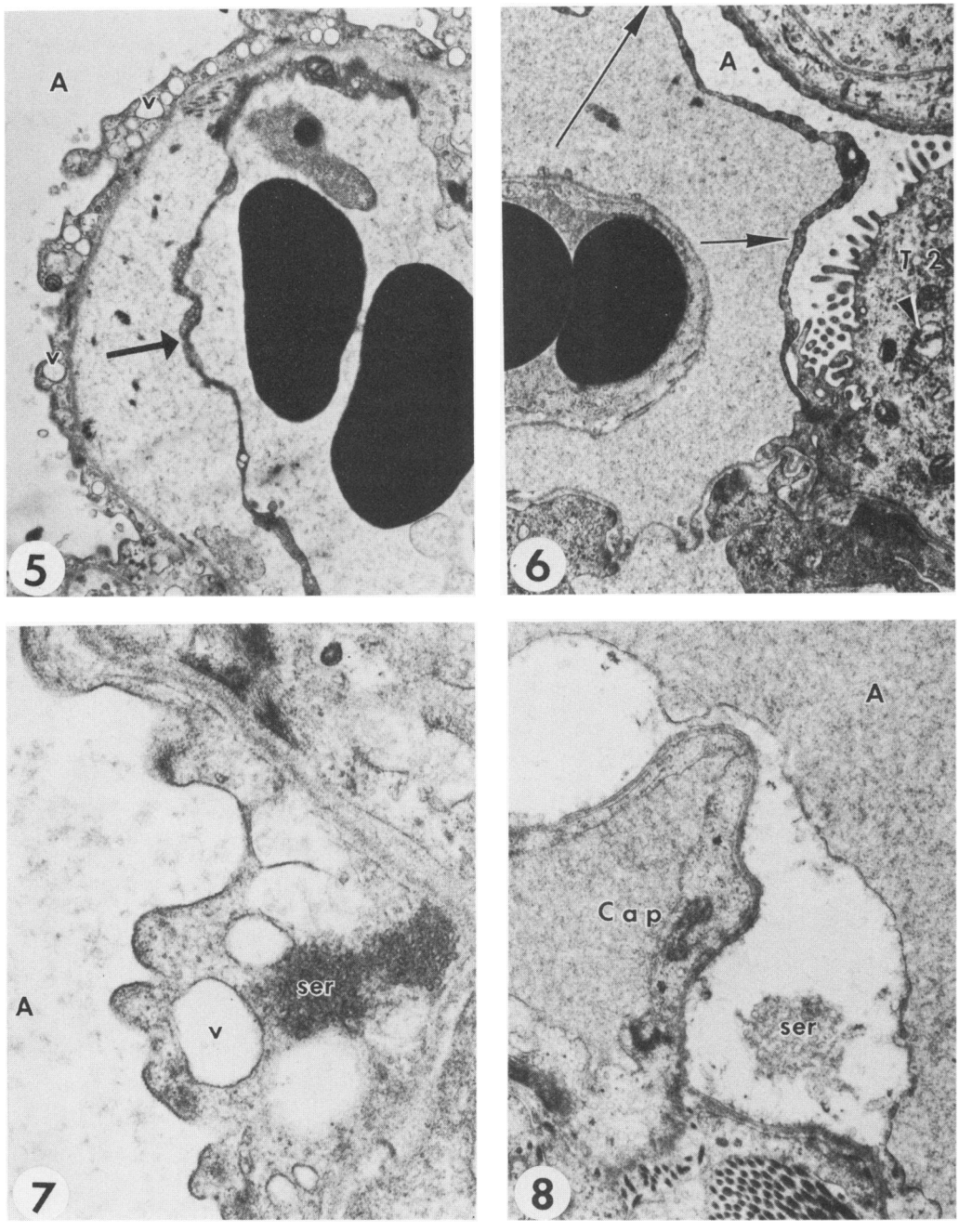
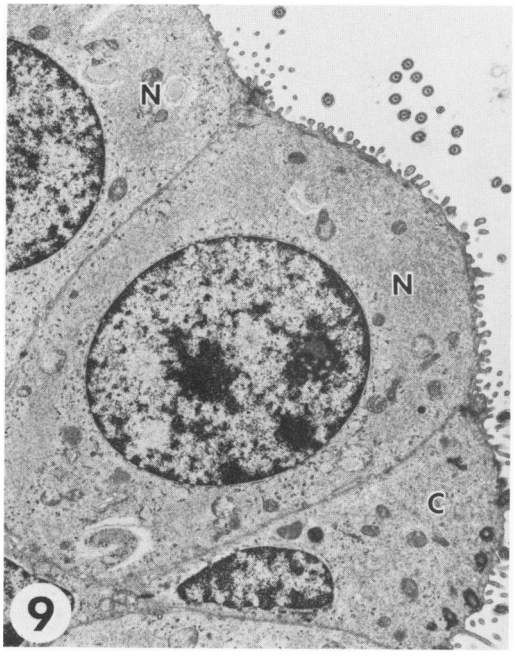


Figure 5—Endothelial blebs or separations from the underlying basement membrane are evident at 2 hours in some interalveolar septal capillaries. The endothelial cell is abnormally electron-dense (*arrow*). Note the numerous swollen vesicles (*v*) in the Type 1 cell lining the alveolus (*A*). (×8400) **Figure 6**—Edematous space separates alveolar Type 1 epithelial cells (*arrows*) from underlying basement membrane by 4 hours after the beginning of the 3MI infusion. A Type 2 (*T2*) cell contains slightly swollen mitochondria (*arrowhead*). Only a small alveolar space (*A*) remains. (×9000) **Figure 7**—Enlarged areas of smooth endoplasmic reticulum (*ser*) are evident in alveolar Type 1 epithelial cells as early as 4 hours after the start of 3MI infusion. Note the dilated intracellular vesicles (*v*) also present. Adjacent alveolar space (*A*) contains electron-dense material indicating the presence of edema. (×36,500) **Figure 8**—By 8 hours, some Type 1 cells appear severely dilated, with only enlarged clusters of smooth endoplasmic reticulum (*ser*) remaining as distinguishable cytoplasmic structures. Note that the dense, proteinaceous material in the alveolar space (*A*) is similar in electron density to that in the capillary (*Cap*). (×16,500)



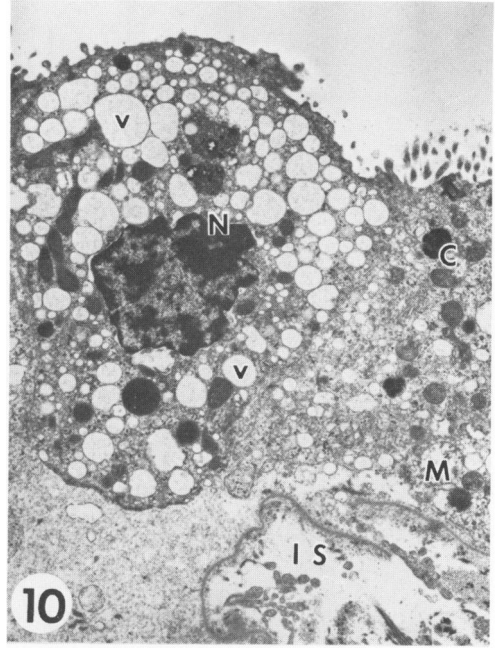


Figure 9—Control goat terminal bronchiole epithelium showing morphologically normal nonciliated cells (*N*) and a more oblique segment for a ciliated (*C*) epithelial cell. (×6000) **Figure 10**—By 0.5 hour, 3MI infusion resulted in severe cytoplasmic damage to nonciliated cells, as evidenced by numerous dilated vesicles (*v*). Less severe changes were present in the ciliated cells (*C*). (×5600)

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