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COLCHICINE INHIBITS ELEVATIONS IN BOTH ALVEOLAR-CAPILLARY MEMBRANE PERMEABILITY AND LAVAGE SURFACTANT AFTER EXPOSURE OF THE RAT TO PHOSGENE

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Colchicine diminishes neutrophil incursion into the lung after $COCl_2$ exposure and reduces lung injury as reflected by lavage protein. Potential sources of lavage protein after phosgene inhalation include the leakage of serum proteins and surfactant accumulation. Permeability characteristics of the alveolar-capillary membrane can be altered by an influx of neutrophils. We tested the hypothesis that colchicine diminishes the neutrophil influx and associated elevations in permeability of the alveolar-capillary membrane but does not affect lavage surfactant accumulation after $COCl_2$ exposure. Rats were treated with either colchicine or saline prior to $COCl_2$ at 0.5 ppm × 60 min. To measure membrane permeability, ^{125}I-labeled bovine serum albumin was injected via the tail vein immediately and 1 day after exposure. After 2 h, radioactivity of blood and lavage fluid was measured. Lavage surfactant was quantified as phospholipid immediately and 1 day after exposure. Permeability was elevated immediately but returned to normal values 1 day after inhalation. Phospholipid in the lavage fluid showed no immediate change but was increased 1 day after $COCl_2$ inhalation. Colchicine inhibited both the immediate rise in the permeability index and the elevation of phospholipid 1 day after exposure to phosgene. We conclude that colchicine inhibited elevations in both alveolar-capillary membrane permeability and lavage surfactant after exposure to phosgene.

INTRODUCTION

Phosgene ($COCl_2$) is a highly toxic gas first used as an agent of warfare in 1915 and then widely applied industrially in the synthesis of isocyanates, polycarbonates, polyurethanes, acid chlorides, dye intermediates, and pesticides (Babad and Zeiler, 1973). As a result, it is estimated

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that a considerable number of workers are at risk of exposure to it (National Institute for Occupational Safety and Health, 1976). Inhalation of a significant concentration × time product of phosgene is followed by a noncardiogenic pulmonary edema and death after a clinical latent phase whose duration is inversely proportional to the extent of the exposure. The mechanism of phosgene toxicity is not known but is hypothesized to result from its low solubility in water (Nash and Pattle, 1971) and its capacity to rapidly acylate many different organic compounds (Potts et al., 1949). Its hydrophobic character allows entry into the lower respiratory tract, where it can react with several functional groups (amino, hydrazino, sulfhydryl, and hydroxyl) of biological importance (Gerard, 1948). These reactions are assumed to result in immediate structural damage to the alveolar-capillary membrane, permitting plasma to enter the interstitium and alveoli. The ability of the lung to clear this fluid is exceeded and edema results. The continued accumulation of this edema fluid eventually becomes clinically apparent and is the terminal event in instances of death (Bruner and Coman, 1945).

Similar to the ozone and nitrogen dioxide, phosgene exposure induces a neutrophil influx (Currie et al., 1987; DeNicola et al., 1979; Seltzer et al., 1986). Recruited neutrophils could further damage the lung tissues through release of endogenous oxidants, hypochlorous acid, cytokines, arachidonic acid products, platelet activating factor, and proteases. Inhibition of this neutrophil influx by colchicine reduced both injury and mortality after inhalation of $COCl_2$ (Ghio et al., 1991). Increases of lavage neutrophils and protein at 24 h after exposure to phosgene at 0.5 ppm × 60 min were suppressed with 1.0 mg colchicine/kg treatment given intraperitoneally (ip) 30 min prior to exposure. Inhibition of the rise in lavage protein after $COCl_2$ exposure by colchicine could reflect a decrement in neutrophil-mediated damage to the alveolar-capillary membrane, as permeability characteristics can be altered by an influx of these cells (Hogg, 1987). However, surfactant is also elevated after phosgene inhalation, and its associated proteins account for some portion of the elevated protein concentrations observed in the lavage fluid (Frosolono and Currie, 1985). Decrements in lavage protein after colchicine treatment could reflect an effect of the medication on surfactant concentrations. We tested the hypothesis that colchicine diminishes the neutrophil influx and associated elevations in permeability of the alveolar-capillary membrane but does not affect lavage surfactant accumulation after $COCl_2$ exposure.

METHODS

Materials included colchicine from Eli Lilly and Company (Indianapolis, Ind.), halothane from Halocarbon Laboratories Inc. (Hackensack,

N.J.), and ^{125}I-labeled bovine serum albumin from New England Nuclear (Cambridge, Mass.). All other reagents were from Sigma (St. Louis, Mo.).

Animals were housed in temperature- and humidity-controlled rooms and fed a standard diet (Ralston Purina Co., St. Louis, Mo.). Food and water were available ad libitum except while in the exposure chambers. Sixty-day-old, male Sprague-Dawley rats (Charles River Breeding Labs, Wilmington, Mass.) were treated with either colchicine 1.0 mg/kg or saline ip 30 min prior to the exposure. Exposures to $COCl_2$ at 0.5 ppm × 60 min and air were accomplished as described previously (Ghio et al., 1991). The resulting groups included phosgene exposed after saline ip, phosgene exposed after colchicine ip, air exposed after saline ip, and air exposed after colchicine ip.

Percent neutrophils and protein in the lavage fluid were quantified immediately (0–2 h) and 1 day (24–26 h) after exposure. A total of 48 animals was used (6 per group at the 2 times specified). Animals were anesthetized with halothane, exsanguinated, and lavaged with a volume of saline that equaled 90% of their total lung capacity (35 ml/kg body weight). Two hundred microliters of lavage fluid was pelleted on a microscope slide using a cytocentrifuge (Shandon Southern Instruments, Inc., Sewickley, Pa.) at 300 g × 3 min, dried, and stained with a modified Wright's stain (Diff-Quick Stain, ASP, McGaw Park, Ill.). As a result of previous investigation demonstrating an increased sensitivity of cellular differentials in reflecting response after $COCl_2$ exposure, the percentage of neutrophils, rather than an absolute cell number in the lavage fluid, was used to measure neutrophil influx (Currie et al., 1987). Cell differential counts were enumerated by counting 500 cells and expressed as a percentage. The remainder of the lavage fluid was centrifuged at 700 × g for 10 min to remove cells. Protein in the supernatant was determined using Lowry's method (Lowry et al., 1951).

Recovery of intravenously ^{125}I-labeled serum albumin from the alveolar space provides a sensitive measure of injury to the alveolar-capillary membrane observed before morphologic changes of lung injury (Alpert et al., 1971). Ten microcuries of ^{125}I-labeled bovine serum albumin in a volume of 0.30 ml was injected via the tail vein immediately (0–1 h) and 1 day (24–25 h) after exposure. Forty-eight animals were used (6 per group at the 2 times specified). The time interval between the injection and sampling was short (2 h) to minimize changes in permeability that can occur during the measurement. Two hours after injection, rats were anesthetized with inhaled halothane (2–5%). The thorax was opened, blood was sampled by cardiac puncture, the abdominal aorta was cut, and the lungs were lavaged. Radioactivity of blood and lavage fluid was measured with a gamma counter (Packard Gamma Counter, Packard Instruments, Downers Grove, Ill.). Transfer of the radiolabeled tracer across the alveolar-capillary membrane was quantified as radioactivity in the lavage fluid. Data are expressed as a permeability index:

$$\frac{\text{(Radioactivity in 1.0 ml of tracheal lavage fluid)}}{\text{(Radioactivity in 1.0 ml of blood)}} \times 100$$

Disaturated phosphatidycholine and phosphatidylglycerol are two phospholipids found in high concentrations in surfactant. Together they serve as a nonspecific marker for surfactant. Lavage surfactant was measured as the total phosphorous immediately (0–2 h) and 1 day (24–26 h) after exposure. Rats were lavaged with saline (35 ml/kg of body weight), the fluid was collected, and the instillation was repeated 4 more times. Lipid was extracted from a 15-ml sample of the lavage fluid employing methanol and chloroform (Bligh and Dyer, 1959). The chloroform layer was collected and dried under nitrogen. Lavage surfactant was quantified as the total phosphorous (Bartlett, 1959). This value was adjusted for the total volume of the collected lavage fluid. Phospholipid amounts were calculated assuming a 4% phosphorus content.

Data are expressed as mean values ± standard error. Analysis of variance was used to determine differences between multiple groups (Colten, 1974). Duncan's multiple range testing was selected as a post hoc test of significance (Duncan, 1955). Significance was assumed at $p < .05$.

RESULTS

Phosgene and colchicine affected neither the weight of the rat (284 ± 3 g) nor lavage return (79 ± 3%). A time-dependent accumulation of neutrophils was apparent after $COCl_2$ inhalation (Fig. 1). This influx of neutrophils did not reach significance immediately but did 1 day following phosgene exposure. There were significant increases in the protein concentrations in the lavage fluid immediately after $COCl_2$ inhalation (Fig. 2). Lavage protein remained elevated 1 day after phosgene.

Coinciding with the increase in lavage protein, the permeability index was elevated immediately after exposure (Fig. 3). However, in contrast to lavage protein concentration, this index returned to a normal value 1 day after inhalation. This change in the permeability index was measured prior to significant increases in percent neutrophils in the lavage fluid. Correction of the index by 1 day provides supportive evidence for the success of repair processes in the lung.

Values of phospholipid in the lavage fluid showed no immediate differences between phosgene- and air-exposed rats (Fig. 4). However, significant increases were observed 1 day after $COCl_2$ inhalation. Transport of ^{125}I-labeled albumin and alveolar surfactant showed different responses to phosgene, with elevations in permeability changes occurring immediately while phospholipids were elevated 1 day after exposure.

Pretreatment of animals with colchicine at 1.0 mg/kg ip inhibited the neutrophil influx after phosgene (Fig. 1). In addition, it prevented increases in lavage protein both immediately and 1 day after $COCl_2$ inhala-

tion (Fig. 2). Corresponding with these effects on protein, colchicine inhibited both the immediate rise in the permeability index (Fig. 3) and the elevation of phospholipid 1 day (Fig. 4) after exposure to phosgene.

DISCUSSION

Our data indicate that (1) injury to the lung, reflected by a rise in lavage protein, occurred within 0–2 h after $COCl_2$ inhalation in rats; (2) increases in alveolar-capillary permeability occurred within 1–3 h after exposure to phosgene and corrected by 27–29 h; (3) concentrations of alveolar surfactant were not affected immediately but were increased by 24–26 h after $COCl_2$ inhalation; and (4) colchicine inhibited the neutrophil influx, the increase in lavage protein accumulation, permeability to albumin, and elevations in lavage surfactant after phosgene exposure.

Corresponding with the immediate elevation in lavage protein after phosgene exposure, there was an increase in the transfer of radiolabeled bovine serum albumin, confirming a permeability change in the alveolar-capillary membrane. The transport of this tracer supports other evidence of damage to the membrane after $COCl_2$ inhalation. In addition to lavage protein, lung injury after phosgene exposure has been measured as in-

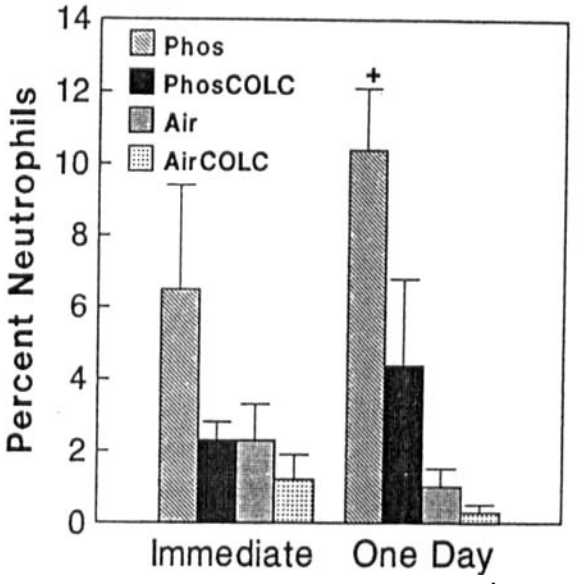

FIGURE 1. Percent neutrophils in lavage fluid of rats exposed to phosgene and treated with colchicine: Phos, phosgene-exposed and injected with saline; PhosCOLC, phosgene-exposed after treatment with colchicine; Air, air-exposed and injected with saline; and AirCOLC, air-exposed after treatment with colchicine. Differences in percent neutrophils among the 4 groups were not observed immediately ($F = 1.87$; $p = .17$) but were present 24 h after phosgene exposure ($F = 6.17$; $p < .05$). Post hoc testing indicated Phos to be greater than all other groups (+).

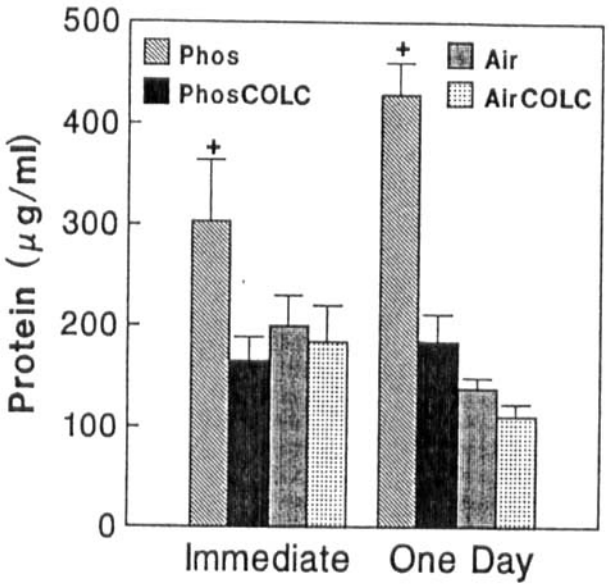

FIGURE 2. Protein in lavage fluid of rats exposed to phosgene and treated with colchicine: Phos, phosgene-exposed and injected with saline; PhosCOLC, phosgene-exposed after treatment with colchicine; Air, air-exposed and injected with saline; and AirCOLC, air-exposed after treatment with colchicine. Disparities in protein among the four groups were found immediately following phosgene exposure ($F = 4.63$; $p < .05$), with Phos being greater than all other groups. Similar differences were observed at 24 h ($F = 13.54$; $p < .05$), with Phos again being greater than all other groups (+).

creases in edema fluid protein (Cameron and Courtice, 1946), lung wet weight to body weight ratio (Coman et al., 1947), lung water (Frosolono and Pawlowski, 1977), lung dry weight (Currie et al., 1987), and lung wet weight (Franch and Hatch, 1986). It is also reflected in abnormalities in pulmonary function including compliance (Rossing, 1964) and diffusing capacity (Long and Hatch, 1961). Finally, edema after $COCl_2$ inhalation can be directly observed histologically (Tobias, 1945; Bruner and Coman, 1945).

Lavage surfactant, as reflected by phospholipid, was not elevated immediately but increased 1 day after $COCl_2$ inhalation. This elevation is consistent with previous observations not only after phosgene but also following exposures of the rat to other oxidant gases (Frosolono and Currie, 1985; Blank et al., 1978; Shelley et al., 1989). The kinetics of surfactant release are slow and an increased release of surfactant is predicted to take hours. While the increased lavage protein immediately after phosgene exposure resulted from a permeability defect, some proportion of the elevated proteins measured 1 day later were associated with surfactant. Calculations of the contribution of phospholipid accumulation to protein elevation in the lavage fluid indicate that only a small

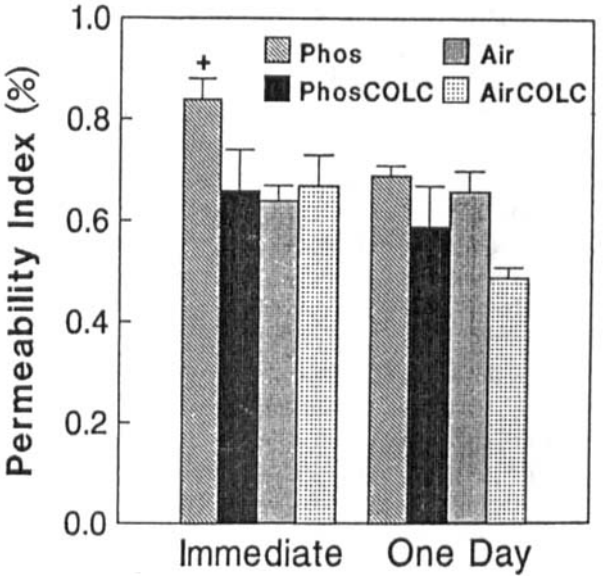

FIGURE 3. Permeability indices of rats exposed to phosgene and treated with colchicine: Phos, phosgene-exposed and injected with saline; PhosCOLC, phosgene-exposed after treatment with colchicine; Air, air-exposed and injected with saline; and AirCOLC, air-exposed after treatment with colchicine. Differences in the permeability index among the four groups were detected immediately after phosgene exposure ($F = 3.94$; $p < .05$), with Phos being greater than all other groups (+). By 24 h after phosgene inhalation, abnormalities in permeability had corrected with no differences noted between the groups ($F = 0.48$; $p = .75$).

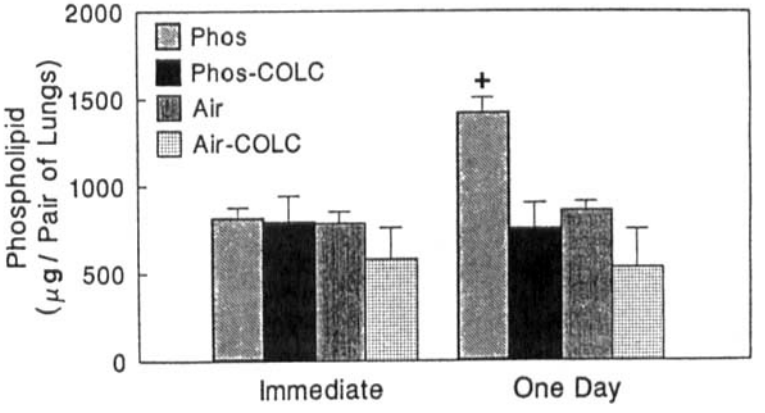

FIGURE 4. Lavage phospholipid of rats exposed to phosgene and treated with colchicine: Phos, phosgene-exposed and injected with saline; PhosCOLC, phosgene-exposed after treatment with colchicine; Air, air-exposed and injected with saline; and AirCOLC, air-exposed after treatment with colchicine. No differences were observed among the four groups immediately after phosgene exposure ($F = 0.69$; $p = .57$). By 24 h after phosgene inhalation, surfactant was found to be significantly elevated in Phos (+) ($F = 6.63$; $p < .05$).

concentration of the protein can be associated with surfactant. Therefore, the transport of serum molecules through the damaged alveolar-capillary membrane immediately after exposure must contribute to the accumulation of protein detected at 24 h.

Colchicine binds tubulin to disrupt formation and function of microtubules (Famey, 1988). It is widely distributed in the body but concentrates in leukocytes. Colchicine inhibited the neutrophil influx, increases in lavage protein, the immediate increase in permeability, and elevations of surfactant 1 day after $COCl_2$ inhalation. The effect of colchicine on permeability and surfactant concentrations could be mediated by its inhibition of the neutrophil influx. Consequences of colchicine treatment on neutrophils include diminished motility, a decreased production of chemotactic factors, and inhibition of both extracellular release of proteases and bursts of respiratory metabolism (Malawista, 1975). Possible mechanisms to explain effects of colchicine on permeability, distinct from those inhibiting neutrophil influx, may include an action on the cytoskeleton. Colchicine inhibits microfilaments whose contractile state affects the permeability of epithelial cells (Bentzel et al., 1980; Welsh et al., 1985). Destabilization of actin by colchicine therefore might inhibit this detrimental reaction to phosgene and oxidant gases.

Neutrophils are also associated with alterations in surfactant (Ryan et al., 1991). Leukocyte products have an in vitro capacity to inhibit surfactant function. A signal for an increased secretion of surfactant by type II epithelial cells to maintain normal lung function could result and an accumulation follow. Inhibition of the neutrophil influx by colchicine could therefore prevent increases in surfactant concentrations. An alternative mechanism for the action of colchicine on surfactant accumulation could involve its interruption of actin polymerization. Cell trafficking and secretion require microtubular function (Kelly, 1990). Disruption of the microfilaments by colchicine could decrease delivery of surfactant to the apical surface and, subsequently, its accumulation in the lower respiratory tract.

We conclude that $COCl_2$ exposure increased alveolar-capillary membrane permeability and lavage surfactant and both were inhibited by colchicine. This suggests a potential role for colchicine in lung injury associated with increased permeability or extracellular surfactant (e.g., adult respiratory distress syndrome and pulmonary alveolar proteinosis, respectively).

REFERENCES

Alpert, S. M., Schwartz, B. B., Lee, S. D., and Lewis, T. R. 1971. Alveolar protein accumulation. A sensitive indicator of low level toxicity. *Arch. Intern. Med.* 128:69–73.

Babad, H., and Zeiler, A. G. 1973. The chemistry of phosgene. *Chem. Rev.* 73:75–91.

Barlett, G. R. 1959. Phosphorus assay in column chromatography. *J. Biol. Chem.* 234:466–468.

Bentzel, C. J., Hainau, B., Ho, S., Edelman, A., Anagnostopoulos, T., and Benedetti, E. L. 1980. Cyto-

plasmic regulation of tight-junction permeability: Effect of plant cytokinins. *Am. J. Physiol.* 239 (*Cell Physiol.* 8): C75–C89.

Blank, M. L., Dalbey, W., Nettesheim, P., Price, J., Creasia, D., and Snyder, F. 1978. Sequential changes in phospholipid composition and synthesis in lungs exposed to nitrogen dioxide. *Am. Rev. Respir. Dis.* 117:272–280.

Bligh, E. G., and Dyer, W. J. 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem.* 37:911–917.

Bruner, H. D., and Coman, D. 1945. The pathologic anatomy of phosgene poisoning in relation to the pathologic physiology. In *Fasciculus on Chemical Warfare Medicine, Respiratory Tract,* ed. Committee on Treatment of Gas Casualties, pp. 234–330. Washington, D.C.: National Research Council.

Cameron, G. R., and Courtice, F. C. 1946. The production and removal of oedema fluid in the lung after exposure to carbonyl chloride (phosgene). *J. Physiol.* 105:175–185.

Colten, T. 1974. *Statistics in Medicine.* Boston: Little, Brown.

Coman, D. R., Bruner, H. D., Horn, R. C., Jr., Friedman, M. D., Boche, R. D., McCarthy, M. D., Gibbon, M. H., and Schultz, J. 1947. Studies on experimental phosgene poisoning. The pathologic anatomy of phosgene poisoning with special reference to the early and late phases. *Am. J. Pathol.* 23:1037–1074.

Currie, W. D., Hatch, G. E., and Frosolono, M. F. 1987. Pulmonary alterations in rats due to acute phosgene inhalation. *Fundam. Appl. Toxicol.* 8:107–114.

DeNicola, D. B., Rebar, A. H., and Henderson, R. F. 1979. Early indicators of pulmonary damage from inhaled nitrogen dioxide gas in Syrian hamsters. *Am. Rev. Respir. Dis.* 119:S211 (abstr.).

Duncan, D. B. 1955. Multiple range and multiple F tests. *Biometrics* 11:1–42.

Famey, J. P. 1988. Colchicine in therapy. State of the art and new perspectives for an old drug. *Clin. Exp. Rheumatol.* 6:305–317.

Franch, S., and Hatch, G. E. 1986. Pulmonary biochemical effects of inhaled phosgene in rats. *J. Toxicol. Environ. Health* 19:413–423.

Frosolono, M., and Currie, W. 1985. Response of the pulmonary surfactant system to phosgene. *Toxicol. Ind. Health* 1:29–35.

Frosolono, M. F., and Pawlowski, R. 1977. Effect of phosgene on rat lungs after single high-level exposures. Biochemical alterations. *Arch. Environ. Health* 32:271–277.

Gerard, R. W. 1948. Recent research on respiratory irritants. In *Science in World War II, Advances in Military Medicine,* ed. E. C. Andrus, pp. 565–587. Boston: Little, Brown.

Ghio, A. J., Kennedy, T. P., Hatch, G. E., and Tepper, J. S. 1991. Reduction of neutrophil influx diminishes lung injury and mortality following phosgene inhalation. *J. Appl. Physiol.* 71:657–665.

Hogg, J. C. 1987. Neutrophil kinetics and lung injury. *Physiol. Rev.* 67:1249–1287.

Kelly, R. B. 1990. Microtubules, membrane traffic, and cell organization. *Cell* 61:5–7.

Long, J. E., and Hatch, T. F. 1961. A method for assessing the physiological impairment produced by low-level exposure to pulmonary irritants. *Am. Ind. Hyg. Assoc. J.* 22:6–17.

Lowry, D. H., Rosenbrough, N. J., Farr, A. L., and Randall, R. J. 1951. Protein measurements with the Folin phenol reagent. *J. Biol. Chem.* 193:265–275.

Malawista, S. E. 1975. The action of colchicine in acute gouty arthritis. *Arthritis Rheum.* 18:835–846.

Nash, T., and Pattle, R. E. 1971. The absorption of phosgene by aqueous solutions and its relation to toxicity. *Ann. Occup. Hyg.* 14:227–233.

National Institute for Occupational Safety and Health. 1976. Criteria for a recommended standard. Occupational exposure to phosgene. U.S. Department of Health, Education, and Welfare, Public Health Service, Centers for Disease Control. Rockville, Md.

Potts, A. M., Simon, F. P., and Gerard, R. W. 1949. The mechanism of action of phosgene and diphosgene. *Arch. Biochem.* 24:329–337.

Rossing, R. G. 1964. Physiologic effects of chronic exposure to phosgene in dogs. *Am. J. Physiol.* 207:265–272.

Ryan, S. G., Ghassibi, Y., and Liau, D. F. 1991. Effects of activated polymorphonuclear leukocytes upon pulmonary surfactant *in vivo*. *Am. J. Respir. Cell. Mol. Biol.* 4:33–41.

Seltzer, J., Bigby, B. G., Stulberg, M., Holtzman, M. J., Nadel, J. A., Ueki, I. F., Leikauf, G. D., Geotzl,

E. J., and Boushey, H. A. 1986. Ozone-induced change in bronchial reactivity to methacholine and airway inflammation in humans. *J. Appl. Physiol.* 60:1321–1326.

Shelley, S. A., Paciga, J. E., Paterson, J. F., and Balis, J. U. 1989. Ozone-induced alterations of lamellar body lipid and protein during alveolar injury and repair. *Lipids* 24:769–774.

Tobias, J. M. 1945. The pathological physiology of the lung after phosgene. In *Fasciculus on Chemical Warfare Medicine. Respiratory Tract,* ed. Committee on Treatment of Gas Casualties, pp. 331–391. Washington, D.C.: National Research Council.

Welsh, M. J., Shasby, D. M., and Husted, R. M. 1985. Oxidants increase paracellular permeability in a cultured epithelial cell line. *J. Clin. Invest.* 76:1155–1168.