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Ultrastructure of acute ammonia toxicity in the human lung*

ABSTRACT A tanker truck carrying anhydrous ammonia (NH₃) fell off a freeway, releasing a dense cloud of NH₃ gas, killing several people. The driver was dead upon impact. To our knowledge, pulmonary NH₃ toxicity in humans has not been studied previously by electron microscopy (EM). Therefore, in two cases, the paraffin-embedded tissue blocks of lung were deparaffinized and reembedded in plastic for 1- μ sections and EM examination. The lung tissue of a third case, the truck driver, was similarly processed as a control. Light-microscopic pulmonary findings in the acute NH₃ deaths included denudation of the tracheobronchial epithelium, edema of the lamina propria, and marked alveolar edema, congestion, and hemorrhage. In contrast, in the truck driver's lungs, the bronchial epithelium was intact, and there was no gross odor of NH₃. Massive pulmonary hemorrhages in his lungs were attributed to trauma rather than NH₃ inhalation. EM examination of the lungs of the truck driver showed no discernible toxic alterations in either the capillary endothelial cells or the Type I or II alveolar epithelial cells, and alveolar and capillary basement membranes were intact. In contrast, EM study of the lungs from two individuals dying acutely of NH₃ inhalation showed marked swelling and imbibitional edema of Type I alveolar epithelial cells; however, alveolar basement membranes and capillary endothelial cells appeared as usual. These electron-microscopic findings demonstrate the Type I epithelial cell to be the target cell of acute alveolar wall injury in NH₃ inhalation.

INTRODUCTION

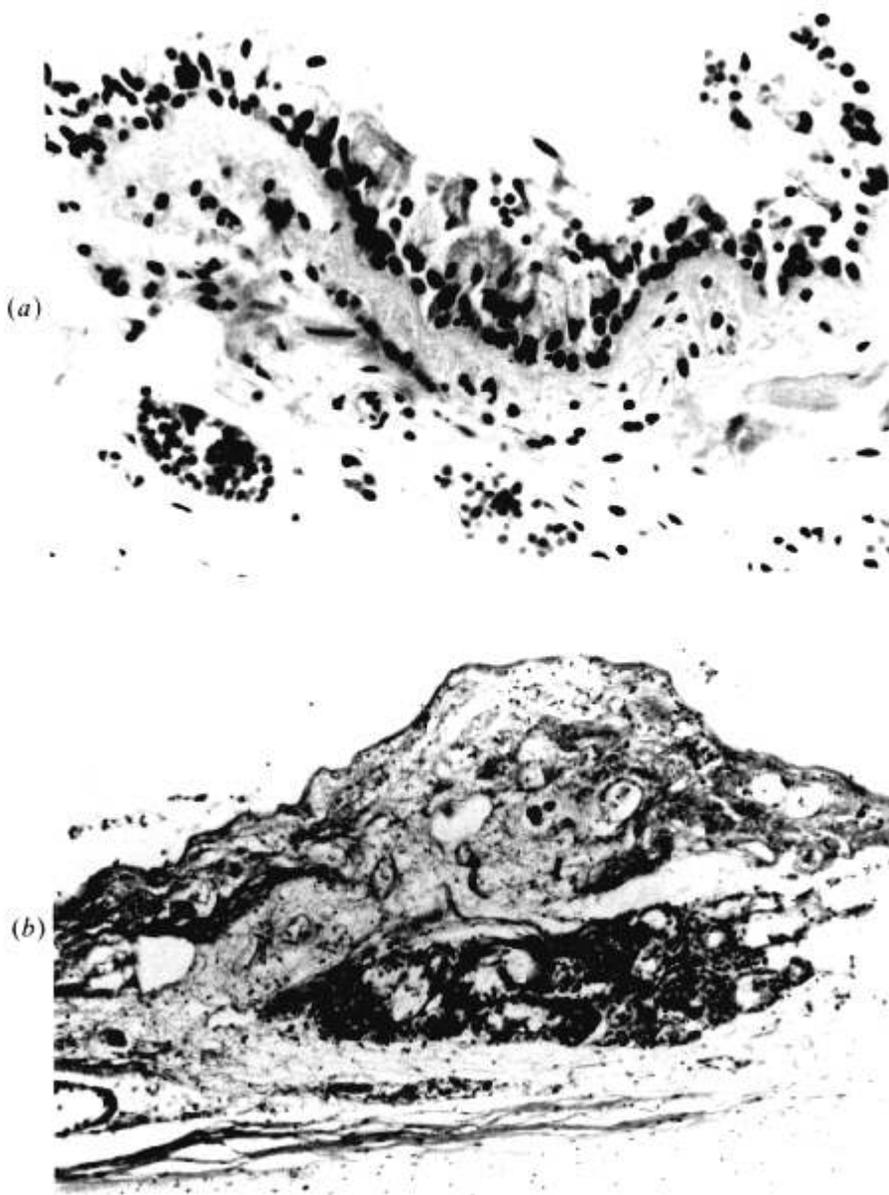
Several case studies and experimental studies of ammonia (NH₃) toxicity have documented the light-microscopic features of NH₃ damage in lungs.⁽¹⁻⁴⁾ However, to our knowledge, no description of ultrastructural findings in acute NH₃ toxicity in humans has been reported. A major traffic accident in Houston, Texas, provided an opportunity for ultrastructural study of acute pulmonary NH₃ toxicity, when a tanker truck carrying 8,000 gallons of anhydrous NH₃ fell off a freeway overpass, exploded and released a dense cloud of NH₃ gas. Six persons died. One hundred fifty people received emergency care, 13 requiring intubation. Sixty were hospitalized. Clinical findings in these patients have been described previously.^(5,6) In cooperation with the Harris County Medical Examiner's Office, autopsy findings were reviewed and samples of lung tissue were reprocessed for electron-microscopic (EM) study.

MATERIALS AND METHODS

Four individuals died at the scene of the accident. The ammonia truck driver, a 28-year-old man, and three other white males, 34-48 years old, were found dead within a few hundred yards of the explosion. A fifth person, a 33-year-old white female, died a few hours later at a local hospital, with a clinical diagnosis of fatal NH₃ inhalation. No autopsy was obtained. A sixth victim of the accident, a 26-year-old black man, died 2½ weeks later, after hospitalization with tracheostomy and mechanical respirator support. The four persons dead at the scene,

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FIGURES 1a-1d

(a) Lung tissue from a 28-year-old truck driver dead of trauma. Bronchial mucosa is intact. (b) Denuded bronchial mucosa in an ammonia victim. (c) Lung tissue from one of the three men dead at the scene after acute ammonia inhalation. Parenchyma is edematous and congested. (d) Lung tissue showing hyaline membranes and acute and chronic inflammation in the 26-year-old man dying 2½ weeks after the accident. Note: a-d are light-microscopic findings.

and the man dying after 2½ weeks hospitalization were autopsied by the Harris County Medical Examiner.

Medical examiner records of gross anatomic findings in the five autopsy cases were reviewed, together with an examination of the routinely processed H&E slides. Paraffin-embedded tissues were available in four of the five autopsy cases (the truck

driver, two of the three white males dead at the scene, and the delayed death). Tissues were reprocessed for electron microscopy by selecting areas of paraffin-embedded lung tissue (using the H&E slides for reference), cutting this tissue from the block, deparaffinizing it overnight in xylene, hydrating through a series of graded ethanols to water, fixing in glutaraldehyde (3%), postfixing in osmium

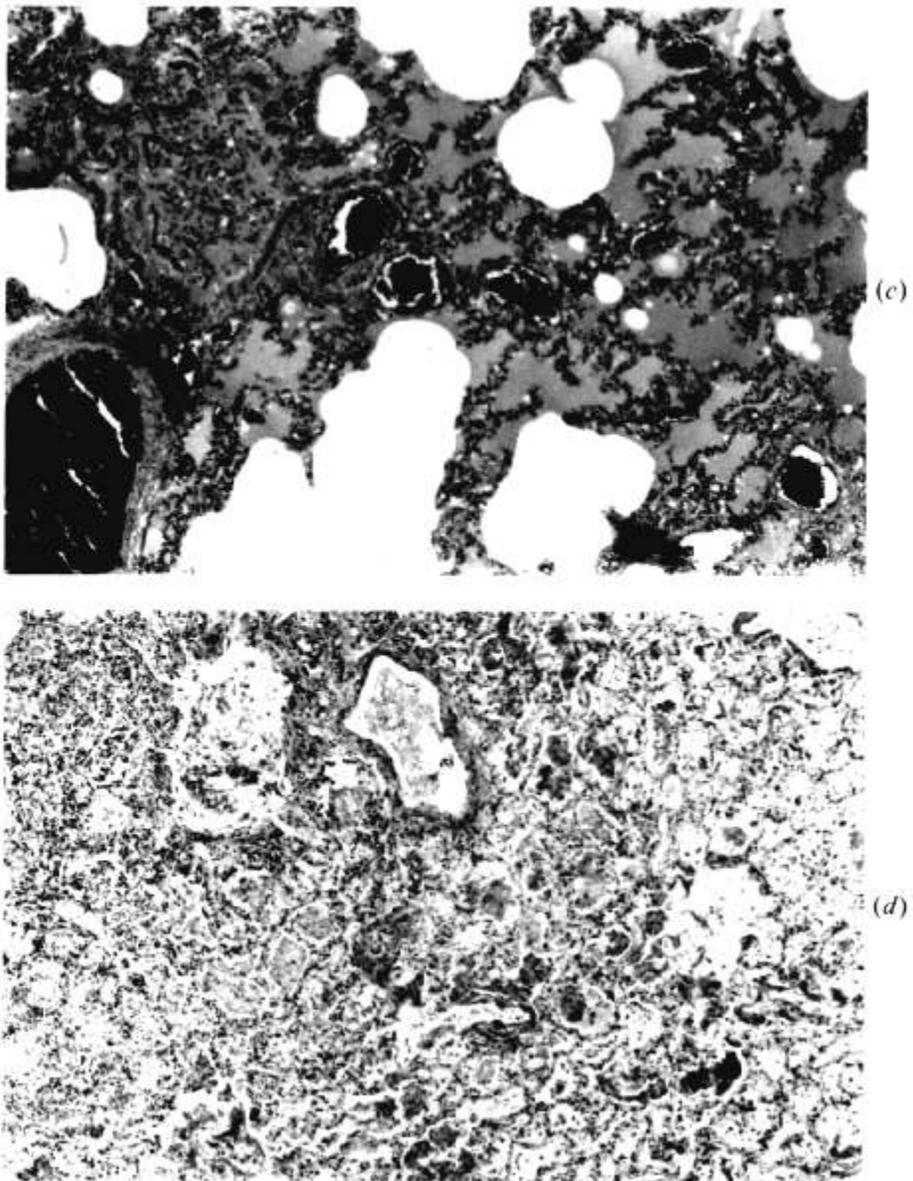


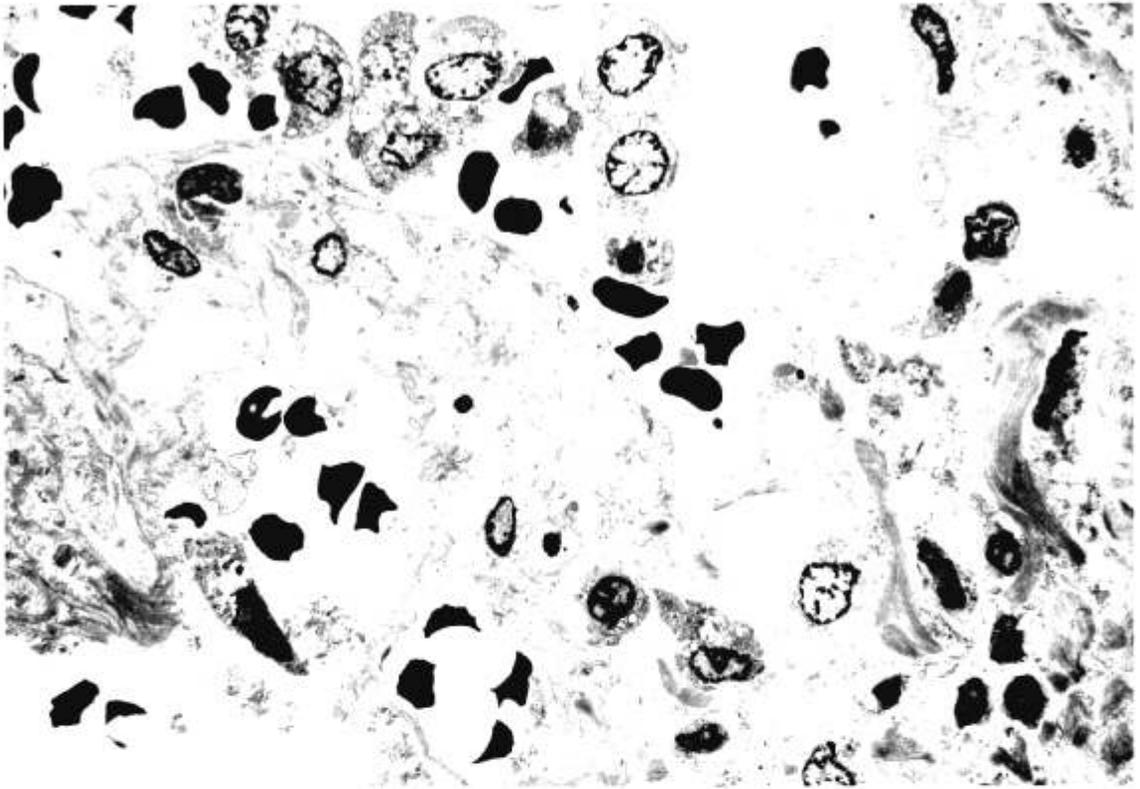
FIGURE 1 (Continued)

tetroxide, dehydrating in ethanol, and embedding in Spurr's plastic. Thin sections were examined with a JEOL 100 C transmission electron microscope.

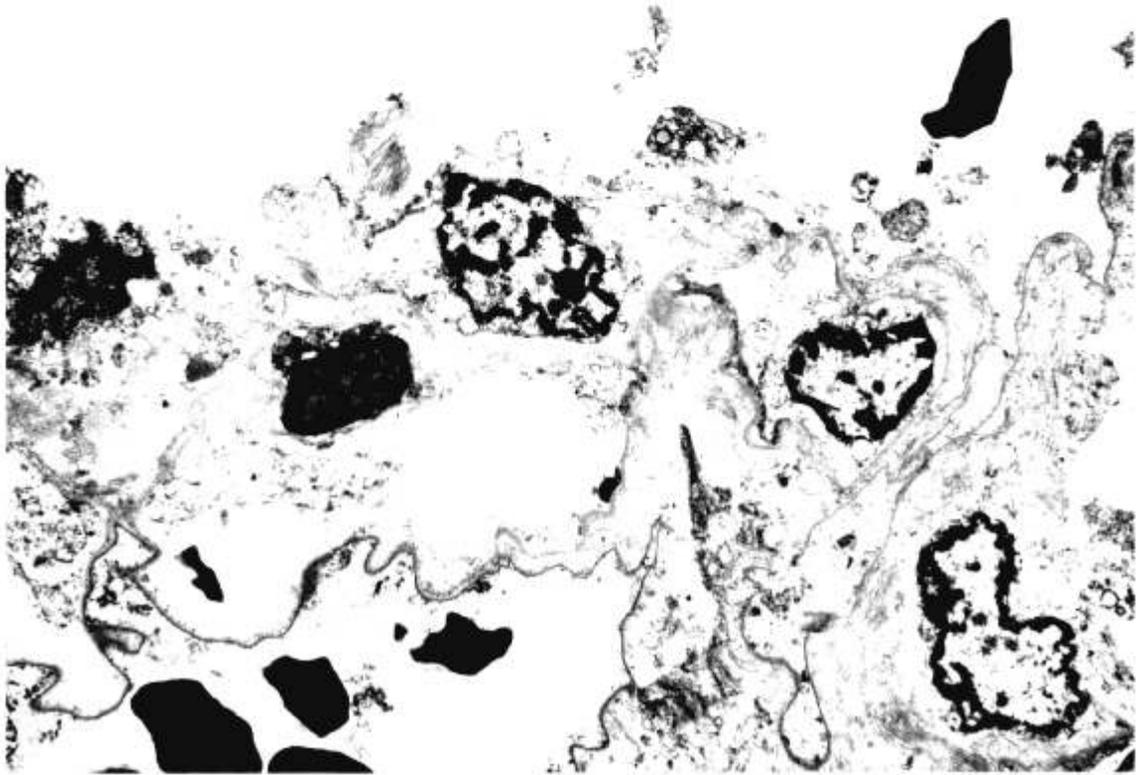
PATHOLOGIC FINDINGS

The driver of the truck died of multiple traumatic injuries received as the truck impacted the guardrail, fell approximately 100 ft. to the freeway below, and exploded. External surfaces revealed a strong NH_3 odor, but no such odor was found upon examination of internal organs, and the tracheobronchial lining

was preserved (Fig. 1a). Pulmonary parenchyma showed patchy atelectasis and hemorrhage. Autopsy examination of the other three white males dead at the scene revealed several features in common. There were second-degree chemical burns over exposed skin areas. Both external surfaces and internal viscera smelled strongly of NH_3 . The tracheobronchial mucosal surfaces were hyperemic and covered with large amounts of frothy red-gray fluid. In one case, hyperemic and edematous vocal cords almost occluded the larynx. In all cases, the lungs smelled strongly of ammonia. The parenchyma was markedly edematous and congested, with combined



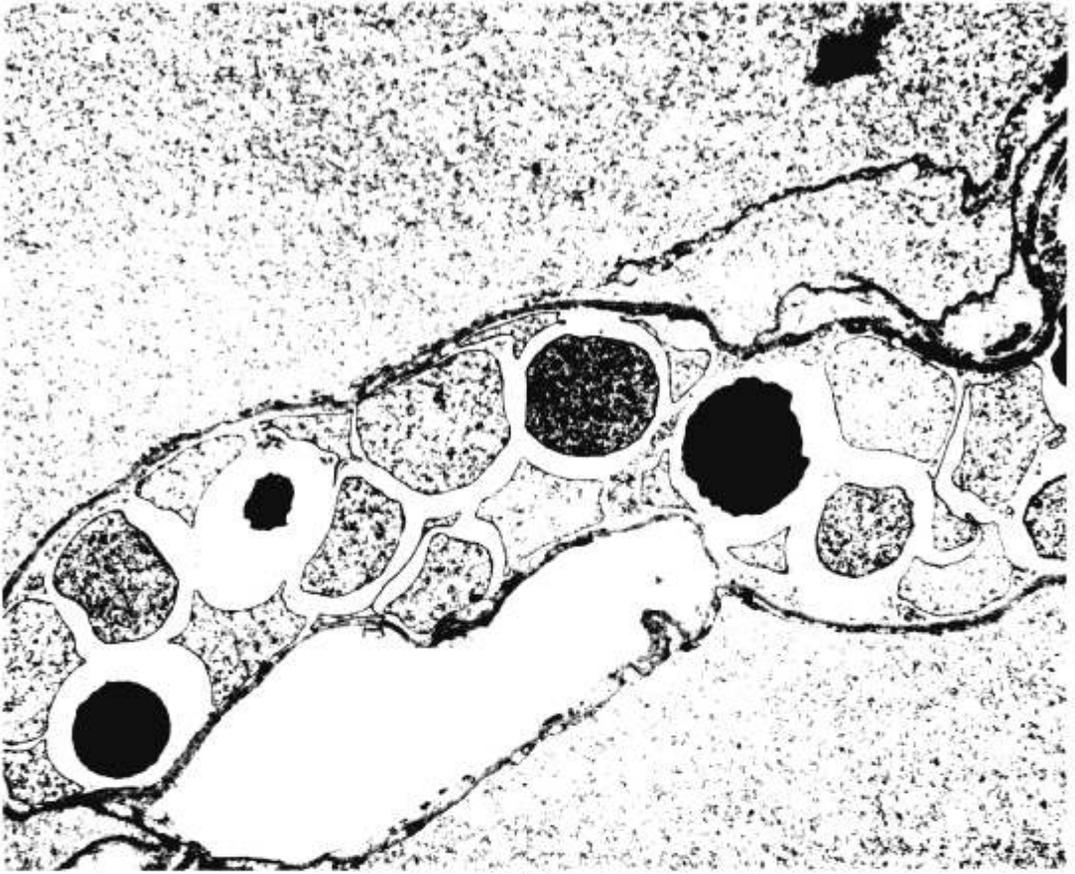
(a)



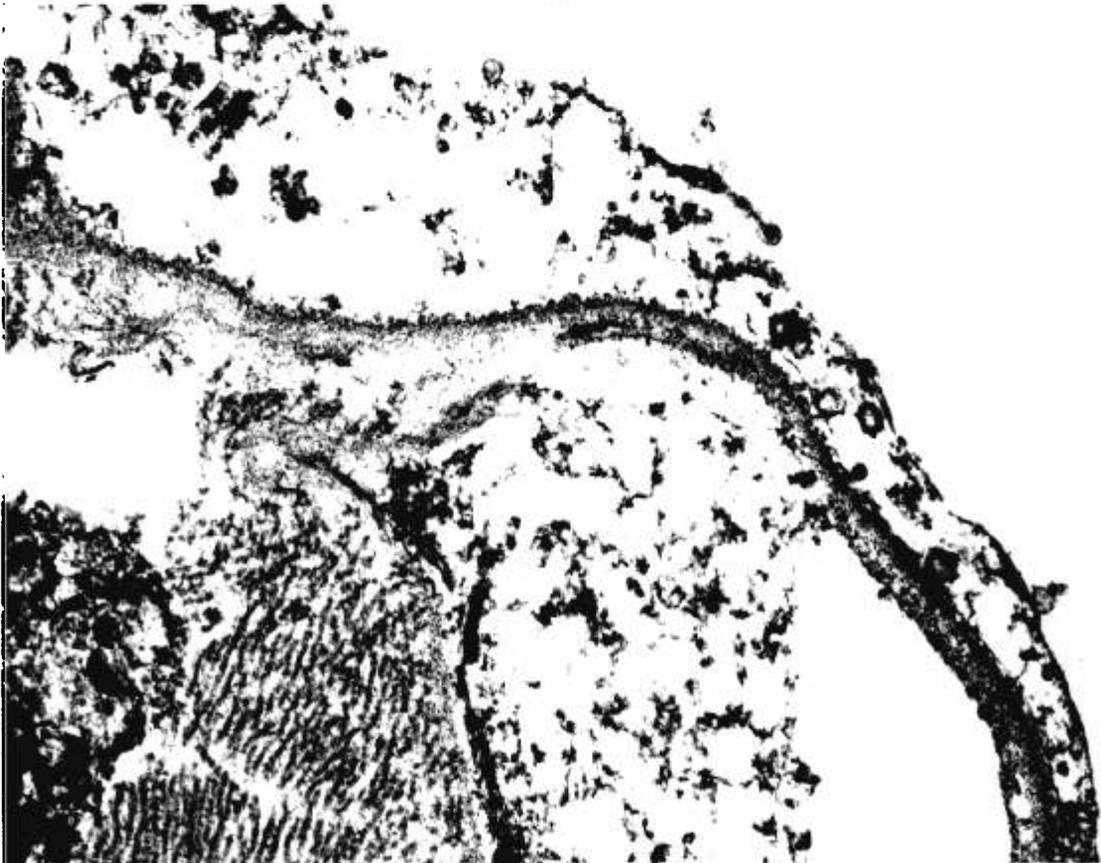
(b)

FIGURES 2a-2d

(a, b) Lung tissue from a 28-year-old truck driver, showing good preservation and thin unremarkable Type I epithelial cells lining the alveoli. (a: original magnification $\times 2600$; b: original magnification $\times 6600$.) (c, d) Lung tissue from two of the three acute inhalational deaths. Alveolar Type I epithelial lining cells are prominent and swollen. Capillary endothelial cells, by contrast are inconspicuous and not swollen, and basement membranes are intact. Edema fluid is also present within the alveolus seen in the lower power. (c: $\times 2000$; d: $\times 8300$.) Note: a-d are electron-microscopic findings.



(c)



(d)

FIGURE 2 (Continued)

lung weights in excess of 1,000 g. On light microscopy (Figs. 1*b* and 1*c*), the tracheobronchial epithelium was sloughed, and extensive edema and congestion were found in the lung parenchyma. The findings in these three patients were consistent with death due to acute NH₃ inhalation. The fifth autopsy of the 26-year-old black man showed healing chemical burns of his various skin surfaces. The acute effects of NH₃ inhalation (edema, congestion, and hemorrhage) were masked by extensive superimposed hyaline membrane formation, autolysis, and areas of acute and chronic inflammation (Fig. 1*d*). His death 2½ weeks after the accident was due to bronchopneumonia and necrotizing tracheobronchitis, secondary to the NH₃ inhalation.

Ultrastructural study of the lungs of the truck driver showed no discernible toxic alterations in either the capillary endothelial cells or the Type I and II alveolar epithelial cells (Figs. 2*a* and 2*b*). Alveolar and capillary basement membranes were intact. In contrast, electron-microscopic examinations of the lung tissue from two of the acute inhalational deaths showed marked swelling and imbibitional edema of the Type I alveolar cells (Figs. 2*c* and 2*d*). Alveolar and capillary basement membranes were intact, and capillary endothelial cells revealed no change. Electron microscopy of the lung in the 26-year-old man dying 2½ weeks after the accident showed hyaline membranes and severe autolysis. Against this background, toxic alterations due to NH₃ could not be discerned.

DISCUSSION

Ammonia is a chemical compound of wide commercial application. It ranked third among the top 50 chemicals produced in the United States in 1972, when production approached 15 million tons at approximately 100 different plants. By 1980, production was estimated to have reached 30 million tons. About 85% of commercial ammonia is used in fertilizer. Ammonia also serves as a coolant in some refrigeration systems and as a volatile alkali in cleaning agents. The National Institute for Occupational Safety and Health (NIOSH), in 1974, estimated that 500,000 United States workers had occupational exposure to ammonia through a wide variety of agricultural and industrial activities.⁽¹⁾

Ammonia is a colorless gas with a pungent odor, high solubility in water, and strong alkali properties. It is ordinarily stored and transported under high pressure as anhydrous liquid ammonia. Most of the accidents involving ammonia have resulted from valve leaks, or tank or pipe explosions with sudden

volatilization of large amounts, forming a dense cloud of highly toxic ammonia gas.⁽¹⁾

The medical hazards of ammonia have been known for over 100 years. Information has accumulated from case reports of accidental ingestions or inhalations, human and animal experiments, and epidemiological studies. The largest series of cases involved 47 people in 1941, in a London air raid shelter where a pipe ruptured releasing NH₃.⁽⁷⁾ Most reports involve only a few individuals. Of 81 cases summarized by NIOSH in 1974, 16 died, nine had clinical evidence of chronic lung disease, and seven had evidence of permanent visual impairment.⁽¹⁾ Toxic effects of NH₃ involved mainly the skin, eyes, and respiratory tract and were produced by direct contact of exposed body surfaces to the gas. Individuals farther away from the released cloud of NH₃ gas suffered less severe consequences than those trapped at the site of initial cloud formation. In only one case of fatal exposure was the air concentration of NH₃ estimated. It was 10,000 ppm; however, the duration of exposure was not given.⁽⁸⁾ Experimental studies have also confirmed the dose and time-dependent nature of NH₃ toxicity.^(1,9) Low level sensitivity of humans to NH₃ odor and irritation is variable, but it appears to average 20–30 ppm, well below the current workplace standard of 50 ppm. At higher concentrations, lacrimation, throat irritation, chest irritation, and cough were observed, with increased respiratory minute volume. Even at 500 ppm exposure levels, BUN and urine ammonia levels remained normal. Higher acute exposures (thousands of ppm) result in immediate severe eye and respiratory tract irritation, with profuse lacrimation, violent coughing, loss of voice, and labored breathing. Animal experiments have shown a LC₅₀ (lethal concentration to 50% of subjects) of approximately 10,000 ppm.⁽¹⁾

The pathology of acute ammonia toxicity has been examined in a number of fatal human cases⁽¹⁻⁵⁾ as well as in laboratory animals.^(1,9) The eyes show corneal edema, with the development of ulcerations and opacities. Second- and third-degree burns can occur on exposed skin areas. Deaths result chiefly from the effects of NH₃ gas on the respiratory tract. Major pathologic light-microscopic changes include edema, congestion, hemorrhage, and liquefactive necrosis. In the larynx, these changes often produce complete glottic closure, necessitating tracheostomy. In the tracheobronchial tree, edema and mucosal sloughing are observed with minimal acute inflammation. The alveolar lung parenchyma typically fills with edema fluid and hemorrhage. Corneal opacities and blindness have been frequent sequelae in survivors, and chronic lung

disease occurs including bronchiectasis, chronic bronchitis, and decreased diffusion capacity. Secondary infection, especially bacterial, is a frequent complication.

The accident of an NH₃ tank truck on a Houston freeway produced one of the largest acute exposures to this chemical on record. Several individuals died and were autopsied. The gross and light-microscopic effects of NH₃ inhalation were consistent with previous reports. The truck driver, dying of traumatic injury, served as our control. Reprocessing of paraffin-embedded tissue for electron microscopy demonstrated adequacy of the technique, with sufficient tissue preservation to permit cautious interpretation. The two acute inhalation deaths were, like the truck driver, autopsied within 24 hours of the accident, and they also showed adequate tissue preservation at the ultrastructural level. The fourth case of the 26-year-old man who died in the hospital was not received and autopsied by the medical examiner until more than 48 hours after death, accounting for the severe autolysis which rendered ultrastructural interpretation impossible. The two acute inhalational deaths stand out ultrastructurally for one prominent feature—the marked edema and swelling of the Type I alveolar epithelial cells. In the truck driver, these cells were flat and appeared as usual. In all three acute cases, alveolar and capillary basement membranes were intact, and capillary endothelial cells were unremarkable. The latter did not show the swelling characteristic of cardiogenic pulmonary edema. These findings suggest that in pulmonary edema of NH₃ toxicity, the initial cell of alveolar injury is the Type I epithelial cell.

The only previous electron-microscopic examination of lungs following NH₃ inhalation was presented by Dr. A. H. Niden at the 11th Aspen Emphysema Conference in 1968.⁽¹⁰⁾ He studied lungs of mice subjected to varying concentrations of ammonia vapors for 3–60 minutes and reported edema of the alveolar epithelium with no changes in the capillary endothelial cells. Alveolar and endothelial basement membranes remained intact. He also described intracapillary thromboses, increased Clara cell secretion, and empty lamellar bodies in the Type II alveolar epithelial cells. In our patients, with probably much higher acute exposures, bronchi and bronchioles were denuded and Clara cells not identified. Alterations in Type II inclusion bodies

could not be distinguished from postmortem changes, and platelet thrombi were not present.

Our findings are thus consistent with the early experimental findings of Niden in mice. They suggest that the Type I alveolar epithelial cell is the initial site of injury in acute NH₃ inhalation. The other changes described by Niden may be related to the relative chronicity of exposure in his experiments. In severe acute inhalational injury, such as was experienced by these patients, the Type I alveolar epithelial swelling is probably dose-related. Such damage induces a marked alveolar edema, followed by intra-alveolar hemorrhage preventing air blood exchange and leading to death. Similar epithelial cell swelling also occurs as an initial event in mucosal surfaces (e.g., cornea, skin, larynx, and trachea) affected by NH₃. □

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