# THE EFFECT OF TACHYKININ DEPLETION ON HYDROGEN SULPHIDE TOXICITY IN RATS

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## INTRODUCTION

Hydrogen sulphide ($H_2S$) toxicity is one of the leading causes of sudden death in the work place. Hydrogen sulphide occurs naturally in coal, oil and natural gas deposits, and is also produced by anaerobic decomposition of sulphur containing organic matter. it is used extensively in industry and more than 70 occupations are potentially exposed to $H_2S$.[9] The problem of $H_2S$ toxicity is particularly acute in Alberta; approximately one in six gas wells emit sour ($H_2S$) gas, and the major emphasis of sulphur containing energy resources in the province has increased the risk of exposure for workers in the petro-chemical industries and the general public.[5]

$H_2S$ is both an irritant and asphyxiant gas which exerts its primary toxic effects on the respiratory and neurologic systems.[4] Fatal cases almost invariably exhibit fulminant hermorrhagic pulmonary edema as well as cerebral edema and severe damage to the conjunctiva, olfactory nasal mucosa and upper respiratory tract.[1,5] Similar findings are observed in experimental animals.[4,14,15,16]

The mechanism for $H_2S$ induced pulmonary edema is not understood. $H_2S$ induced paralysis of the respiratory control centre and/or stimulation of carotid body receptors, may be involved.[4,3,9] There is also evidence that $H_2S$ is directly toxic to the lungs. $H_2S$ is only moderately soluble, and is able to penetrate to the lung periphery. Injury to the alveolar/capillary membrane would result in increased vascular permeability and edema.[10] The high protein and cellular content in the alveolar fluid in experimental $H_2S$ exposure support the latter possibility.[14] A direct toxic effect on the respiratory system is also indicated by the observation that pulmonary edema occurs at exposure levels below those associated with severe central nervous system depression.

A further possible mechanism for $H_2S$ induced pulmonary edema might involve stimulation and release of vasoactive neuropeptides from vagal nerve fibres. Unmyelinated postganglionic nerves of the C-fibre group contained in the vagus nerve, are important mediators of neurogenic inflammatory responses in the lung. This response is mediated by a specific neurotransmitter known as substance P which, in part, is responsible for increased vascular permeability and edema occurring during the acute stages of lung inflammation.[18,19,11,6] In addition to modulating vascular permeability in the respiratory tract, substance P is a potent constrictor of bronchial smooth muscle, stimulates mucociliary activity and promotes mucous secretion in the airways.[23,26,28] Immunohistochemical studies have revealed a rich plexus of substance P containing nerve fibres within and beneath airway epithelium, and around blood vessels and seromucous glands.[20,21] Capsaicin, the main pungent ingredient of hot peppers, is a vanilly/amide derivative that produces selective depletion of tachykinins, including substance P, in C-afferent fibres.[12]

In view of the important role of substance P in airway inflammatory responses, we decided to study the effects of hydrogen sulphide in animals previously depleted of substance P. This report will focus on the pathophysiology of the airway lesions. The vascular and edemogenic component will be published in detail elsewhere. In addition, the histological changes induced in the lungs of animals exposed to $H_2S$ were compared with those observed in the lungs of human cases of fatal hydrogen sulphide exposure.

## REVIEW OF WORKPLACE EXPOSURES IN ALBERTA, 1977-1986

One hundred and sixty two lost-time workman's compensation cases were recorded in Alberta in the decade 1977–1986.[2] The majority of these (68%) involved exposures in the oil and gas industry. 79% were aged 34 or less. There were 21 fatalities; most of these occurred in facilities where the dangers were known and protective equipment was available. Eight fatalities were a direct result of failure to follow correct safety practices. 87% of all workers exposed to $H_2S$ developed respiratory system symptoms. In all cases where $H_2S$ intoxication was the primary cause of death, autopsy revealed pulmonary edema. (Figure 1)

## ANIMAL STUDIES

### Materials and Methods

Thirty-six male, Fischer-344 (CDF24Cr1BR), eight week old rats (Charles River, Inc., Quebec) were obtained for this study and acclimatized for ten days under carefully controlled conditions.[24] The guidelines provided by the Canadian Council of Animal Care, were followed throughout all phases of the study.[7] At the time of exposure, the rats weighed

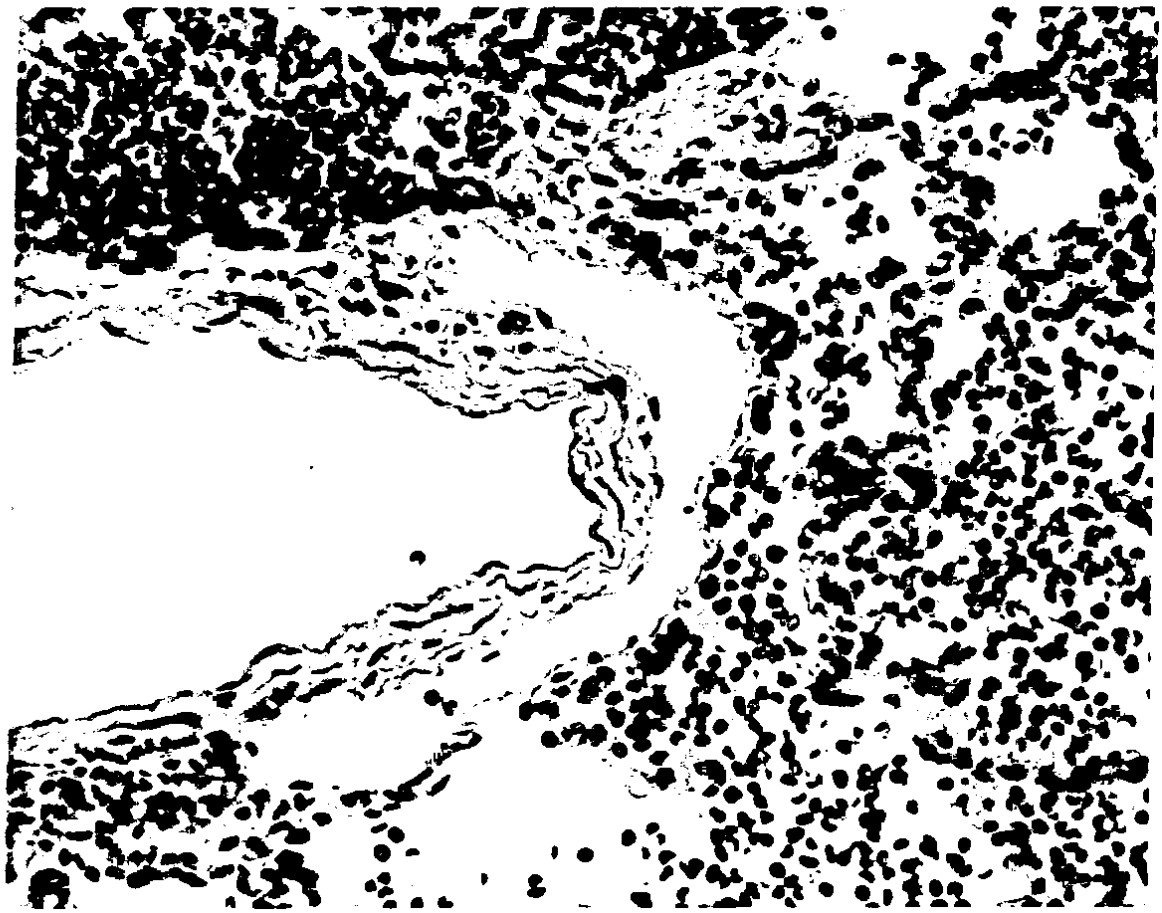


Figure 1. Microscopic appearance of lungs from human fatal case of acute $H_2S$ intoxication. A. The alveoli are flooded with hemorrhagic edema fluid. Polymorphonuclear cells are noted in the alveoli and marginating along vessels in the alveolar interstitium. (Hematoxylin and eosin x 150)

138.4±4.8 gms. Rats were assigned to one of four different groups using a randomized model (Table I). Within one hour of termination of exposure, rats were anesthetized with Halothane (5%) and exsanguinated by incising the abdominal aorta.

### Depletion of Substance P

Capsaicin (Rotichrome[R] Carl Roth) was dissolved in 10% alcohol and 10% tween 80. In the treated group, capsaicin was administered to a total dose of 150 mg/kg subcutaneously, in eight divided doses over a period of two days. The acute effects of capsaicin were reduced by pretreatment with aminophylline 10 mg/kg IP. Rats of the control group received physiologic saline and aminophylline.

### Hydrogen Sulphide Exposures

Fourteen days after the last injection of capsaicin or saline, rats were divided into four groups and exposed for four consecutive hours to either air or $H_2S$. The concentration of gas in the two $H_2S$ chambers is shown in Table I. The $H_2S$ exposure system has been described in detail elsewhere.[24] A schematic diagram of the exposure system is shown in Figure 2. The chamber atmosphere was sampled every two minutes and analyzed by gas chromatography (Model 5790A, Hewlett Packard[R]).

### Bronchoalveolar Lavage and Protein Determination

The left lung was cannulated and three consecutive bronchoalveolar lavages performed. The protein concentration (g/l) in the lavage fluid supernatant was determined using methods previously reported.[14]

### Light and Electron Microscopy

The tracheas of subgroups of rats were cannulated and the lungs inflated with 2.5% glutaraldehyde (320 M Osmol) at a constant pressure of 20 cm of water for 30 minutes in situ. They were then removed from the thoracic cavity and allowed to fix for twenty-four hours. Following fixation, blocks were processed for routine light microscopy and 5 μ sections were mounted on glass slides and stained with hematoxylin-eosin.[17]

For scanning electron microscopy and morphometry, the intrapulmonary portion of the left main bronchus was excised with adjacent lung and dehydrated in graded concentrations

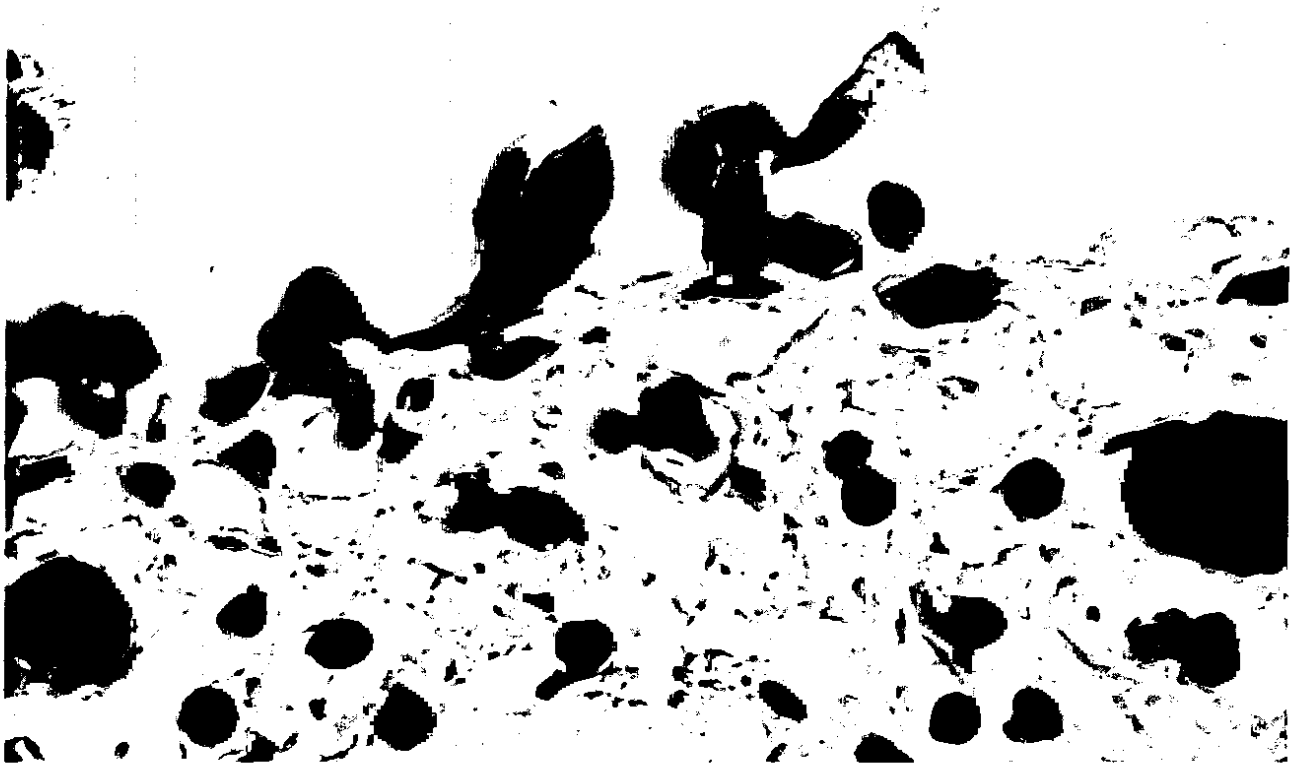


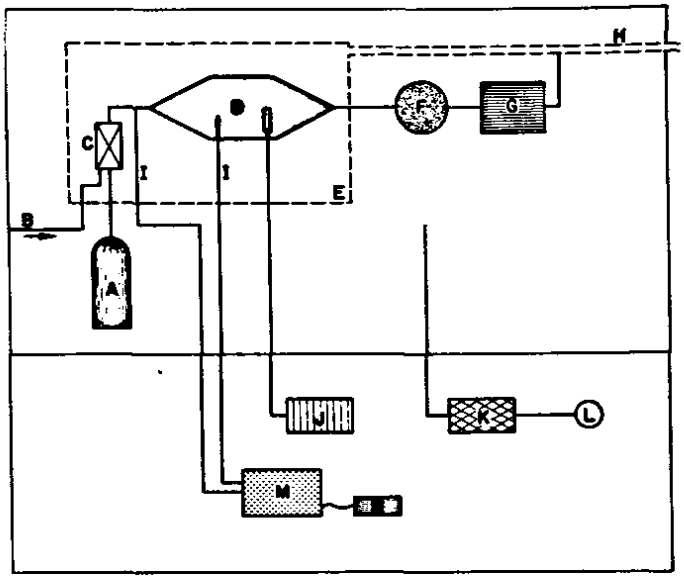
Figure 1. Microscopic appearance of lungs from human fatal case of acute $H_2S$ intoxication. B. Section of main bronchus showing ciliated epithelial cell exfoliation and mucosal edema. (Hematoxylin and eosin x 400)

Table I
Experimental Design

| Treatment | Hydrogen sulphide (mg $m^3$) | | |
|---|---|---|---|
| | 0 | $H_2S$ (1) | $H_2S$ (2) |
| Capsaicin | 6* | 6* | 6** |
| Saline | 6* | 6* | 6** |

Total number of rats = 36

(1) actual concentration 559 ± 144 mg $m^3$
(2) actual concentration 525 ± 87 mg $m^3$
* for bronchoalveolar lavage
** for histopathology




LEGEND

A - GAS CYLINDER
B - AIR
C - FLOW CONTROLLERS
D - EXPOSURE CHAMBER
E - FUME HOOD
F - VACUUM PUMP
G - SCRUBBER
H - EXHAUST TO OUTSIDE
I - SAMPLE LINE
J - RELATIVE HUMIDITY & TEMPERATURE MONITOR
K - $H_2S$ MONITOR
L - ALARM
M - GAS CHROMATOGRAPH
N - COMPUTER

Figure 2. Diagram of the $H_2S$ exposure system.
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of ethyl alcohol, critical point dried and coated with gold/palladium. The specimens were examined in a Hitachi S-450 scanning electron microscope. Five approximately equally spaced photographs were obtained of the proximal 3 mm of the left intrapulmonary bronchus at 1000 x magnification and constant working distance. The density of ciliated and nonciliated epithelial cells, expressed as a percent of the total area, was determined for each photograph using a Dapple[R] image analysis system.

### Detection of Substance P

Substance P was detected in tissues using an indirect immunofluoresence technique[27] and examined using a Reichert-Jung Polyvar microscope, equipped with filter system Bl (excitation wavelength 450-495 nm).

### Statistical Analysis

The effect of capsaicin and/or $H_2S$ on mortality was tested by the Fischer Exact Test. The effects of capsaicin and $H_2S$ on airway epithelial cells was tested by analysis of variance.[25]

## RESULTS

Sections of lung and trachea stained by immunofluorescence for substance P, showed staining of nerve fibres in the mucosa of the trachea and within the walls of small airways and around blood vessels in the lung.The density of fibres was greatest in the trachea and least in the peripheral lung. Animals treated with capsaicin showed almost complete depletion of substance P containing nerve fibres.

Animals pretreated with capsaicin showed normal weight gain and exhibited no signs of toxicity or mortality prior to exposure. Exposure to $H_2S$ for four hours, produced 100% mortality in the capsaicin treated animals and 20% mortality in the saline treated controls. (Table II) At postmortem examination, frothy blood-stained fluid was noted to exit from the mouths and noses of all animals dying from hydrogen sulphide exposure. The lungs of these animals were deeply congested and failed to collapse when the thorax was opened. Histological examination of the affected lungs revealed large quantities of hemorrhagic and highly proteinaceous fluid within the alveolar spaces. Edema fluid was also noted in perivascular and interstitial locations.

Animals exposed to $H_2S$ alone, showed significantly more protein in bronchoalveolar lavage fluid than was seen in air exposed saline or capsaicin pretreated animals. This effect was even greater in animals pretreated with capsaicin and then exposed to $H_2S$. Animals pretreated with saline and exposed to $H_2S$, showed a significant increase in lung wet weights. This effect was even greater in animals pretreated with capsaicin.

Table II

Mortality (%), and Substance P in Rats Pretreated with Saline Solution or Capsaicin and Exposed to Hydrogen Sulphide

| Variable | Air | | $H_2S$ | |
|---|---|---|---|---|
| | Saline | Capsaicin | Saline | Capsaicin |
| Substance P | + | - | | |
| Mortality (%) | 0 | 0 | 20 | 100* |

* $P<0.05$

Examination of large and small conducting airways by light and scanning electron microscopy revealed evidence of severe mucosal damage following $H_2S$ exposure. The changes were most marked proximally and occasional areas of ulceration were noted in the mucosa of the trachea but not in the major bronchi. (Figure 3) The primary finding in the major conducting airways was wide-spread exfoliation of epithelial cells with lateral spreading of basal and intermediate cells. (Figure 4) The bronchioles were relatively spared of toxic effects. The effects noted above were much more severe in animals pretreated with capsaicin and subsequently exposed to $H_2S$. (Figure 5) The results of morphometric eval-

Figure 3. Scanning electron micrograph of area of ulceration in trachea from saline pretreated rats exposed to $H_2S$ for 4 bours. (x 2000)

uation of the extent of loss of ciliated epithelial cells in the left main bronchus, are shown in Figure 6. There is evidence of an additive effect attributable to capsaicin alone p=0.0003 and an additive effect attributable to hydrogen sulphide p=0.003. There was no evidence that the effect of capsaicin depended on the presence or absence of hydrogen sulphide p=0.61.

Figure 4. Scanning electron micrograph of left main bronchus from saline pretreated rat exposed to 559 mg $M^3$ $H_2S$ for 4 hours. There is exfoliation of ciliated epithelial cells with lateral spreading of basal and intermediate cells. (x 2000)

## DISCUSSION

These experiments confirm the previously reported findings concerning the toxic effects of $H_2S$ on the lungs. Animals exposed to $H_2S$ without capsaicin pretreatment, had a 20% mortality at an average concentration of 542 mg $M^3$. These results are similar to those previously reported from this laboratory with this strain of rat where the LC50 and LC10 values for four hours of exposure were 701 and 591 mg per $m^3$, respectively.[24] The exposure concentration was selected such that there would not be significant mortality in normal

Figure 5. Scanning electron micrograph of left main bronchus from: (A) air-exposed, saline pretreated rat. Approximately 50% of the mucosal surface is ciliated.

rats. Animals pretreated with capsaicin and exposed to the same concentration of hydrogen sulphide, showed 100% mortality. In addition, the animals died at an earlier time during exposure than animals pretreated with saline and then exposed to $H_2S$. Animals pretreated with capsaicin and subsequently exposed to $H_2S$, also had more severe pulmonary edema with greater concentrations of protein in the lavage fluid and heavier lungs postmortem. Although to

Figure 5. Scanning electron micrograph of left main bronchus from: (B) saline pretreated $H_2S$ exposed rat showing loss of ciliated epithelium.

some extent these changes could reflect transudation of fluid into the lungs postmortem, we consider this unlikely in view of the magnitude of the effect.

The airway lesions noted in the $H_2S$ exposed rats are similar to those reported for sulphur dioxide.[22] The lesions were most severe in the proximal airways with relative sparing

Figure 5. Scanning electron micrograph of left main bronchus from: (C) capsaicin pretreated $H_2S$ exposed rat showing greater loss than is seen in B. (x 1000)

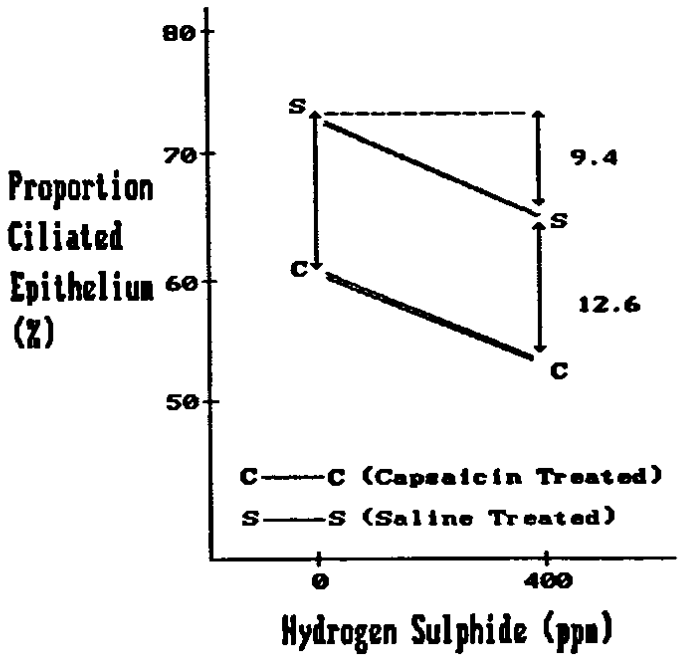


Figure 6. Proportion of airway mucosa occupied by ciliated epithelium by group. $H_2S$ exposure alone and capsaicin pretreatment alone result in loss of ciliated epithelium. Capsaicin plus $H_2S$ has an additive effect.

of the bronchioles and alveolar ducts. Previous studies have shown that the respiratory and olfactory epithelial cells of the nasal mucosa are also very sensitive to $H_2S$ induced injury.[15] This pattern of injury is consistent with the moderate solubility of $H_2S$ in water. Scrubbing of $H_2S$ (as $H_2SO_3$) in the nasal passages and upper airways should result in maximal concentrations of gas proximally with greatest injury to this site.

The mechanism of irritant and oxidant gas injury of the upper airway has been extensively studied. (reviewed in 22) Ciliated epithelial cells appear to be more susceptible to injury than non-ciliated cells. This is true for $SO_2$, $O_3$ and $NO_2$[22,13] and based on this study, appears to be also true for $H_2S$ induced injury. Lipid peroxidation is probably the primary biochemical mechanism for cell injury due to oxidant gases. The mechanism of cellular toxicity due to $H_2S$ is likely to be different. $H_2S$ is toxic to a number of cellular systems and biochemical pathways,[3,4] however the ability of $H_2S$ to react with metal ion-containing proteins is probably of primary importance. $H_2S$ is able to reduce one of the hemes of the intracellular mitochondrial enzyme cytochrome C oxidase, thus interfering with oxidative metabolism. (reviewed in 3) $H_2S$ is reported to be a more potent inhibitor of cytochrome oxidase than hydrogen cyanide.[8] $H_2S$ also interacts with succinic dehydrogenase, catalase and peroxidase and these interactions may also be important in promoting epithelial injury.

Injury of airway epithelium results in cytoskeletal abnormalities and disruption of the tight junctions between the epithelial cells. This leads to an increase in paracellular permeability and exfoliation of the ciliated cells. (reviewed in 22) The repair process is initiated immediately as the remaining viable non-ciliated epithelial cells spread laterally to maintain the epithelial barrier. The data presented in this paper indicate that a similar sequence of events occurs following $H_2S$ injury.

An unexpected finding in this study was the demonstration of a potentiating effect of neuropeptide depletion on $H_2S$ induced airway injury. Thompson *et al.*[29] have demonstrated that airway responsiveness to toluene diisocyanate in guinea pigs is mediated by capsaicin sensitive afferent nerves. These findings indicate a role for tachykinins in pulmonary defense mechanisms against inhaled toxic agents and in the maintenance of structural integrity of the airway mucosa. Studies are underway to fully characterize this phenomenon and elucidate the mechanisms.

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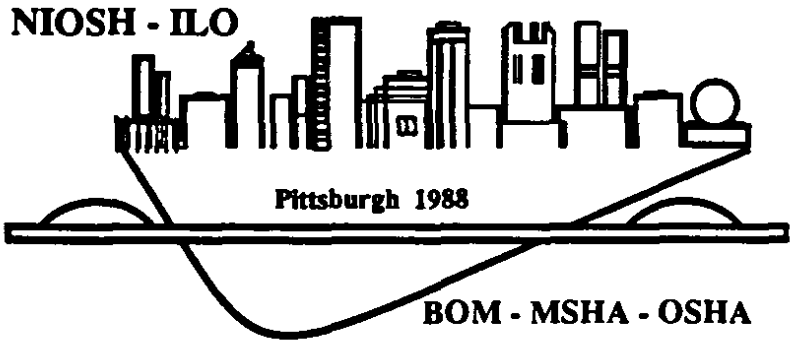


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