

**ULTRASTRUCTURAL ASSESSMENT OF BRONCHIAL GLAND CELL INJURY AND EXFOLIATION IN SHEEP AFTER EXPOSURE TO TOXIC SMOKE.** Robert A. Cox (1), Ann S. Burke (2), Mokhtar Maamar (1), Daniel L. Traber (1), and Hal K. Hawkins (1). (1) University of Texas Medical Branch, Galveston, TX. 301 University Blvd. Galveston, Texas 77550, (2) Shriners Hospital for Children, 815 Market St. Galveston, Texas 77550. Email:roc@utmb.edu

Smoke inhalation injury increases the mortality and morbidity of burn victims<sup>1</sup>. Previous studies have shown that an ovine model of cooled cotton smoke inhalation models the pathophysiology of these injuries in humans<sup>2</sup>. In the ovine model, inhalation of smoke causes exfoliation of the lining epithelium<sup>3</sup> and mucous secretion<sup>4</sup>. Together, these conditions, along with the acute inflammatory response, contribute to life-threatening, airway obstruction. The loss of the mucociliary escalator and enhanced mucous secretion may contribute to the high incidence of pneumonia in burn patients with inhalation injury<sup>1</sup>. Although studies have described the exfoliation of the lining epithelium, no studies have assessed the effects of smoke inhalation on bronchial submucosal glands. The aims of this study are to use transmission electron microscopy (TEM) to assess secretion, cell injury, exfoliation and apoptosis in acinar gland cells in sheep after exposure to cooled cotton smoke. **Methods:** These studies were approved by the animal care and use committee of the University of Texas Medical Branch and were conducted in compliance with guidelines for the use and care of laboratory animals of the National Institutes of Health and the American Physiology Society. Sheep received 4 x 12 breaths of cooled cotton smoke exposure per protocol. To assess the effects of toxic smoke on gland cells, lung tissue from the main right upper lobe bronchus was taken from sheep at 1 (n=2), 3(n=3), 6 (n=3) and 12 (n=3) hours after injury. Sampled tissue from uninjured sheep (n=3) served as controls.

Following animal sacrifice, bronchial tissue was fixed in 2% glutaraldehyde in 0.08 M cacodylate buffer, pH 7.4. Following fixation, tissue was post fixed in 1% osmium tetroxide and dehydrated and embedded in Spurr epoxy resin. Ultra-thin sections, stained with uranyl acetate and lead citrate, were examined with a JEOL 100CX transmission electron microscope. **Results:** In contrast to acinar cells in uninjured tissue, Figure 1A, analysis of injured tissue showed acinar lumens full of secreted mucus in tissue one through twelve hours after injury. In the acinar lumens, occasional nuclei and cytoplasmic fragments and debris were seen, suggesting exfoliation of gland cells, Figure 1B. Over time, gland cells contained less mucous granules in their cytoplasm and showed evidence of sublethal injury and attenuation in cell height, Figure 1C. No ultrastructural evidence of apoptosis was seen up to 12 hours post injury. **Conclusion:** Gland cell exposure to toxic smoke induced a rapid secretory response that was accompanied by a decrease in cytoplasmic mucous granules and attenuation in cell height. Some evidence of cell death and exfoliation was seen, although the glandular epithelium remained basically intact. No evidence of apoptosis was seen.

## Reference List

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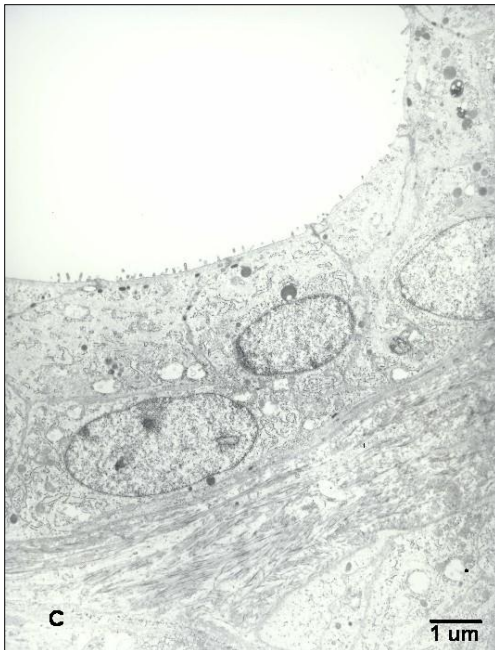
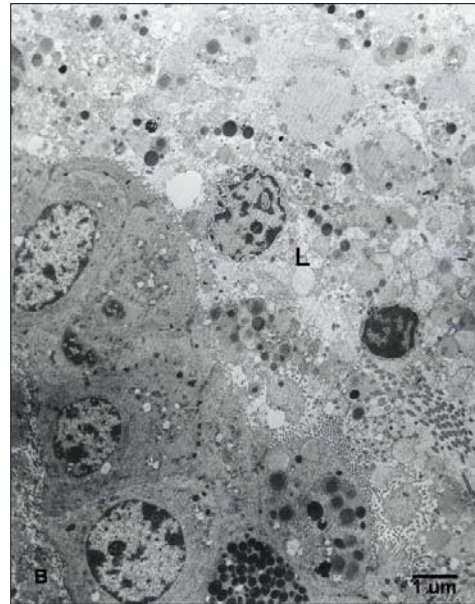
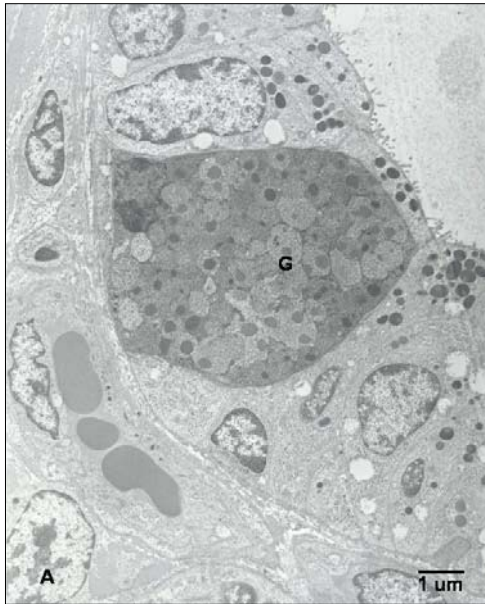


Figure 1. A) Micrograph showing a goblet cell (G), in a bronchial gland from an uninjured sheep. B) Micrograph showing the acinar lumen in a sheep 3 hours after exposure to cotton smoke. Lumen (L) contains nuclei and cytoplasmic debris suggesting cell death and exfoliation. C) Micrograph showing attenuated acinar cells, virtually absent of cytoplasmic mucous granules, from a sheep 12 hours after injury.