Taurine Protects Hamster Bronchioles From Acute NO_2-Induced Alterations

A Histologic, Ultrastructural, and Freeze-Fracture Study

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In this study the authors describe the use of dietary taurine to protect hamster lung epithelium from acute nitrogen dioxide (NO_2) injury. The conclusions were based on histologic, ultrastructural, and freeze-fracture analyses. Hamsters were pretreated for 14 days with 0.5% taurine in their drinking water. They were then exposed to either 7 or 30 ppm NO_2 for 24 hours. The lungs from animals of these experimental groups were compared with those from hamsters treated with only NO_2, and those given only taurine and with untreated controls. After treatment, hamsters were anesthetized and perfusion-fixed through the right side of the heart with a solution containing 1% glutaraldehyde, 4% paraformaldehyde, and 0.2 M cacodylate. The trachea and lungs were removed *en bloc* and stored overnight in cacodylate buffer at 4 C. Terminal and respiratory bronchioles, including alveolar ducts and peribronchiolar alveoli, were dissected from each lobe and processed for embedding in Epon and freeze-fracture replication. Light and transmission electron microscopy revealed the typical inflammatory cell infiltrate in the bronchiolar and alveolar duct regions in the lungs of hamsters exposed to NO_2. The bronchiolar epithelium appeared flattened because of loss and breakage of cilia on ciliated cells and apical protrusions of Clara cells. Clara-cell secretory granules were reduced or absent. Freeze-fracture replicas of tight junctions of bronchiolar epithelium analyzed by morphometric techniques demonstrated a reduction and fragmentation of fibrils. Only animals exposed to 30 ppm NO_2 exhibited physiologic intercellular penetration of horseradish peroxidase. Hamsters pretreated with taurine and then exposed to NO_2 showed none of these alterations. They exhibited the same morphologic features as the untreated controls and the hamsters treated only with taurine. On the basis of this evidence, it is suggested that prophylactic dietary taurine can prevent acute NO_2-induced morphologic lung injury. Taurine may also be effective in preventing lung injury induced by other oxidant gases. (Am J Pathol 1986, 125:585–600)

NITROGEN DIOXIDE (NO_2) is a ubiquitous noxious gas found in significant concentration in the urban environment. It is also a component of tobacco smoke and automobile exhaust and a by-product of the burning of fossil fuels in many industries.[1,2] Examination of human lung tissues from occupational exposures[3–6] and accidents[7,8] have shown that acute high concentrations of NO_2 will cause pulmonary edema and asphyxia.[9] Acute and chronic effects of exposure to low NO_2 concentrations in man have only been implicated because the gas is rarely present in its pure form at these levels, usually mixed with other injurious gases. Low-dose exposures generally go undetected because clinical symptoms are negligible.

Many studies in lower mammals have been performed to investigate the mechanisms involved in high and low dose NO_2 injury. These studies have revealed a variety of injurious effects that presumably apply to man. Chronic exposure to NO_2 has been shown to produce morphologic emphysema in hamsters[10] and rats.[11] In acute studies, it has also been shown to induce an inflammatory response in bronchioles, alveolar ducts, and peribronchiolar alveoli,[12,13] as well as alterations and proliferation of epithelial cells[14–18] and disruption of epithelial barrier function.[19–22]

The precise mechanism(s) of NO_2 injury in the lungs remains unknown, however, many of the current theories suggest that oxidant gas interactions directly with

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tissue or free radicals produced by resident inflammatory or epithelial cells may be the initiating and promoting factor.[23,24] Neutralizing or blocking the effects of these radicals and oxidants may prevent the lung injury.[25,27] Selenium and vitamin E (α-tocopherol) have recently been used as antioxidants, to protect against oxidant lung injury.[25-27] The biochemical data[25-26] and the report of a single morphologic study[27] indicate that these agents may have the potential to protect alveolar epithelium from NO_2 injury. The effectiveness of prophylactically administered antioxidants remains unclear.

Recently, there has been excitement about a molecule naturally occurring in most tissues of most species, 2-aminoethanesulfonic acid (taurine). Taurine, apparently by its molecular structure, has detoxifying, antioxidant, and membrane-stabilizing properties[28-30] and is found in higher concentrations in tissues that have the potential for oxidant exposure.[31] Orally administered taurine has been used as a means of protecting against oxidant injury in many tissues other than the lungs.[32-34] Biochemical and morphologic data presented in these[32-34] and other studies have indicated that taurine was effective in preventing such injury.

In this article we report the protective effects of orally administered taurine on the small-airway epithelium of hamsters exposed acutely to low and high concentrations of NO_2. The evidence for its protective capacity was based on histologic, ultrastructural, and freeze-fracture analysis of the appropriate lung tissue.

Materials and Methods

Animal Treatment and Exposure Protocols

Thirty six male Syrian Golden hamsters weighing 90–100 g were randomly divided into two groups of 18 animals each. Both groups were kept in separate cages and fed with Purina hamster chow. Group 1 received drinking water enriched with 0.5% taurine, and Group 2 received tap water with no additives. The animals were kept on this diet for 14 days. On Day 15, 6 animals from each group were transferred to chambers and exposed to NO_2 for 24 hours at a concentration of 30 ppm, and 6 animals from each group were exposed at 7 ppm. Six animals from each group acted as untreated controls, breathing room air for the same 24-hour period. The NO_2 concentration was monitored by CSI 1600 NO_2 analyzer (Columbia Scientific Industries) and charted on a Brown-Honeywell recorder (Brown-Honeywell, Denver, Colorado). The NO_2 analyzer was intermittently calibrated to the chamber NO_2 concentrations according to the method of Saltzman.[35]

Tissue Preparation

All animals were anesthetized immediately after their removal from the NO_2 chamber by intraperitoneal injection of 0.2 ml of a 1:1 dilution of 0.5 mg/ml sodium pentobarbital in normal saline. The trachea was exposed by dissection and retraction of the covering neck organs and clamped closed upon inspiration. The peritoneal cavity was opened. While the heart was still beating, the chest cavity was opened for exposure of the heart and lungs. The right ventricle was cannulated, and the vascular system was irrigated with 15 ml of normal saline instilled at 20 cm H_2O pressure. At the time of cannulation the renal vein was cut to release the blood. The animals were then perfused with a fixative solution containing 1% glutaraldehyde and 4% paraformaldehyde in 0.2 M sodium cacodylate buffered at pH 7.2 via the cannula. Immediately after 10 minutes of perfusion fixation the lungs were removed *en bloc* by the trachea and immersed in the fixative solution for an additional 1.5 hours. The lungs were washed overnight in 0.2 M sodium cacodylate buffer at 4 C.

Twenty-four randomly selected areas were dissected from each lung for each of the following procedures. Four specimens were taken from each lobe. Each specimen included a terminal and respiratory bronchiole, an alveolar duct, and surrounding alveoli.

Transmission Electron Microscopy

One group of lung specimens was processed for standard transmission electron microscopic examination of thin sections. The specimens were dissected from each lung under a stereo microscope at high enough power for visualization of the terminal bronchiole, the respiratory bronchiole, and the site of bifurcation into the alveolar ducts. These structures were oriented and systematically dissected out from the lobe of the lung. The specimens were sequentially treated with 1% OsO_4 for 1 hour, dehydrated in graded ethanol steps through propylene oxide, and embedded in Epon 812. During embedding, the block containing the terminal and respiratory bronchioles and the alveolar ducts along with the surrounding lung parenchyma were oriented so that cross-sections for light and electron microscopy could be cut at each level as the specimen was skip-sectioned through its entirety. In this way we were able to control for differences along the bronchioles or the degree of inflation described by Becci et al.[36] One-micron sections stained with toluidine blue from each bronchiole from each animal were evaluated by light microscopy in a single blind experiment without prior knowledge of experimental group or duration of NO_2 exposure, as de-

scribed by Case et al.[20] The bronchiolar epithelium was rated for number of ciliated cells (as a percentage of the total) and degree of ciliary loss. Qualitative assessment of epithelial "flattening" was obtained by determining the percentage of nonciliated cells lacking normal apical cytoplasmic protrusions. Miscellaneous observations include presence or absence of inflammatory cells, epithelial hyperplasia, and nonciliated cell hypertrophy. Ultrathin sections from each selected region from each block were cut, stained with uranyl acetate and lead citrate, and observed with a JEM 100CX transmission electron microscope.

Freeze-Fracture Procedure

Tissue cubes containing cross-sectional areas of bronchioles were obtained as described above. These blocks were transferred from cold cacodylate buffer to a 25% glycerine/0.2 M sodium cacodylate buffer mixture at 4 C. After 90 minutes of glycerine treatment with gentle agitation, tissue blocks were transferred to nickel–gold specimen holders and rapidly frozen in a Balzers double-replica device immersed in Freon 22 cooled by liquid nitrogen. Frozen specimens were transferred to a precooled stage in a Balzers 301 freeze-fracture plant. Vacuum was increased to 10^{-6} torr, and lung tissue samples were fractured at a stage temperature of −110 C. Instantaneously after fracture, the specimens were shadowed with platinum–carbon at 45 degrees and carbon at 90 degrees. Freeze-fracture replicas were removed, lung tissue was digested from the replicas by successive washing in decreasing concentrations of sodium hypochlorite, rinsed in three changes of triple distilled filtered water, and mounted on 300-mesh copper grids.

All replicas were examined and photographed in a JEM 100CX electron microscope equipped with a goniometer stage. A minimum of 10 freeze-fracture replicas from 10 different bronchioles were examined from each animal. Junctional areas within the airway epithelium were defined as areas of cleaved membrane surface exceeding 0.5 μ in horizontal dimension, bearing tight-junctional fibrils.

Each junction photographed was evaluated by two observers, one of whom had no prior knowledge of animal exposure status. Description of tight junctions included integrity of tight-junctional strands, presence of bare membrane areas, obvious breaks of linear particle arrays, and depth of distribution of junctional material. Photographs of randomly selected interalveolar cell junctions were also evaluated in each animal. Micrographs were obtained with a goniometer stage, with the electron beam striking the membrane surfaces at an incident angle as close to 90 degrees as possible. Only specimens where the replicas from both fracture surfaces were preserved were evaluated morphometrically.

Double replicas were initially treated as individual observations, and then photographs of each pair of matched replicas were measured and analyzed for number and continuity of fibrils. In the case of the NO_2-exposed and recovery airway specimens, where the tight junctions were disrupted, lines were drawn through the fragments to be analyzed for number and depth of fibrils by the method previously described by Schneeberger[37] in the rat and Marin et al in hamsters.[38] A grid with 0.25-μ spacing was placed on micrographs of equal magnification (50,000 diameters) so that the lines were perpendicular to the axis of the tight-junctional complex. The number of tight-junctional strands were determined by counting the intersections between the junctional fibrils or the line drawn through the fragments and the lines of the grid. In all cases corners where two or more cells intersected one another were not evaluated because of the different ultrastructural appearance. At the same time, the mean total lateral plasma membrane depth of tight-junctional complex was calculated. To evaluate the fragmentation of fibrils, each micrograph and specimen was analyzed for continuity with the assistance of a Numonics digitizing graphics calculator by techniques originally described by Walker et al[39] and modified by Gordon et al.[22] For analysis of fibril continuity, randomly chosen 0.5-μ areas of micrographs of matched replicas enlarged to 100,000 diameters were traced on separate transparent plastic, then overlaid to correspond to one another. The tracings were graphically calculated for length of fragments and length of breaks. Each measurement was categorized into one of three groups: >0.2 μ, <0.2 μ, but >0.05 μ and <0.05 μ. The number of the measurements for each category was graphically demonstrated. Each measurement graphed was based on the mean and standard error of the mean number of measurements per 0.5-μ tight-junctional area. The values graphed were calculated from the following minimum number of measurements: 5 animals, 3 distal airways per animal, 10 different cells per airway, and at least one 0.5-μ length of tight junction per cell. All the calculations for fibril number and depth described above were made from the same minimum number of measurements described above.

Horseradish Peroxidase (HRP) Procedure

Two animals from each of the above described groups were anesthetized prior to peroxidase installation by an intraperitoneal injection of 0.2 ml of a 1:1 dilution of

0.5 mg/ml sodium pentobarbital in normal saline. The ventral aspect of the neck of each animal was dissected open exposing the trachea, and an 18-gauge intracatheter needle was introduced into the trachea. The needle was withdraw, and the catheter was left in place. An insulin syringe was attached to the catheter, and 0.2 ml of a 125-mg/ml solution of HRP (R2 0.6, activity 350–500 U/mg) in 0.9% NaCl was introduced into the trachea over a 5-minute period. After 30 minutes the trachea was clamped and the rib cages of the NO_2-exposed and control animals were dissected open and perfusion-fixed as previously described. After 10 minutes of perfusion fixation *in situ* and 1 hour immersed in fixative after removal from the animal, the tissue was washed in cacodylate buffer and sections of lung immediately treated by the method of Graham and Karnovsky[38] so that we could visualize the peroxidase reaction product by electron microscopy. With a dissecting microscope, the percentage of bronchioles containing reaction product was determined, and at least 10 bronchioles with reaction product on their surface were cut into small blocks and processed for embedding in plastic. Thick and thin sections were prepared from representative areas and viewed by light and electron microscopy unstained, and the proportion of intracellular regions containing reaction product was calculated.

Results

The gross appearance of the hamster lungs from all the groups before and after fixation was found to be normal. There was no evidence of pneumonia or large areas of inflammatory foci.

Light-Microscopic Findings

Most of the light-microscopic findings are summarized in Table 1. The table generally shows that the animals exposed to NO_2, whether 7 or 30 ppm, exhibited alterations of the bronchiolar epithelium and inflammatory cell infiltration. The magnitude or degree of alterations was greater in the lungs of animals exposed to 30 ppm NO_2. In terms of the condition of the bronchiolar epithelium and the inflammatory response, the groups of NO_2 exposed hamsters pretreated with taurine were not significantly different from the unexposed and untreated controls.

When the lungs of animals from each group were examined by light microscopy, it was noted that exposure to NO_2 at 7 and 30 ppm without any other treatment induced inflammatory cell infiltrates in the lungs. The inflammatory cell infiltrate was restricted to the areas directly surrounding the terminal bronchioles and the alveolar ducts (Figures 1 and 2). The infiltrate was composed mostly of neutrophils and a few macrophages. Many inflammatory cells were observed within the mucus layer of terminal bronchioles, continuous with inflammatory cells in the alveolar ducts and peribronchiolar alveoli. Specimens from the group of hamsters exposed to the 30 ppm were qualitatively more severe (Table 1) (Figure 2). The most significant observation was the absence of infiltrative cells in hamsters pretreated with taurine and then exposed to NO_2 (Figure 3). Specimens from the untreated group (Figure 3) and those treated with taurine alone showed no inflammatory infiltrate.

The bronchiolar epithelium of hamster lungs is normally composed of ciliated and Clara cells (Figure 4). The Clara cells have large protruding apical cytoplasm

Table 1—Light- and Electron-Microscopic Changes in Bronchiolar Epithelium*

Treatment group	Number of ciliated cells (% of total cells counted)	Degree of ciliary loss (0 to + + +)	Epithelial "flatness" index† (%)	Apical Membrane damage and miscellaneous ultrastructural changes‡ (0 to 0+ + +)	Degree of inflammatory infiltrate§
Untreated control	58 ± 3.5	0	35 ± 17	0	0
Taurine	57 ± 3.7	0	32 ± 10	0	0
NO_2					
7 ppm	38 ± 3.1	+ +	51 ± 13	+ +	+ +
30 ppm	26 ± 4.2	+ + +	68 ± 12	+ + +	+ + +
Taurine + NO_2					
7 ppm	54 ± 3.9	+	37 ± 11	+	+
30 ppm	51 ± 3.6	+	39 ± 12	+	+

* Determined by light microscopy (n > 100 cells) and confirmed by electron microscopy (n > 15 cells).
† Percentage of total nonciliated cells lacking apical protrusions by light microscopy.
‡ Qualitative determination from electron microscopy.
§ Inflammatory cells observed in bronchiolar walls, lumen, and surrounding alveolar parencyma confirmed by electron microscopy.

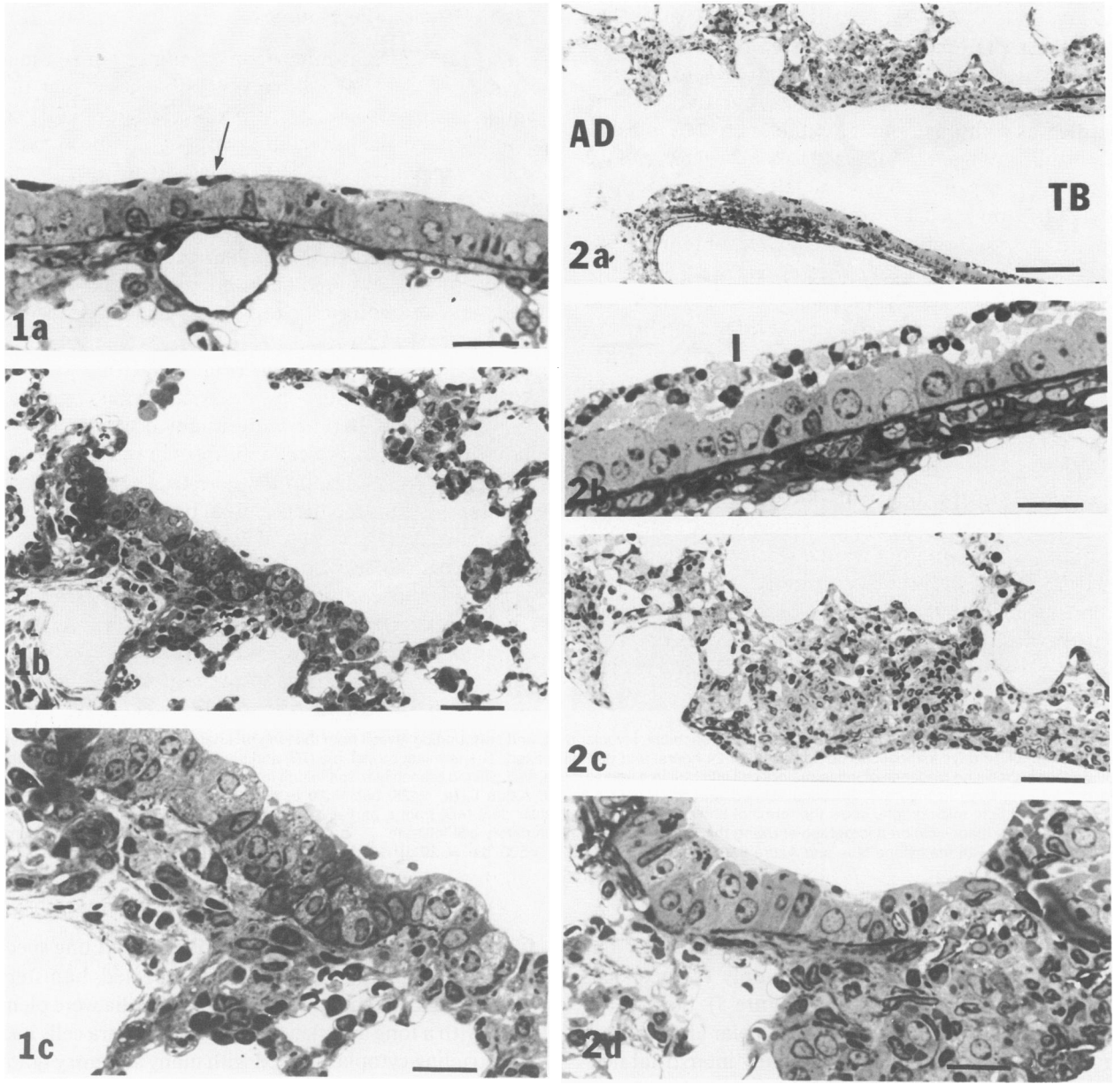


Figure 1a–c—This series of light micrographs of 1-μ plastic sections shows the bronchiolar and alveolar epithelium from a hamster exposed to 7 ppm NO_2 for 24 hours and perfusion-fixed. **a**—The flattened bronchiolar epithelium with a few inflammatory cells (*arrow*) in the mucus layer. **b** and **c**—The alveolar duct at low and high magnification exhibiting a slight cell infiltrate. Sections were stained with methylene blue and Azure II. (**a**, ×500, bar = 20 μ; **b**, ×250, bar = 40 μ; **c**, ×500, bar = 20 μ) **Figure 2a–d**—This series of light micrographs shows the bronchiolar alveolar duct and surrounding alveoli from the lung of a hamster exposed to 30 ppm NO_2 for 24 hours and perfusion-fixed. **a**—A low-magnification micrograph showing the terminal bronchiole (*TB*) and alveolar duct (*AD*). The inflammatory cell infiltrate (*I*) in the mucus layer of the bronchiole is seen in higher magnification in **b** and in the alveolar duct and peribronchiolar alveoli in **c** and **d**. Sections are stained with methylene blue and Azure II. (**a**, ×125, bar = 80 μ; **b**, ×500, bar = 20 μ; **c**, ×250, bar = 40 μ; **d**, ×500, bar = 20 μ)

which contains secretory granules (Figure 4). Bronchiolar epithelium from NO_2-exposed hamsters appeared flattened (Figures 1 and 2). Apical protrusions and cilia were absent. The NO_2-exposed groups also showed a qualitative decrease in the number of secretory granules in Clara cells. None of the above changes were observed in the specimens from the groups pretreated with taurine before exposure to NO_2 (Figure 3). The bronchiolar epithelium was normal in its histologic appearance. The specimens from animals treated with taurine alone could not be differentiated from untreated controls.

Electron-Microscopic Findings

Ultrathin sections of terminal bronchioles, alveolar ducts, and peribronchiolar alveoli corresponded with

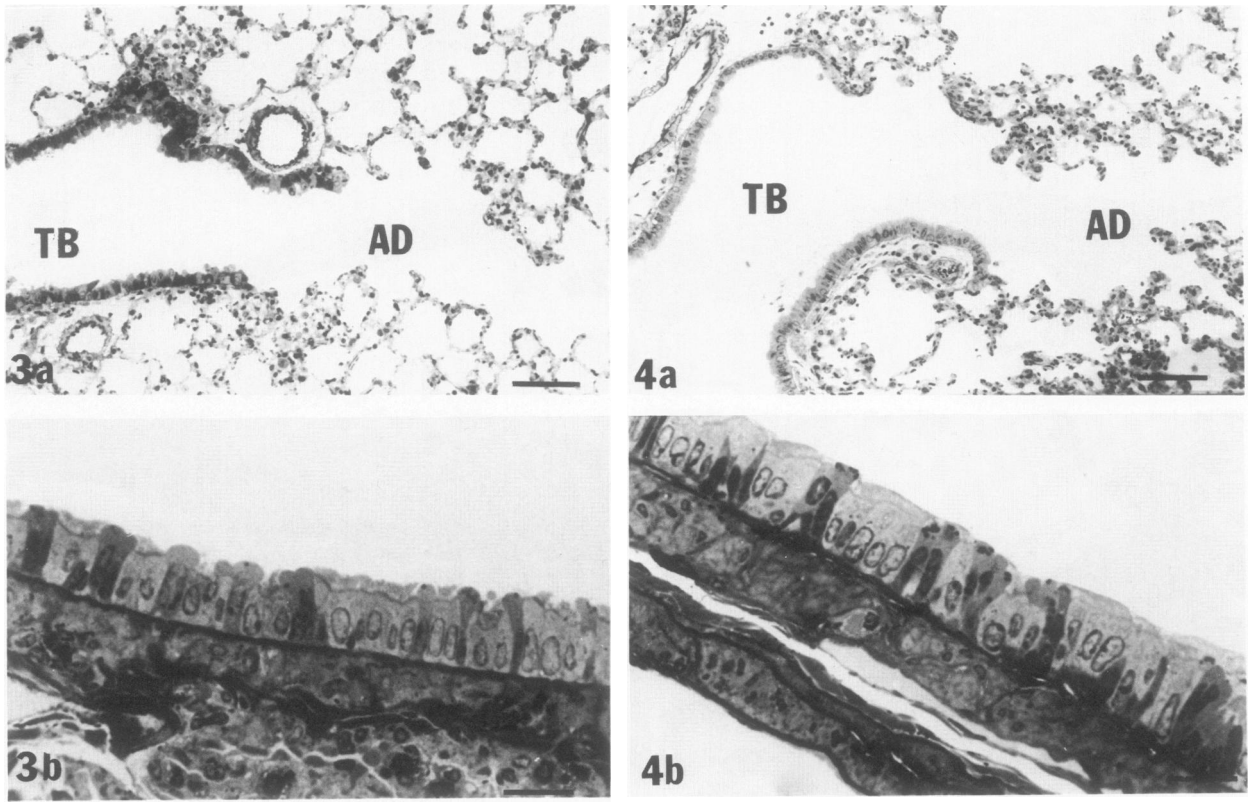


Figure 3a and b—These light micrographs show the bronchiole, alveolar duct, and surrounding alveoli from the lung of a hamster that had been treated with taurine for 14 days, exposed to 30 ppm NO_2 for 24 hours, and perfusion-fixed. The terminal bronchiole (*TB*) and alveolar duct (*AD*) in this partially inflated lung shows no evidence of inflammatory cell infiltrate in **a** and a normal well-ciliated bronchiolar epithelium in **b**. This lung resembles the untreated control lung seen in Figure 4. Sections stained with methylene blue and Azure II. (**a**, ×125, bar = 80 μ; **b**, ×500, bar = 20 μ) **Figure 4a and b**—These light micrographs show the terminal bronchiole (*TB*) and alveolar duct (*AD*) from a partially inflated lung of an untreated control hamster. **a**—Low-magnification micrograph showing the absence of any inflammatory cell infiltrate. **b**—Normal well-ciliated bronchiolar epithelium. Sections stained with methylene blue and Azure II. (**a** ×125, bar = 80 μ; **b**, ×500, bar = 20 μ)

the light-microscopic observations (Table 1). The inflammatory reaction was found only in specimens from the NO_2-exposed groups (Figure 5). Sections of peribronchiolar (Figure 5) and alveolar duct alveoli from the 30 ppm group showed many interstitial neutrophils. The lungs from hamsters exposed to 7 ppm had few if any interstitial neutrophils (Figure 5). The infiltrative cells were also observed within the alveolar spaces and in the mucus and epithelial layer of the terminal bronchioles, particularly, in sites adjacent to alveolar ducts. The inflammatory cell infiltrate was absent in the lungs of NO_2 exposed hamsters pretreated with taurine (Figure 6). The bronchiolar epithelium, most disturbed by the NO_2 exposure, showed loss, breakage, and disorganization of cilia. Clara cells had a flattened apical surface with few if any secretory granules (Figure 5). Intracellular organelles did not appear changed. The bronchiolar epithelium from lungs of hamsters pretreated with taurine and exposed to NO_2 had none of the alterations described for NO_2-treated hamster lungs (Figure 6). They resembled specimens from untreated control hamsters (Figure 6). Lung specimens from the NO_2 and taurine-treated hamsters showed tall ciliated and Clara cells. The cilia were plentiful, with a long and slender shape, and Clara cells had a protruding cytoplasm filled with many secretory granules and smooth endoplasmic reticulum.

The bronchiolar epithelium from hamsters treated with taurine alone could not be differentiated from that of the untreated control hamster lungs.

Freeze-Fracture Analysis

Freeze-fracture of tight-junctional regions from hamsters exposed to either 7 or 30 ppm NO_2 exhibited disruption of the number, depth, and continuity of fibrils, 30 ppm being more severe (Figure 7). The number of fibrils and depth were reduced, and each fibril was composed primarily of individual particles and short linear aggregates from replicas of bronchiolar epithelium of hamsters exposed to 30 ppm NO_2 (Figure 7). Figure 8 and Table 2 summarize the results of morphometric

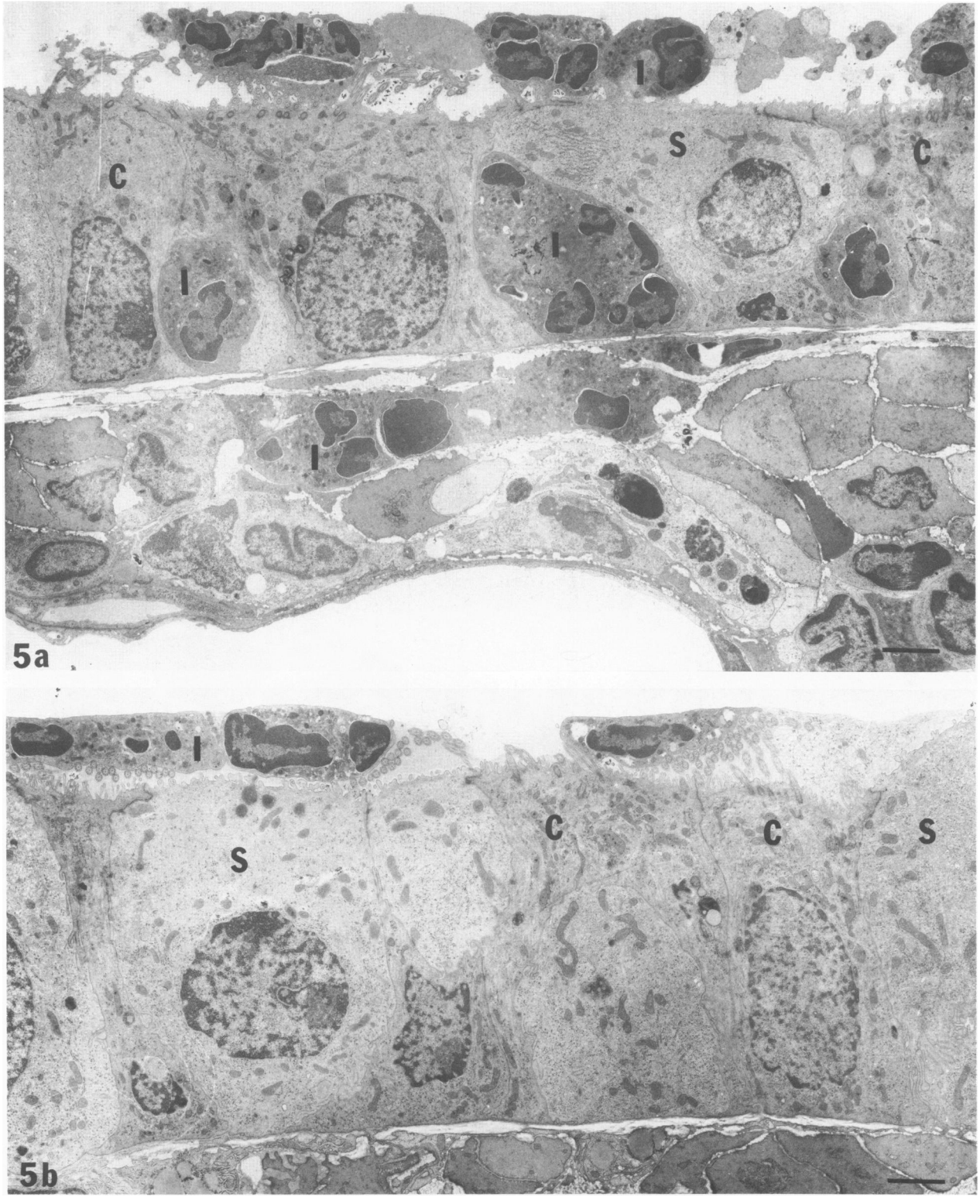


Figure 5a and b—Transmission electron micrographs of thin sections of bronchiolar epithelium from hamster lungs exposed to 30 ppm NO_2 (**a**) and 7 ppm NO_2 (**b**) for 24 hours before perfusion fixation. The epithelium from both exposures exhibits a flattened appearance apparently due to breakage and reduction of cilia on ciliated cells (*C*) and the absence of apical protrusions and paucity of granules in Clara cells (*S*). There are many neutrophils (*I*) in the bronchiolar interstitium, within the epithelium, and in the mucous layer from hamsters exposed to 30 ppm NO_2. Bronchioles from hamsters treated with 7 ppm exhibit only a mild inflammatory response with only a few neutrophils in the mucous layer. Sections stained with uranyl acetate and lead citrate. (**a**, ×3700, bar = 2.7 μ; **b**, ×4700, bar = 2.1 μ)

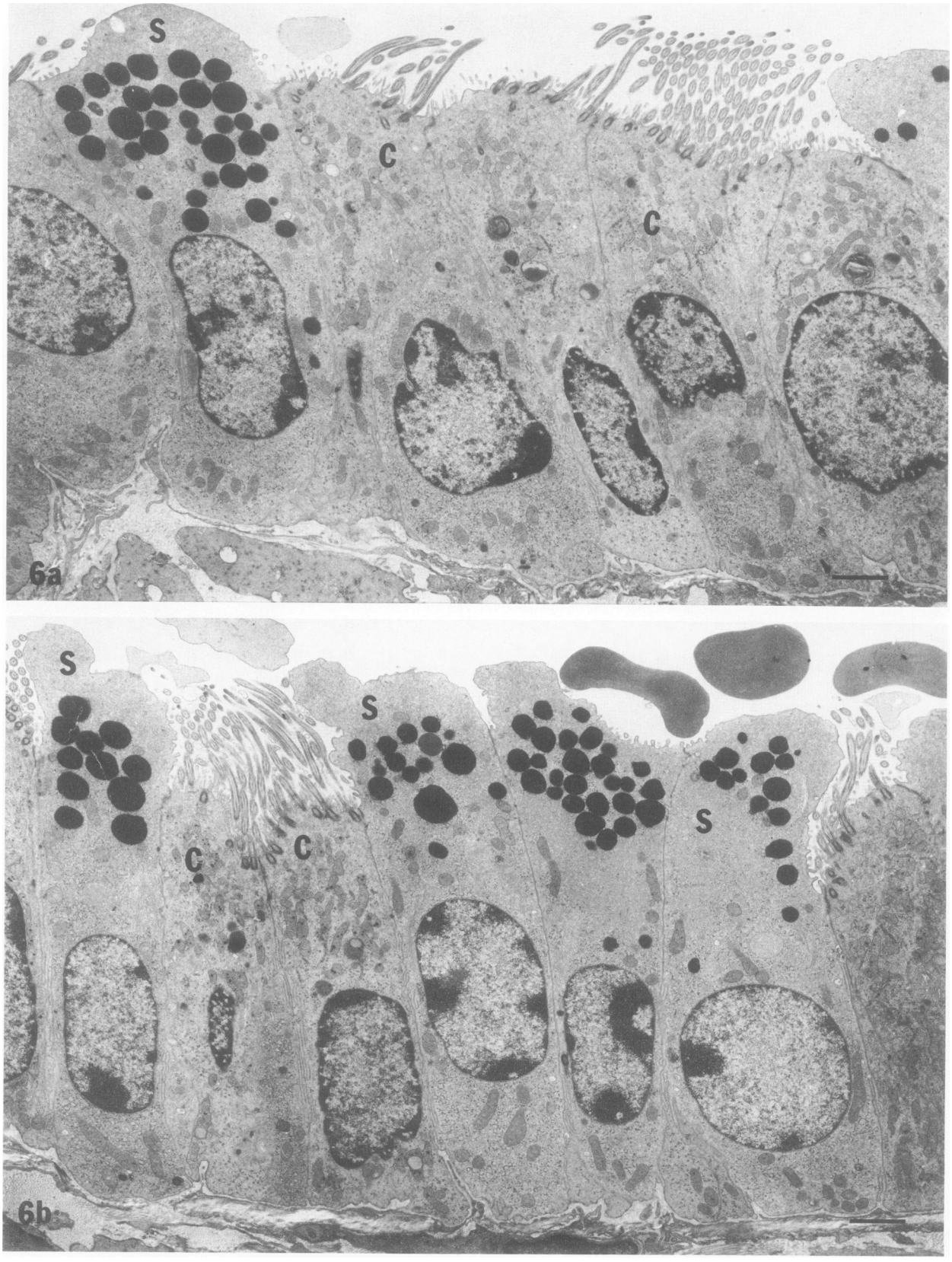


Figure 6a—Electron micrograph of a section of a bronchiole from the lung of a hamster treated with taurine and then exposed to 30 ppm NO_2 for 24 hours. This section shows well-ciliated cells (*C*) and Clara cells (*S*) with apical protrusions and many electron-dense granules. There is no evidence of inflammatory cell infiltrate. The bronchioles from these animals were comparable to those of the normal untreated controls seen in **b**. **b**—Classic appearnce of ciliated cells (*C*) and Clara cells (*S*) from untreated control hamsters. Sections stained with uranyl acetate and lead citrate. (**a**, ×3700, bar = 2.7 μ; **b**, ×5100, bar = 2.0 μ)

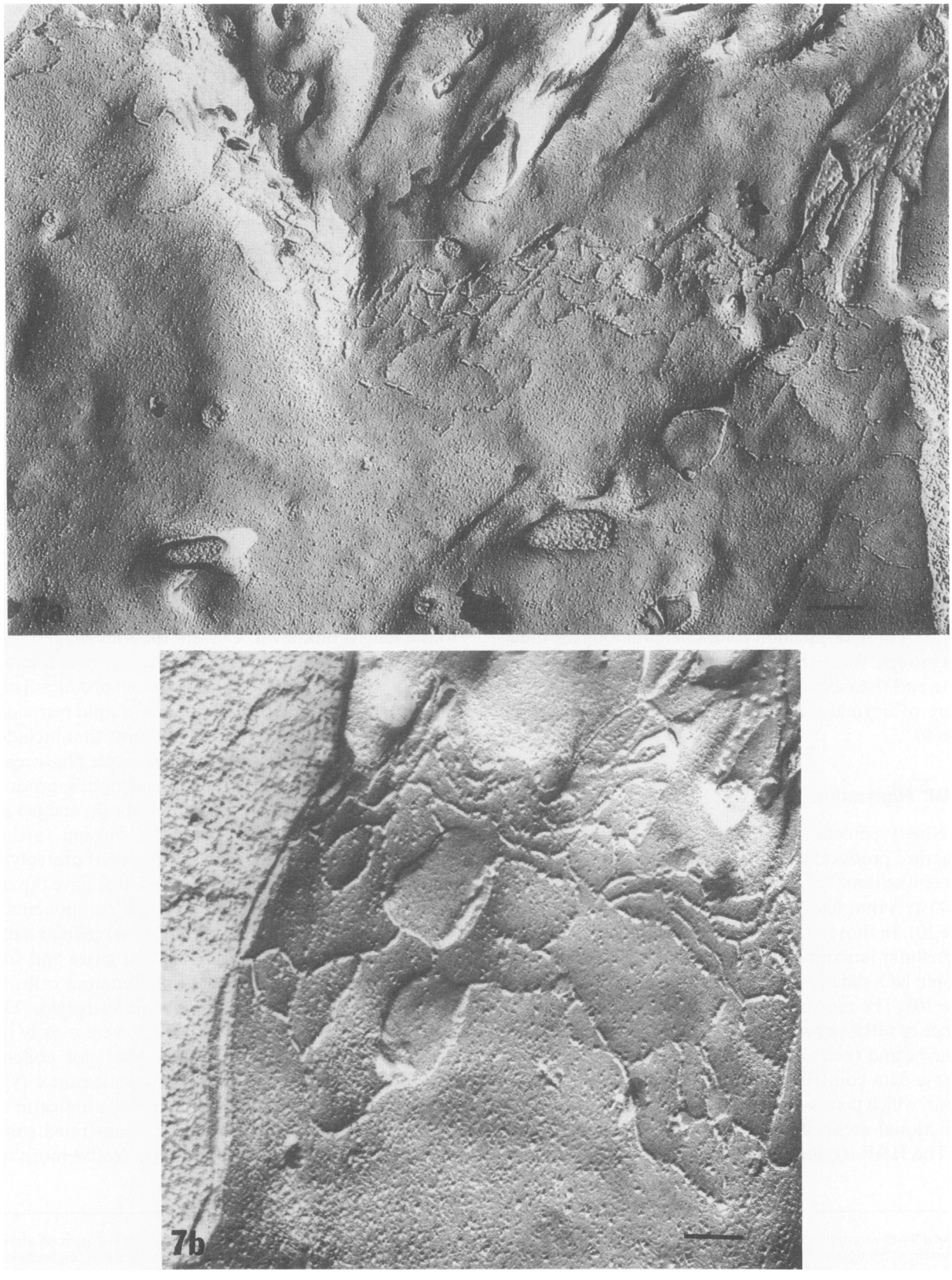


Figure 7a and b—Electron micrographs of platinum replicas of freeze-fractured bronchiolar epithelium from an adjacent area to that seen in Figure 5. The tight-junctional region from the hamster exposed to 30 ppm NO_2 (**a**) shows extensive reduction in fibril number and continuity. Most fibrils are fragmented and no longer run parallel to the lumen surface. **b**—Tight-junctional region of a bronchiolar epithelial cell from a hamster exposed to 7 ppm NO_2. The fibrils exhibit a loss of the normal parallel fibril organization and a significant degree of fragmentation, but not as extensive as in the hamster exposed to 30 ppm NO_2. (**a**, ×97,500, bar = 0.1 μ; **b**, ×97,500, bar = 0.1 μ)

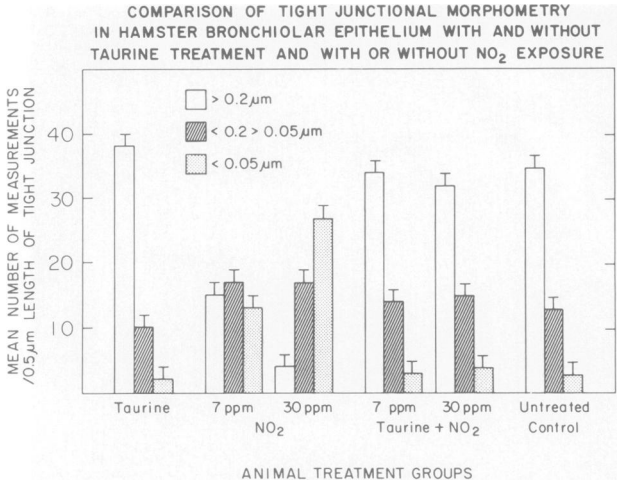


Figure 8—The normal tight-junctional complex consists primarily of many long uninterrupted fibrils. The composition of the tight-junctional complex during NO_2 exposure shows a significant increase in the number of very short fibrils and a nearly proportional decrease in long fibrils, indicating a great deal of fibril fragmentation. It is also obvious that the animals treated with the higher concentration of NO_2 exhibited a greater degree of fragmentation. The bronchiolar tight junctions from animals pretreated with taurine and exposed to NO_2 at the high or low concentrations are not significantly different from those of untreated controls. The tight junctions from animals treated with taurine alone demonstrated a slightly significant increase in fibril tight-junctional fibril length.

analysis of tight-junction components among the different groups. When the hamsters were pretreated with taurine and then exposed to NO_2, tight-junctional fibrils were of normal number, depth, and continuity (Figure 9).

HRP Penetration

Ninety percent of the bronchioles contained HRP reaction product in their lumens. The only bronchiolar epithelium that showed HRP penetration was from hamsters that had been exposed to 30 ppm NO_2 (Figure 10). In those bronchioles examined, 75% of the intercellular junctions were penetrated. Those exposed to 7 ppm NO_2 did not show any epithelial penetration (Figure 10). The most important observation was the absence of HRP penetration in specimens pretreated with taurine and then exposed to 30 ppm NO_2 (Figure 10). These data coincide with the freeze-fracture observations, which demonstrated preservation of normal tight-junctional architecture.

The HRP section of the study, although not quantitative, does contribute significantly, in that it allows us to correlate physiologic function of the epithelial barrier with the morphologic freeze-fracture ultrastructure of tight junctions. The data clearly demonstrate that at the higher NO_2 exposure levels taurine prevented the penetration of HRP (60,000 mol wt) between adjacent bronchiolar cells, corresponding to the maintenance of normal tight-junctional morphologic characteristics.

Table 2—General Morphologic Characteristics of Tight Junctions of Hamster Bronchioles Among Treatment Groups

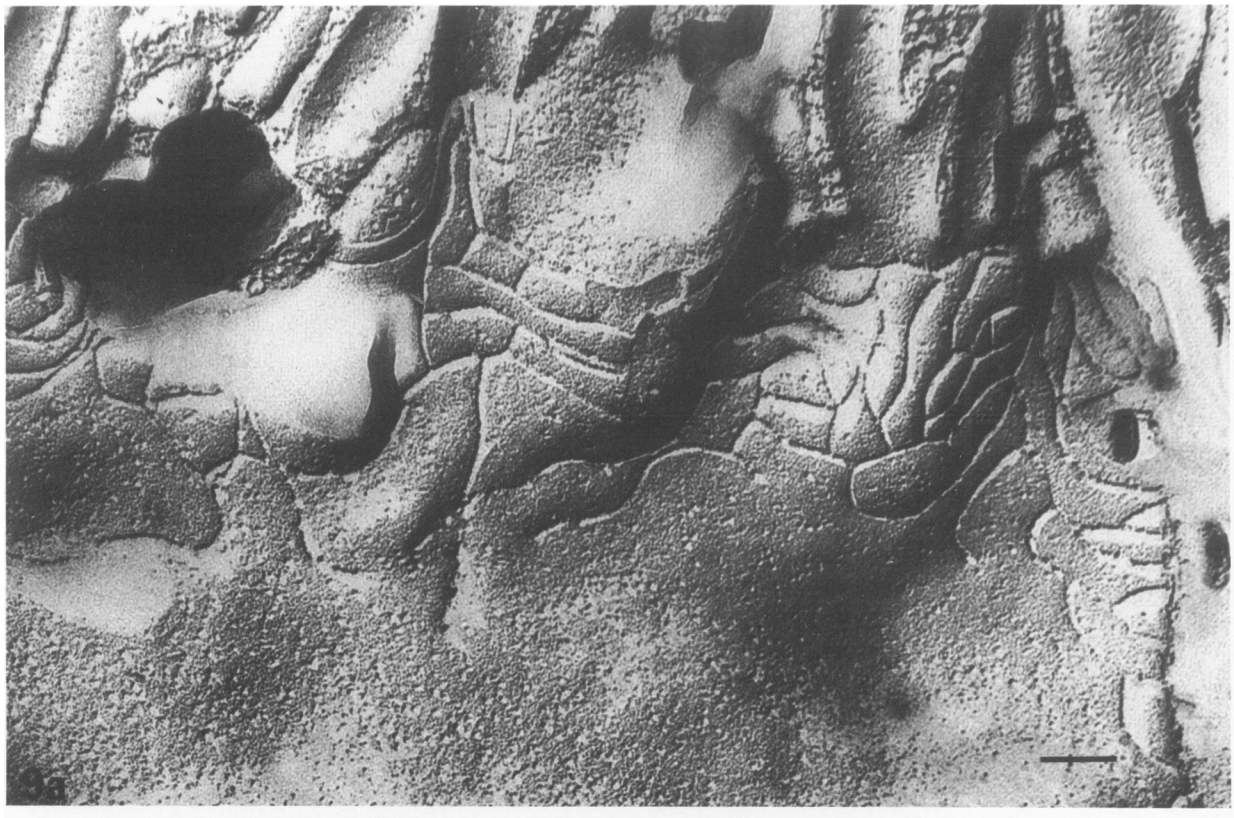
Treatment group	Number of fibrils		Mean depth of fibrils (μ)
	Range	Mean ± SE	
Control (untreated)	4–10	6.5 ± 0.1	0.40
Taurine	5–11	7.1 ± 0.2	0.50
NO_2			
7 ppm	2–6	4.2 ± 0.4	0.30
30 ppm	2–4	2.9 ± 0.1	0.20
Taurine + NO_2			
7 ppm	4–10	6.5 ± 0.1	0.40
30 ppm	4–10	6.2 ± 0.2	0.40

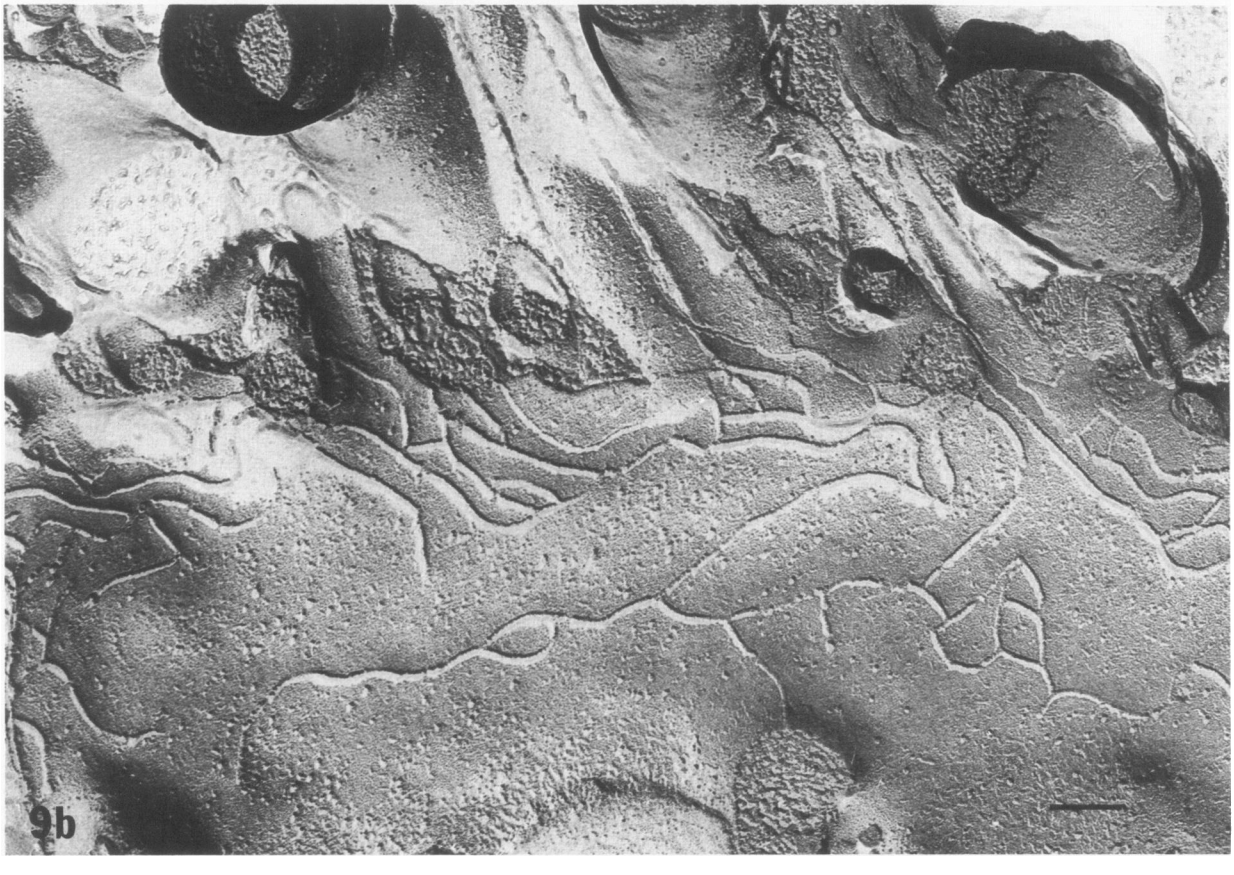
Discussion

The precise molecular mechanism(s) of NO_2 injury to the lung is unknown. Current theories suggest that the NO_2 and its highly reactive oxidant species interact directly with plasma membranes or cell products (surfactant), possibly via the mechanism of lipid peroxidation,[41-43] triggering a sequence of events that includes the release of chemotactic factors and acute phase reactants responsible for the influx of neutrophils; production and release of superoxides, free radicals, and peroxide from these inflammatory cells, causing further epithelial damage; and finally the release of proteolytic enzymes from the scavenging cells, which have the capacity for altering alveolar interstitial components.

In an attempt to short circuit the initial cellular damage to the lung epithelium by oxidant gases and free radicals released from resident inflammatory cells, α-tocoferol and selenium have been investigated. The results of these investigations, which were mostly indirect biochemical measurements were not conclusive.[25-27] The single morphologic study measured Type II pneumocyte proliferation as an indirect indicator of Type I pneumocyte injury[27] and demonstrated some protective capabilities. The overall protective morpho-

Figure 9—Electron micrographs of a platinum replica of freeze-fractured bronchiolar epithelium from adjacent regions described in Figure 6A shows a section of the tight-junctional region of bronchiolar epithelium from a hamster given taurine and NO_2. The fibrils are of normal number, organization, and continuity, as compared with **b**, of a region of tight junction from the bronchiolar epithelium of a normal untreated control. (**a**, ×95,000, bar = 0.11 μ; **b**, ×70,000, bar = 0.14 μ)

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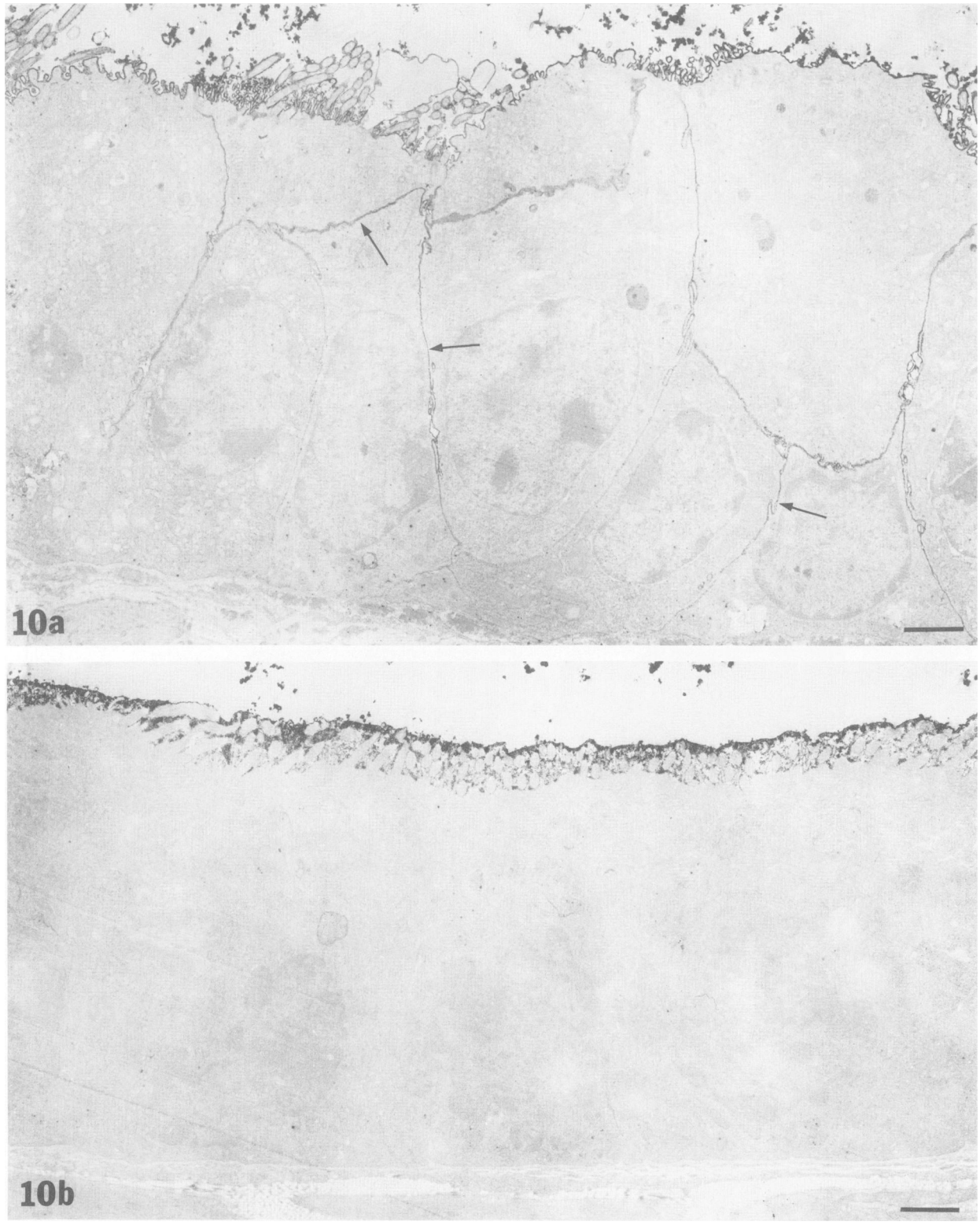
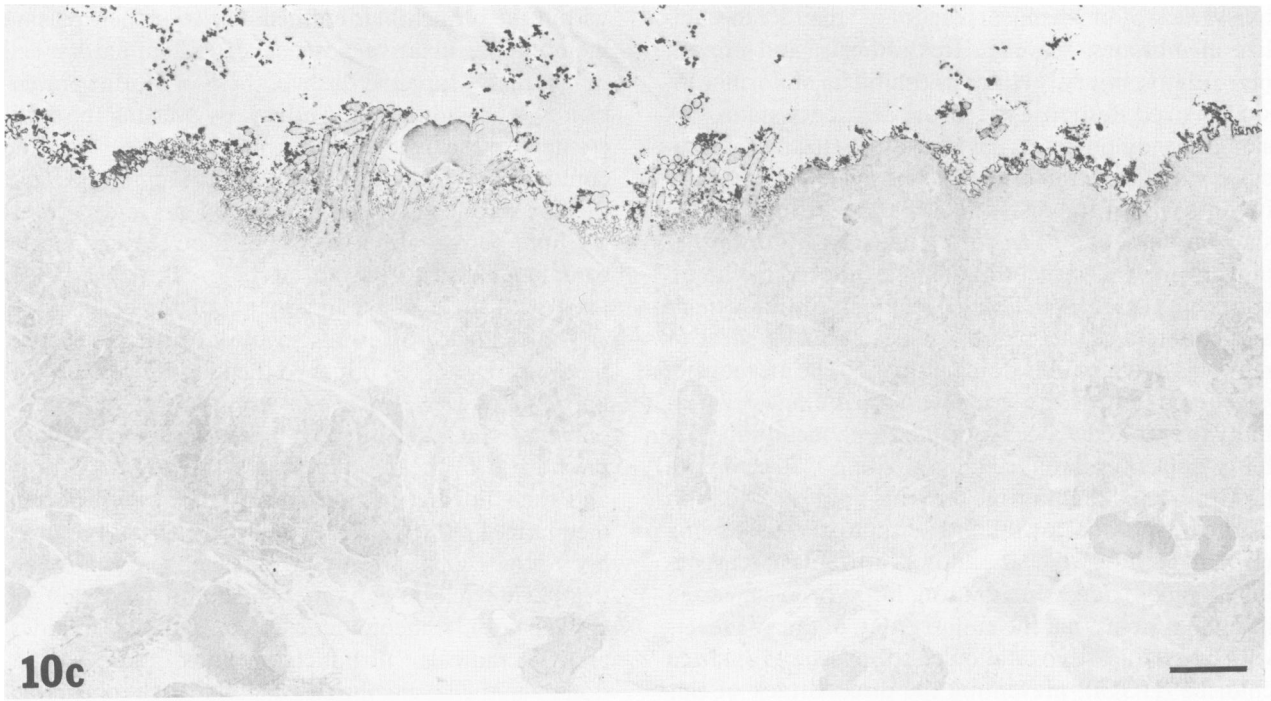
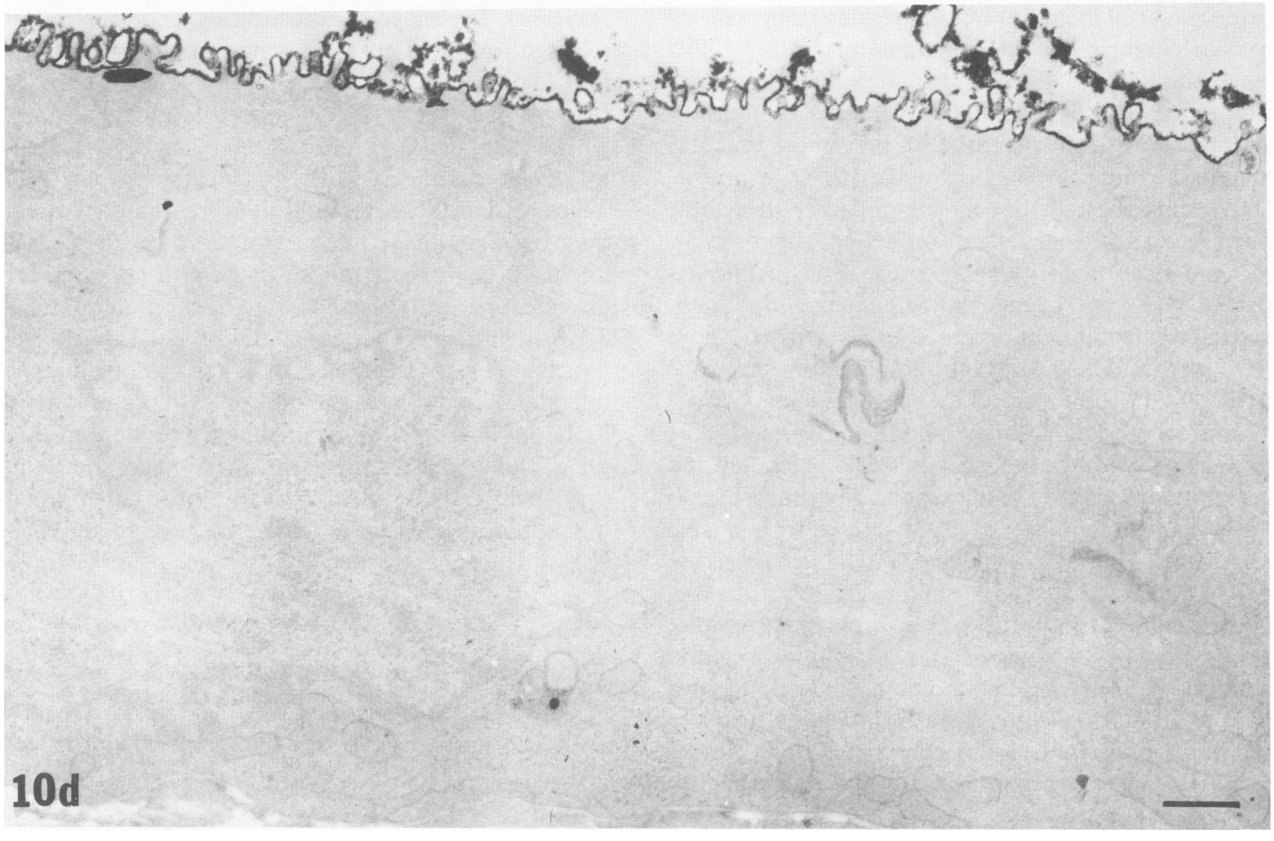


Figure 10—Electron micrographs of thin sections of bronchiolar epithelium 30 minutes after tracheal instillation of HPR. **a**—Intercellular penetration (*arrows*) of the HRP in a bronchiole from a hamster exposed to 30 ppm for 24 hours. **b**—Absence of penetration of the bronchiolar epithelium from a hamster exposed to 7 ppm NO_2. **c**—There is no intercellular penetration in the bronchiolar epithelium from a hamster pretreated with taurine and then exposed to 30 ppm NO_2 for 24 hours. **d**—Section treated with HRP from an untreated control hamster. No penetration is observed. Sections viewed and photographed unstained with uranyl acetate and lead citrate. (**a**, ×6000, bar = 1.67 μ; **b**, ×6600, bar = 1.53 μ; **c**, × 3700, bar = 2.7 μ; **d**, ×12,000, bar = 0.83 μ)





logic effects of these antioxidants on lung tissue remain unclear. In addition, plasma membranes, implicated as the initial cellular site of oxidant damage to epithelium and inflammatory cells, have not been explored except superficially for alterations in ionic surface charge of epithelial cells.[14,44]

Recent reports have indicated that taurine, by its distribution through mammalian tissues and participation

in a variety of biochemical reactions,[28] may act to stabilize membranes, scavenge free radicals, and prevent peroxidative injury.[29] Humans exhibit an abnormal Visual Evoked Response in taurine deficiency states.[45] A similar condition in cats has been attributed mechanistically with disruption of the plasma membranes, resulting in death of the disk cells. The process may be the same in humans.[46,47] *In vitro* studies have shown that taurine prevents structural damage induced by illumination and oxidants in isolated segments of frog retina[48] and counteracts the membrane-destabilizing effect of retinoids in lymphoblastoid cultures.[49] The mechanism is believed to involve a decrease in membrane permeability to ions and water, associated with membrane stability, thus preventing cellular swelling.[49] Recent data have indicated that taurine prevents lipid peroxidation of the membranes of rabbit spermatozoa, preserving normal motility, which the authors attribute to scavenging of superoxide.[32] In addition, it has been suggested in some systems that the amino group of taurine reacts with by-products of oxidation reactions such as oxidized chlorine (HOCl), preventing the direct attack of this oxidant on cell membranes, suppressing or preventing lysis of parenchymal and inflammatory cells.[31,41,50,51]

On the basis of these and many other supportive studies, we hypothesized that pretreatment of hamsters with taurine had the potential for preventing injury to lung tissue induced by the sequelae of NO_2 exposure. Our results showed that morphologically detectable damage to epithelial cells in bronchioles and the associated inflammatory response were absent in taurine-treated hamsters. The morphologic changes in the non-taurine-treated animals exposed to 30 ppm or 7 ppm NO_2 were similar to those described in previous studies.[12,20,21] These changes included inflammatory cell infiltrates, marked disorganization and breakage of cilia, secretory cell flattening with surface blebbing and depletion of secretory granules, and disruption of epithelial tight junctions. The group treated with taurine whether alone or followed by NO_2, exhibited preservation of normal ciliated and secretory cell structure. The preservation of tight junctions of bronchiolar epithelium in the taurine-treated NO_2-exposed group clearly indicated a protective membrane effect. In many instances, the taurine-treated group exhibited even greater continuity and regularity of fibril numbers in freeze-fracture replicas than did the unexposed untreated control group.

The ultrastructural evidence presented indicates that taurine is capable of preventing acute NO_2-induced morphologic damage. The data strongly support the hypothesis that taurine prevents the earliest events in the process of NO_2-induced injury. The basis of these conclusions is the absence of any ultrastructural changes within the bronchiolar epithelial cells or their plasma membranes. Relative absence of inflammatory cell influx further supports the hypothesis of taurine prevention of NO_2-induced lung injury. By averting the earliest steps in the injury process, mechanistically taurine could be intercalating into plasma membranes and thereby stabilizing the lipid components and preventing lipid peroxidation. It may also be scavenging the oxidant gas and its free radicals before these agents can react with molecular components of the cell.

The sequence of events involved in the molecular mechanisms of NO_2 injury to the lung is relatively unknown. However, evidence exists to support the hypothesis that the NO_2 directly injures lung epithelium, presumably by the free radicals and superoxides it forms and their interaction, via peroxidation, with plasma membrane lipids.[41,52,53] The same mechanisms presumably injure resident macrophages, which may initiate activation and release of a variety of enzymes (eg, elastase, collagenase),[54] chemotactic factors,[55,56] superoxides, and free radicals.[57] Products of direct lipid peroxidation of epithelial membranes may also act as chemotactic agents. The enzymes, free radicals, and superoxides released from resident macrophages could potentially induce damage to the epithelium, but such is unlikely because of the relative low concentrations. However, with recruitment and influx of many more inflammatory cells and the potential inactivation of enzymes which neutralize the free radicals and enzymes released, there would be adequate concentrations to cause injury to the epithelium. If the insult were to continue for extended periods, there is significant potential for accessibility[58] and damage to the underlying connective tissues. Although speculative, it is possible that the interaction and binding of taurine with plasma membranes of lung epithelium and normal resident macrophages may protect against the initial stages of cell injury from NO_2 or other oxidant gases and prevent inflammatory cell recruitment and activation and their consequences.

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