FINAL REPORT

Role of Alveolar Cell Interactions in the Pathogenesis and Evolution of Silicosis

Biochemistry Section, LIB, DRDS, NIOSH

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16. Abstract (Limit: 200 words)

Development of assays to monitor functions of alveolar macrophages, neutrophils, and type-II epithelial cells, to characterize variables which modify phagocytic activity of alveolar macrophages and neutrophils, to construct dose response curves for macrophages exposed to crystalline silica (14808607), and to test response of neutrophils to a possible mediator were reported. Assays were developed to monitor membrane integrity, viability, phagocytic activity, and oxidant release by macrophages. Viability and oxidant release in neutrophils were successfully assayed, along with water movement, surfactant synthesis, and xenobiotic metabolism of type-II cells. Release of potentially harmful oxidants and lysosomal enzymes from macrophages resulted from in-vitro silica exposure. Intratracheal instillation of silica activated oxidant production by macrophages. Silica could become coated with surfactants making it less toxic. Formation of radicals on the surface of freshly cut quartz contributed to overall toxicity. No change in-vivo was noted in activity of cytochrome-P450 enzymes in type-II cells. Tetrandrine, an antifibrosis drug, inhibited production of oxidants by macrophages and was effective in-vitro and in-vivo. Activity of leukocytes and macrophages was enhanced by platelet activating factor, suggesting a role in stimulating release of oxidants. Toxicities of coal dust, diesel exhaust, cotton dust, mycotoxins, asbestos (1332214), wollastonite (13983170), and metal ions were also determined.

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18

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ROLE OF ALVEOLAR CELL INTERACTIONS IN THE PATHOGENESIS AND EVOLUTION OF SILICOSIS

FINAL REPORT

IDENTIFICATION #: VKC BAB 118

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INTRODUCTION

The lung is composed of more than 40 different types of cells (1). Therefore, the lungs' response to any occupationally related dust exposure is the result of the sum of responses of individual cell types to the particulate as well as the effects of complex interactions among different types of pneumocytes in response to exposure. To date, the majority of toxicological studies with airborne pollutants have concerned the effects of particulates on single types of lung cells. It is clear that further understanding of respiratory disease processes requires investigation of interactions which occur among lung cells in response to exposure.

Upon contact with inhaled particulate, alveolar macrophages release reactive forms of oxygen (i.e., O_2^- , H_2O_2, $\cdot OH$, etc.) and lysosomal enzymes which act to detoxify foreign particles and kill bacteria (2). Although activation of macrophages is a normal defense mechanism, hyperactivation can damage the lung. Such hypersecretion by macrophages has been demonstrated for exposures to cigarette smoke (3,4) and coal dust (5). Hypersecretion of reactive compounds would damage lung tissue and lead to emphysema (6,7). Particle-induced activation of alveolar macrophages can also result in secretion of fibrogenic factors (8,9). Therefore, interaction between macrophages and other lung cells may be involved in the development of pulmonary diseases such as emphysema and/or fibrosis.

Alveolar macrophages may also exhibit several other interactions with lung cells. Macrophages may secrete substances which are chemotactins for neutrophils (10). Macrophages also may secrete prostaglandins leading to inflammation (11), leukotrienes causing airway constriction (12), platelet activation factor (13), and phytohemaglutinin affecting lymphoid cells (14).

The role of these mediators in disease processes has not yet been completely defined.

Occupational exposure to crystalline silica is associated with both chronic and acute pulmonary disease. Chronic silicosis becomes manifest 20-40 years after initial exposure and is characterized by the development of concentric hyalinized nodular lesions in the lung and by dyspnea. Acute silicosis, on the other hand, occurs within a few years following exposure and is characterized by the accumulation of amorphous granular lipoprotein material in the air spaces and by the rapid development of respiratory disability (15-17). Acute silicosis is commonly associated with sandblasting, rock drilling, tunnelling, and silica mill operations (15-18).

It is clear that the fibrotic reaction of chronic silicosis and the lipidotic response in acute silicosis result from complex interactions among various pulmonary cell types. To date the responses of individual cell types to silica exposure as well as the interactions among cell types has not been completely understood. Therefore, the objectives of this project were to:

1) develop assays to monitor various functional parameters of several types of lung cells, i.e., alveolar macrophages, neutrophils, and alveolar type II epithelial cells;
2) characterize variables which modify the phagocytic activity of alveolar macrophages and neutrophils and the functions of alveolar type II cells;
3) construct dose-response curves for the response of alveolar macrophages to silica exposure;
4) test the response of neutrophils to a possible mediator, platelet activating factor (PAF), released from silica-exposed macrophages.

OUTPUTS

This project was scheduled to begin on 10/1/83. However, due to congressional delays in the budget, headquarters did not approve funds for this project until well into the 3rd quarter of FY'84. In addition, FY'87 funding for this project was so low that allocations were expended after the 2nd quarter. Therefore, work on this 4 year project was in reality limited to 3 years, i.e., the normal span for an LIB project. In spite of allocations for this project being far below requested levels, all milestones were met. This was made possible by collaboration with WVU and the use of WVU-supported personnel, equipment, animals, and supplies. In addition, we were able to collaborate with Dr. Chaolin Li from the Peoples Republic of China. His expertise enabled us to investigate the actions of tetrandrine, a Chinese anti-fibrotic drug, on silica-induced activation of alveolar macrophages.

This project resulted in the publication of 40 manuscripts and 22 abstracts which were presented at national and international meetings.

Publications:

1. Castranova, V., J.R. Wright, H.D. Colby, and P.R. Miles. Ascorbate uptake by isolated rat alveolar macrophages and type II cells. J. Appl. Physiol. 54:208-214, 1983.
2. Castranova, V., G.S. Jones, and P.R. Miles. Transmembrane potential of isolated rat alveolar type II cells. J. Appl. Physiol. 54:1511-1517, 1983.
3. Miles, P.R., J.R. Wright, L. Bowman, and V. Castranova. Incorporation of ^{3}H-palmitate into DSPC in alveolar type II cells isolated by centrifugal elutriation. Biochimica et Biophysica Acta. Vol. 753:107-118, 1983.

4. Van Dyke, K, G. Pekoe, D. Peden, H. Mengoli, V. Castranova, and D. English. Multipurpose radiomatic automated flow liquid scintillation counter system for measurement of burst or delayed chemiluminescence reactions: Model-drug inhibition with lumino-dependent myeloperoxidase. Microchem J. 27:277-284, 1982.

5. Van Dyke, K, M. Matamoros, C. Van Dyke, and V. Castranova. Calcium ionophore-stimulated chemiluminescence (CL) from human granulocytes: Evidence that A23187-induced chemiluminescence originates from arachidonic acid metabolism. Microchem J. 28:568-579, 1983.

6. Castranova, V., G.S. Jones, J.R. Wright, H.D. Colby, L. Bowman, and P.R. Miles. Transport properties of isolated type II alveolar epithelial cells. Am Rev. Respir. Dis. 127:S28-S33, 1983.

7. Castranova, V., and K. Van Dyke. Analysis of oxidation of the membrane potential probe, Di-S-C_3(5), during granulocyte activation. Microchem Journal. 29:151-161, 1984

8. Peden, D., K. Van Dyke, G. Peden, and V. Castranova. Temporal sequence of chemiluminescence and aggregation of human neutrophils and platelets after stimulation by arachidonic acid. Microchem. J. 28:91-98, 1983.

9. Castranova, V., L. Bowman, M.J. Reasor, T. Lewis, J. Tucker, and P.R. Miles. The response of rat alveolar macrophages to chronic inhalation of coal dust and/or diesel exhaust emissions. Mortality and Morbidity Weekly Report. 33:101-102, 1984.

10. Pailes, W.H., D.J. Judy, H. Resnick, and V. Castranova. Relative effects of asbestos and wollastonite on alveolar macrophages. J. Tox. Environ. Health 14:497-510, 1984.

11. Sorenson, W.G., J. Simpson, and V. Castranova. Toxicity for the mycotoxin patulin in rat alveolar macrophages. Environ. Research 38:407-416, 1985.

12. Van Scott, M.R., P.R. Miles, and V. Castranova. Respiratory burst activity in rat alveolar macrophages: particle-stimulated oxygen consumption independent of superoxide anion release. In Cellular Chemiluminescence, Vol II, K. Van Dyke and V. Castranova (eds). CRC Press, Boca Raton, Chapter 2, 1987.

13. Edinboro, L.E., K. Van Dyke, D. Peden, V. Castranova, and D. Wierda. Studies of luminol-dependent-whole-blood-chemiluminescence induced by platelet-activating factor (PAF). Microchem. J. 31:261-271, 1985.

14. Van Dyke, K. and V. Castranova. Effects of platelet activating factor on membrane potential and respiratory burst activity of human granulocytes. In: Cellular Chemiluminescence, Vol. II, K. Van Dyke and V. Castranova (eds.). CRC Press, Boca Raton, Chapter 8, 1987.

15. Castranova, V., L. Bowman, M.J. Reasor, T. Lewis, J. Tucker, and P.R. Miles. The response of rat alveolar macrophages to chronic inhalation of coal dust and/or diesel exhaust. Environ. Research 36:405-419, 1985.

16. Miles, P.R., M.J. Reasor, C.A. Glance, and V. Castranova. Effects of chemicals associated with cotton dust on alveolar macrophage function. Proc. Eight Cotton Dust Res. Conf., eds. P.J. Wakelyn and R.R. Jacobs, pp. 121-124, 1984.

17. Van Dyke, K, V. Castranova, C.J. Van Dyke, J. Ma, K. Michaux, K.W. Mollison, and G.W. Carter. Granulocyte response to oxidized FMLP: evidence for partial inactivation of FMLP. Inflammation. 8:87-99, 1984.

18. Parker, J.C. and V. Castranova. Volume-activated sodium and proton movements in dog red blood cells. J. Gen. Physiol. 84:379-401, 1984.

19. Castranova, V., L. Bowman, J.R. Wright, H.D. Colby, and P.R. Miles. Toxicity of metallic ions on the lung: Effects on alveolar macrophages and type II cells. J. Tox. Environ. Health 13:845-856, 1984.

20. Van Scott, M.R., P.R. Miles, and V. Castranova. Direct measurement of hydrogen peroxide release from rat alveolar macrophages: artifactual effect of horseradish peroxidase . Exp Lung Res. 6:103-114, 1984.

21. Miles, P.R., L. Bowman and V. Castranova. Incorporation of ^{3}H-palmitate and ^{14}C-choline into disaturated phosphatidylcholines in rat alveolar macrophages. Biochimica et Biophysica Acta. 833:342-350, 1985

22. Castranova, V., P. Lee, J.Y.C. Ma, K.C. Weber, W.H. Pailes and P. R. Miles. Chemiluminescence from macrophages and monocytes. In: Cellular Chemiluminescence, Vol. II, K. Van Dyke and V. Castranova (eds). CRC Press, Boca Raton, Chapter 1, 1987.

23. Van Scott, M.R., T.D. Sommerville, R. C. Lantz, P.R. Miles, and V. Castranova. Heterogenicity of rat pulmonary macrophages: relationship between cellular properties and lavage number. In: Cellular Chemiluminescence, Vol. III, K. Van Dyke and V. Castranova (eds). CRC Press, Boca Raton, Chapter 4, 1987.

24. Van Dyke, K., M.R. Van Scott, and V. Castranova. Phagocytes and cell mediated cytotoxicity and it relationship to chemiluminescence. Methods in Enzymol. 132:498-507, 1986.

25. Peden, D., K. Van Dyke, L. Edinboro, D. Wierda, and V. Castranova. Measurement of chemiluminescence, aggregation, and 5-HETE production of FMLP-stimulated human polymorphonuclear neutrophils. Microchem. J. 31:22-28, 1985.

26. Wallace, W.E., Jr., M.J. Keane, V. Vallyathan, T. Ong, and V. Castranova. Pulmonary surfactant interaction with respirable dust. Proc. Coal Mine Dust Conference, ed. S. Peng, 1984.

27. Castranova, V., M.R. Van Scott, and K. Van Dyke. Isolation and identification of phagocytic cells. In: Cellular Chemiluminescence, Vol. I, K. Van Dyke and V. Castranova, eds. CRC Press, Boca Raton, Chapter 2, 1987.

28. Castranova, V., M.R. Van Scott, and K. Van Dyke. Stimulus secretion coupling in phagocytes. In: Cellular Chemiluminescence, Vol. I, K. Van Dyke and V. Castranova, eds. CRC Press, Boca Raton, Chapter 12, 1987.

29. Sorenson, W.G., G.F. Gerberick, D.M.Lewis, and V. Castranova. Toxicity of mycotoxins in the rat pulmonary macrophage in vitro. Environ. Health Perspectives 66:45-53, 1986.

30. Lee, P., E. R. Walker, P. R. Miles, and V. Castranova. Differential generation of chemiluminescence from various cellular fractions obtained by dog lung lavage. In: Cellular Chemiluminescence, Vol. III, K. Van Dyke and V. Castranova (eds). CRC Press, Boca Raton, Chapter 3, 1987.

31. Pailes, W., J. Rabovsky, D. Judy, J. Tucker, V. Castranova, and R.C. Lantz. Effect of silica inhalation on pulmonary alveolar macrophages (PAM) and microsomal cytochrome P450-activities. Proc. Int. Symp. on Respir. Dust in Min. Indus. In Press.

32. Miles, P.R., V. Castranova, and L. Bowman. Catabolism of rat surfactant disaturated phosphatidylcholines during incubation of alveolar lavage materials in vitro at 37°C. Biochim. Biophys. Acta 836:39-44, 1985,

33. Castranova, V., V.A. Robinson, J.H. Tucker, D. Schwegler, D.A. Rose, D.S. DeLong, and D.G. Frazer. Time course of pulmonary response to inhalation of cotton dust in guinea pigs and rats. In: Proceedings of the Eleventh Cotton Dust Research Conference. R.R. Jacobs and P.J. Wakelyn (eds), National Cotton Council, Memphis, TN, pp. 79-83, 1987.

34. Wallace, W.E., M.J. Keane, C.A. Hill, V. Vallyathan, F. Saus, V. Castranova, and D. Bates. The effect of lecithin surfactant and phospholipase enzyme treatment on some cytotoxic properties of respirable quartz and kaolin dusts. Proc. Int. Symp. on Respir. Dust in Min. Indus. In Press.

35. Van Dyke, K., R. Burrel, C. S. Van Dyke and V. Castranova. Luminol dependent chemiluminescence from human granulocytes activated by immune complexes with and without complement. In: Cellular Chemiluminescence, Vol. II, K. Van Dyke and V. Castranova (eds). CRC Press, Boca Raton, Chapter 5, 1987.

36. Van Dyke, K. and V. Castranova. Detection of receptor and synthesis antagonists of platelet activating factor in human whole blood and neutrophils using luminol-dependent chemiluminescence. Proceedings of New Horizons in Platelet Activating Factor Research, M. L. Lee and C. M. Winslow (eds), Wiley & Sons, NY. pp. 181-188, 1987.

37. Wallace, W. E., M. J. Keane, V. Vallyathan, P. Hathaway, E. D. Regad, V. Castranova and F.H.Y. Green. Suppression of inhaled particle cytotoxicity by pulmonary surfactant and retoxification by phospholipase: distinguishing properties of quartz and kaolin. Proceedings of 1985 Inhaled Particles Conference. In Press.

38. Brestel, E.P., E.J. McClain, and V. Castranova. Carrageenan stimulates reduction of nitroblue tetrazolium by human neutrophils without membrane depolarization, myeloperoxidase secretion, or increased oxygen consumption. Inflam. 10:425-434, 1986.

39. Castranova, V., J. Rabovsky, J.H. Tucker, and P.R. Miles. The alveolar type II epithelial cell: a multifunctional pneumocyte. Funda. Appl. Pharmacol. In Press.

40. Vallyathan, V., X. Shi, N.S. Dalal, W. Irr, and V. Castranova. Potential role of reactive silicon-oxygen radicals in acute silica-induced lung injury. Submitted to Am. Rev. Respir. Dis.

Abstracts

1. Castranova, V., G.S. Jones, and P.R. Miles. Toxicity of metallic ions to isolated rat alveolar macrophages and alveolar type II cells. Tox. Soc. 1983.

2. Pailes, W.H., D.J. Judy, H. Resnick, and V. Castranova. Comparison of the effects of asbestos and wollastonite on alveolar macrophages. Tox. Soc. 1983.

3. Bowman, L., P.R. Miles, M.J. Reasor, T. Lewis, and V. Castranova. The effects of diesel exhaust and/or coal dust on alveolar macrophages. Tox. Soc. 1983.

4. Fedan, J.S., M.S. Franczak, C. Kosten, J.F. Cahill, D.G. Frazer, T. Lewis, and V. Castranova. Effects of chronic inhalation of coal dust and/or diesel exhaust on responses of rat airway smooth muscle to agonists. Tox. Soc. 1983.

5. Castranova, V., et al. Biological effects of chronic inhalation of coal dust aerosol (CDA) and/or diesel exhaust (DE). Tox. Soc. 1983.

6. Castranova, V. G.S. Jones, and P.R. Miles. Transmembrane potential of isolated rat alveolar type II cells. Fed. Proc. 1983.

7. Miles, P.R., L. Bowman, and V. Castranova. Incorporation of ^{3}Hpalmitate into disaturated phosphatidylcholines (DSPC) in rat alveolar macrophages. Fed. Proc. 1983.

8. Van Scott, M.R., P.R. Miles, and V. Castranova. Direct measurement of hydrogen peroxide release from rat alveolar macrophages: artifactual effect of horseradish peroxidase. Fed. Proc. 1983.

9. Van Dyke, K., V. Castranova, and C.J. Van Dyke, Antiinflammatory drug effects on luminol-dependent chemiluminescence (CL) from human granulocytes stimulated by Ca^{++} ionophore (A23187) and by the reduced or oxidized forms of a formyl peptide (FMLP or FMoxLP). Fed. Proc. 1983.

10. Castranova, V., C.A. Glance, and M.J. Reasor. Effects of chemicals associated with cotton dust on rat alveolar macrophage function. The Physiologist. 26:A-125 1983.

11. Van Scott, M.R., Miles, P.R., and V. Castranova. Respiratory burst activity in rat alveolar macrophages: stimulated O_2 consumption independent of superoxide and hydrogen peroxide release. Am. Rev. Respir. Dis. 129:A32, 1984.

12. Sommerville, D., M.R. Van Scott, P.R. Miles, and V. Castranova. Heterogenicity of alveolar macrophages: relationship between cellular properties and lavage number. Am. Rev. Respir. Dis. 129:A6, 1984.

13. Edinboro, L., K. Van Dyke, and V. Castranova. Effects of platelet activating factor on membrane potential and respiratory burst activity of human granulocytes. Fed. Proc. 43:1030, 1984.

14. Miles, P.R., L. Bowman, and V. Castranova. Catabolism of rat surfactant disaturated phosphatidylcholines (DSPC) during in vitro incubation at $37^{o}C$. Fed. Proc. 43:3191, 1984.

15. Miles, P.R., W. Cheng, V. Castranova, J. Tucker, M.J. Reasor, and L. Bowman. Possible mechanisms by which chlorphentermine (CP) causes phospholipidosis in rat alveolar lavage materials. Fed. Proc. 44:3580, 1985.

16. Wallace, W.E., M.J. Keane, V. Vallyathan, T-M. Ong, V. Castranova. Pulmonary surfactant interaction with respirable dust. Coal Mine Dust Conf. October 1984.

17. Van Dyke, K., and V. Castranova. Detection of receptor and synthesis antagonists of platelet activating factor in human whole blood and neutrophils using luminol-dependent chemiluminescence. New Horizons in Platelet Activating Factor Research. October 1985.

18. Castranova, V., M. R. Van Scott and P. R. Miles. Respiratory burst in rat alveolar macrophages. 30th International Congress of Physiological Sciences, July 1986.

19. Rabovsky, J., N.A. Sapola, D.J. Judy, W.H. Pailes, and V. Castranova. 7-ethoxyresorufin deethylase activity in rat alveolar type II cells. Fed. Proc. 45:2806, 1986.

20. Pailes, W., J. Rabovsky, D. Judy, J. Tucker, V. Castranova, and C. Lantz. Effect of silica inhalation on pulmonary alveolar macrophages and microsomal cytochrome P450 activities. Respirable Dust in the Mineral Industry, Oct. 1986.

21. Rao, K.M.K. and V. Castranova. Phenylmethyl sulfonyl fluoride (PMSF) inhibits chemotactic peptide-induced actin polymerization and the respiratory burst in human neutrophils. Fed. Proc. 46:3907, 1987.

22. Wallace, W.E., M.J. Keane, C.A. Hill, V. Vallyathan, F. Saus, V. Castranova, D. Bates. The effect of lecithin surfactant and phospholipase enzyme treatment on some cytotoxic properties of respirable quartz and kaolin dusts. Respiratory Dust in the Mineral Industry, Oct. 1986.

RESULTS

I. Development of functional assays for alveolar macrophages -

Our laboratory has developed the expertise to isolate and identify alveolar macrophages (publication - 27). We have developed techniques to harvest alveolar macrophages by pulmonary lavage from rats, guinea pigs, rabbits, and humans. We have mastered techniques to identify the cells by light microscopy using an elastase stain and by transmission or scanning electron microscopy.

We have perfected several functional assays for determining the toxicity of various agents on alveolar macrophages. These techniques include measurement of particle-induced activation of these cells [publication - 22, 24 (chemiluminescence); 12 and 20 (oxygen consumption, superoxide anion release, and hydrogen peroxide secretion)]. We have also determined that alveolar macrophages harvested by pulmonary lavage are heterogeneous with regard to morphology, size, and phagocytotic activity (publications - 23,30). Older macrophages are more vacuolated, larger and less active than younger macrophages. They are also most easily removed from the lungs by lavage.

II. Toxicity of non-silica material on alveolar macrophages -

During the course of this project, we were able to test the effects of several non-silica materials on alveolar macrophages.

1) Dose-response curves were obtained for the effects of a variety of metal ions on oxygen consumption, trypan blue exclusion, and chemiluminescence of alveolar macrophages as well as on oxygen consumption and membrane integrity of alveolar type II cells (publication - 19). In general, metals decreased oxygen consumption

and membrane integrity of both types of lung cells as well as particle-stimulated chemiluminescence of alveolar macrophages. Type II cells seemed more resistant to the harmful effects of metals than were alveolar macrophages.

2) Exposure of alveolar macrophages to asbestos in tissue culture results in a decrease in the viability of these cells. The time course of this decline in viability was monitored over 4 days in the presence of various doses of asbestos by measuring trypan blue exclusion, or the release of cytosolic and lysosomal enzymes. Wollastonite, a possible asbestos substitute, was not toxic to alveolar macrophages (publication - 10).

3) Fungal mycotoxins have been shown to decrease viability of alveolar macrophages (publication - 11,29). Such mycotoxins can contaminate grains and be associated with air conditioning or humidification systems.

4) In vivo exposure to coal dust has been shown to increase the phagocytotic activity of alveolar macrophages measured as increased secretion of reactive forms of oxygen and increased surface ruffling and cellular spreading. In contrast, in vivo exposure to diesel exhaust decreases phagocytotic activity (publication - 9, 15). These data are consistent with coal exposure leading to emphysema and diesel exposure leading to susceptibility to pulmonary infection.

5) The effects of cotton dust and chemicals associated with the cotton plant on alveolar macrophages was tested (publication -16, 33). In vitro exposure of macrophages to gossypol, rutin, and catechin decreased the viability and activity of these phagocytes. In

contrast, in vivo exposure to cotton dust increased the phagocytotic activity of alveolar macrophages and increased the number of neutrophils in the air spaces. These responses were both time and dose dependent.

III. Characterization of stimulus-secretion coupling in neutrophils -

Influx of neutrophils into the airspaces and the activation of these cells is a common response to inhalation of dusts. This inflammation and increased oxidant burden could result in pulmonary damage to the lung.

We have developed methods to isolate neutrophils by centrifugal elutriation and identify these cells by light (peroxidase stain) and electron microscopy (publication - 27). We have found that oxidants can be generated by stimulated neutrophils. These oxidants may be due to the secretion of reactive forms of oxygen, myeloperoxidase (publication - 4) or arachidonic acid metabolites (publication - 8). A good deal of effort has been spent in characterizing the cellular events which occur during stimulus-secretion coupling (publication - 5,7,28) and the cellular responses to chemoattractants, immune complexes and inflammatory agents have been investigated (publications - 17, 25,35,36).

IV. Characterization of alveolar type II cells -

It is well documented that type II cells synthesize and secrete pulmonary surfactant which decreases the surface tension of the air-liquid interface of the lung. Therefore, type II cells may be involved in the lipidotic response to silica exposure.

We have developed methods to isolate and identify alveolar type II cells. We have characterized the ionic transport properties and membrane potential of

their plasma membrane (publication - 2). We have found that these ionic transport properties play an important role in prevention of edema (publication - 6) and the accumulation of vitamin C by type II cells (publication - 1). This ability to accumulate vitamin C may explain the resistance of type II cells to oxidant injury. In addition, we have developed methods to study xenobiotic metabolism in type II cells (publication - 39). In this system, cytochrome P_{450} enzymes act to detoxify inhaled organic chemicals. Lastly, we have characterized the synthesis and degradation of surfactant (publication - 3, 32).

V. Effects of silica on lung cells (published data) -

In vitro exposure of alveolar macrophages to silica can decrease membrane integrity and release lysosomal enzymes. These enzymes may damage the alveolar walls, and lead to fibrosis. Evidence suggests that lecithin, the major component of pulmonary surfactant, decreases the toxicity of silica. This effect can be reversed when lipid-coated quartz was treated with phospholipases (publication - 26, 34, 37). Data also suggest that radicals associated with fresh cleavage planes may be particularly damaging to lung cells (publication - 40). Lastly, intratracheal instillation of silica does not affect the ability of alveolar type II cells to metabolize organic chemicals (publication - 34).

VI. Investigation of possible mediators -

Interaction among lung cells may be critical to the disease process. Recently, investigators have described a lipid secreted from activated alveolar macrophages which can stimulate a number of lung cells. We have

shown that this lipid mediator, platelet activating factor (PAF), is a potent stimulant of neutrophils (publication - 13,14,36). Therefore, PAF may play an important role in increasing the oxidant burden in lungs exposed to silica.

VII. Effects of silica on lung cells (unpublished data) -

Alveolar macrophages were exposed in vitro to various doses of silica and dose-response curves constructed for oxygen consumption (Fig. 1), superoxide anion release (Fig. 2), hydrogen peroxide release (Fig. 3), and chemiluminescence (Fig. 4). In general exposure of alveolar macrophages to low-doses of silica increased the phagocytotic activity of these cells. This hypersecretion of oxidants may play a role in silica-induced lung injury. At silica exposures above 2 mg/ml, there were decreases in secretory activity consistent with cell death. The superoxide assay was the least sensitive for monitoring these effects.

Tetrandrine is a drug currently being used in China to treat fibrosis. Therefore, we tested the effects of in vitro treatment of alveolar macrophages with this drug on the cellular responses to in vitro exposure to silica. Tetrandrine, at doses up to 75 µg/ml, was not toxic to alveolar macrophages; i.e., tetrandrine did not inhibit oxygen consumption of otherwise untreated alveolar macrophages (Fig. 5). However, tetrandrine did depress the activation of alveolar macrophages by zymosan particles or silica. Tetrandrine decreased silica-induced oxygen consumption by 95% (Fig. 6); decreased zymosan-induced superoxide anion secretion by 90% (Fig. 7); decreased zymosan-induced hydrogen peroxide release by 80% (Fig. 8); and decreased silica-induced hydrogen peroxide release by 87% (Fig. 9).

Tetrandrine was also effective *in vivo*. That is, oral administration of tetrandrine for 1 month (7.5 mg/day) effectively inhibited activation of alveolar macrophages in response to intratracheal instillation of silica (40 mg/rat prior just prior to drug treatment) (Table 1).

SUMMARY

1) Assays have been developed to monitor membrane integrity, viability, phagocytotic activity, and oxidant release by alveolar macrophages.
2) Assays have been developed to monitor viability and oxidant release in neutrophils.
3) Assays have been developed to monitor H_2O movement, surfactant synthesis, and xenobiotic metabolism of alveolar type II cells.
4) *In vitro* exposure to silica causes the release of potentially harmful oxidants and lysosomal enzymes from alveolar macrophages.
5) In some cases, intratracheal instillation of silica also activates oxidant production by alveolar macrophages.
6) Surfactant can coat silica and make it less toxic.
7) Part of the toxicity of silica is due to radicals of the surface of freshly cut quartz.
8) Intratracheal instillation of silica does not alter the activity of cytochrome P450 enzymes in alveolar type II cells. These enzymes have been associated with detoxication of carcinogens.
9) An anti-fibrosis drug used in China, i.e., tetrandrine, inhibits the production of oxidants from alveolar macrophages. This drug is effective both *in vitro* and *in vivo*.

10) PAF, platelet activating factor, enhances the activity of leukocytes and alveolar macrophages. Therefore, it may be a mediator which stimulates the release of oxidants by these cells.

11) Assays developed in this project have been used to determine the toxicity of coal dust, diesel exhaust, cotton dust, mycotoxins, asbestos, wollastonite, and metal ions in addition to determining the toxicity of silica.

REFERENCES

1. Sorokin, S.P. The cells of the lungs. In: Morphology of Experimental Respiratory Carcinogenesis. P. Nettesheim, M.G. Hanna, and J.W. Deatherage. Washington, D.C. AEC (conf. 700501) pp. 3-43, 1970.

2. Green, G.M. Amerson Lecture: In defense of the lung. Am. Rev. Respir. Dis. 102:691-700, 1970.

3. Hoidal, J.R., Fox, LeMarbe, Peri, and Repine. Altered oxidative metabolic responses in vitro of alveolar macrophages from asymptomatic cigarette smokers. Am. Rev. Respir Dis. 123:85-89, 1981.

4. Hoidal, J.R. and D.E. Niewoehner. Lung phagocyte recruitment and metabolic alterations induced by cigarette smoke in humans and in hamsters. Am. Rev. Respir. Dis 126:548-552, 1982.

5. Castranova, V., L. Bowman, M.J. Reasor, T. Lewis, J. Tucker, and P.R. Miles. The response of rat alveolar macrophages to chronic inhalation of coal dust and/or diesel exhaust. Environ. Res. In press.

6. Goldstein, E. Hydrolytic enzymes of alveolar macrophages. Rev. Inf. Dis. 5:1078-1092, 1983.

7. Fridovich, I. The biology of oxygen radicals: The superoxide radical is an agent of oxygen toxicity; superoxide dismutases provide an important defense. Sci. 201:875-880, 1975.

8. Heppleston, A.G. and J. A. Styles. Activity of a macrophage factor in collagen formation by silica. Nature 214:521-522, 1967.

9. Bitterman, P.B., S.I. Rennard, G.W. Hunninghake, and R.G. Crystal. Human alveolar macrophage growth factor for fibroblasts: regulation and partial characterization. J. Clin. Invest. 70:806-822, 1982.

10. Gadek, J.E., G.W. Hunninghake, R.L. Zimmerman, and R.G. Crystal. Regulation of the release of alveolar macrophage-derived neutrophil chemotactic factor. Am. Rev. Respir. Dis. 121:723-734, 1980.

11. Sirosis, P., A. Cadieux, M. Rola-Pleszcynski, and R. Begin. Perifused alveolar macrophages. A technique to study the effects of toxicants on prostaglandin release. Exp. 38:1125-1127, 1981.

12. Scott, W.A., C.A. Rouzer, and Z.A. Cohn. Leukotriene c release by macrophages. Fed. Proc. 42:129-133, 1983.

13. Pinckard, R.N. Platelet-activating factor. Hosp. Prac. 35:67-76, 1983.

14. Brain, J.D., D.W. Golde, G.W. Green, D.J. Massaro, P.A. Valberg, P.A. Ward, and Z. Werb. Biologic potential of pulmonary macrophages. Am. Rev. Respir. Dis. 118-435-443, 1978.

15. Ziskind, M., R.N. Jones, and H. Weill. Silicosis. State of the art. Am. Rev. Respir. Dis. 113:643-665, 1976.

16. Parkes, W.R. Occupational Lung Disorders, 2nd Edition, Butterworth, Boston, 1982.

17. Kleinerman, J. and J.A. Merchant. In: Textbook of Pulmonary Diseases, G.L. Baum and E. Wolinsky (eds), Little Brown and Co., Boston, 3rd Edition, 1983.

18. Banks, D.E. Acute silicosis. In: Occupational Respiratory Diseases. J. A. Merchant (ed), U. S. Dept. of Health Publication No. 86-102, U. S. Government Printing Office. Washington, D. C. pp. 239-241, 1986.

19. Heppleston, A.G. Silicotic fibrogenesis: A concept of pulmonary fibrosis. Ann. Occup. Hyg. 26:P449-462, 1982.

20. Davis, G.S. The pathogenesis of silicosis. State-of-the-art. Chest 89:166-195, 1986.

TABLE 1

Effects of In Vivo Treatment

with Tetrandrine*

Treatment	Hydrogen Peroxide Secretion
Unexposed - Untreated	4.86 ± 1.68
Unexposed - Drug treated	4.72 ± 1.74
Silica Exposed - Untreated	9.72 ± 3.84
Silica Exposed - Drug Treated	5.90 ± 1.62

*Tretrandrine (7.5 mg/day) administered orally for month following intratracheal instillation of silica (40 mg/rat).

Fig 1

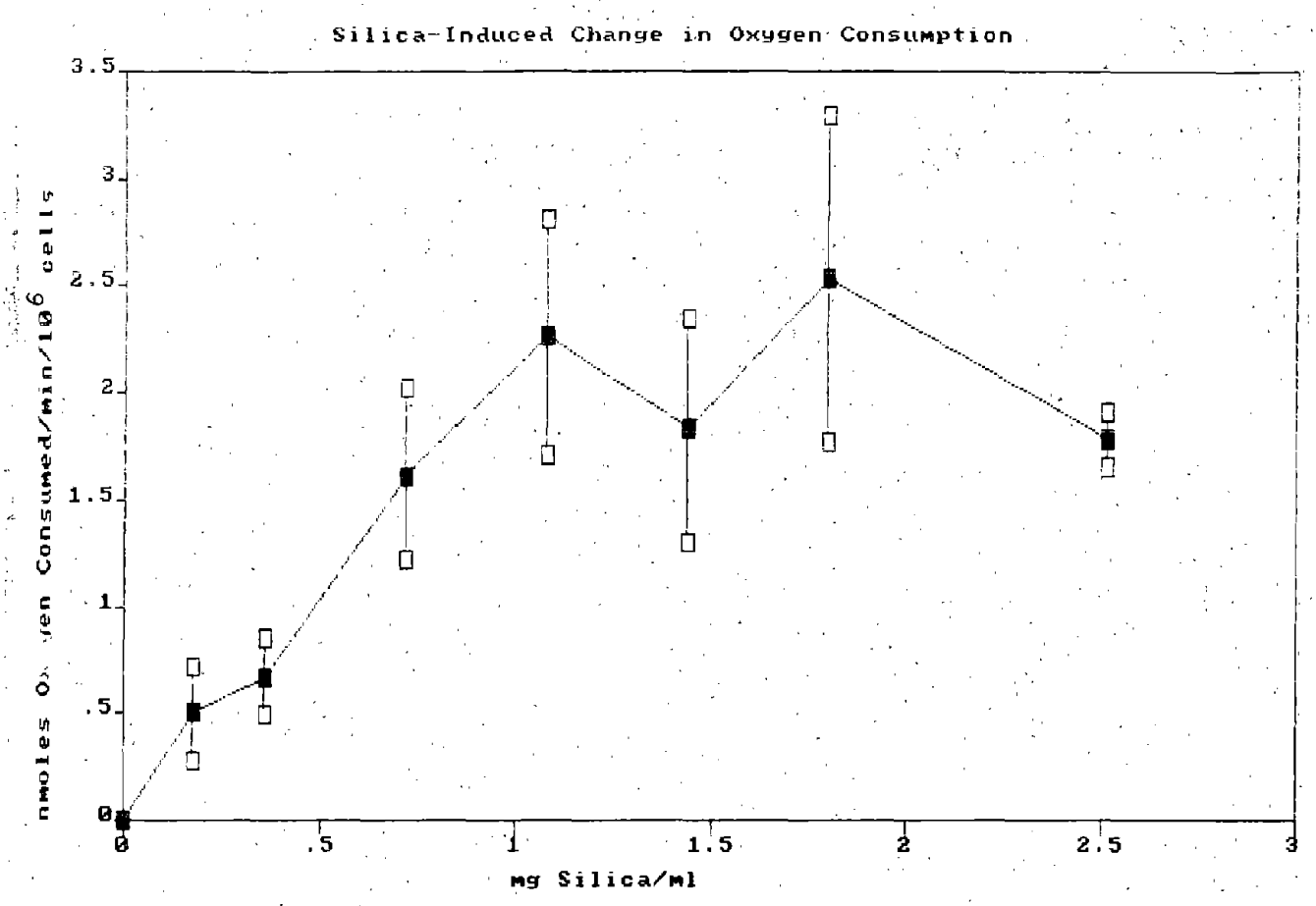

Silica-Induced Change in Oxygen Consumption
nmoles Oxygen Consumed/min/10^6 cells
mg Silica/ml
0
.5
1
1.5
2
2.5
3
3.5

Fig 2

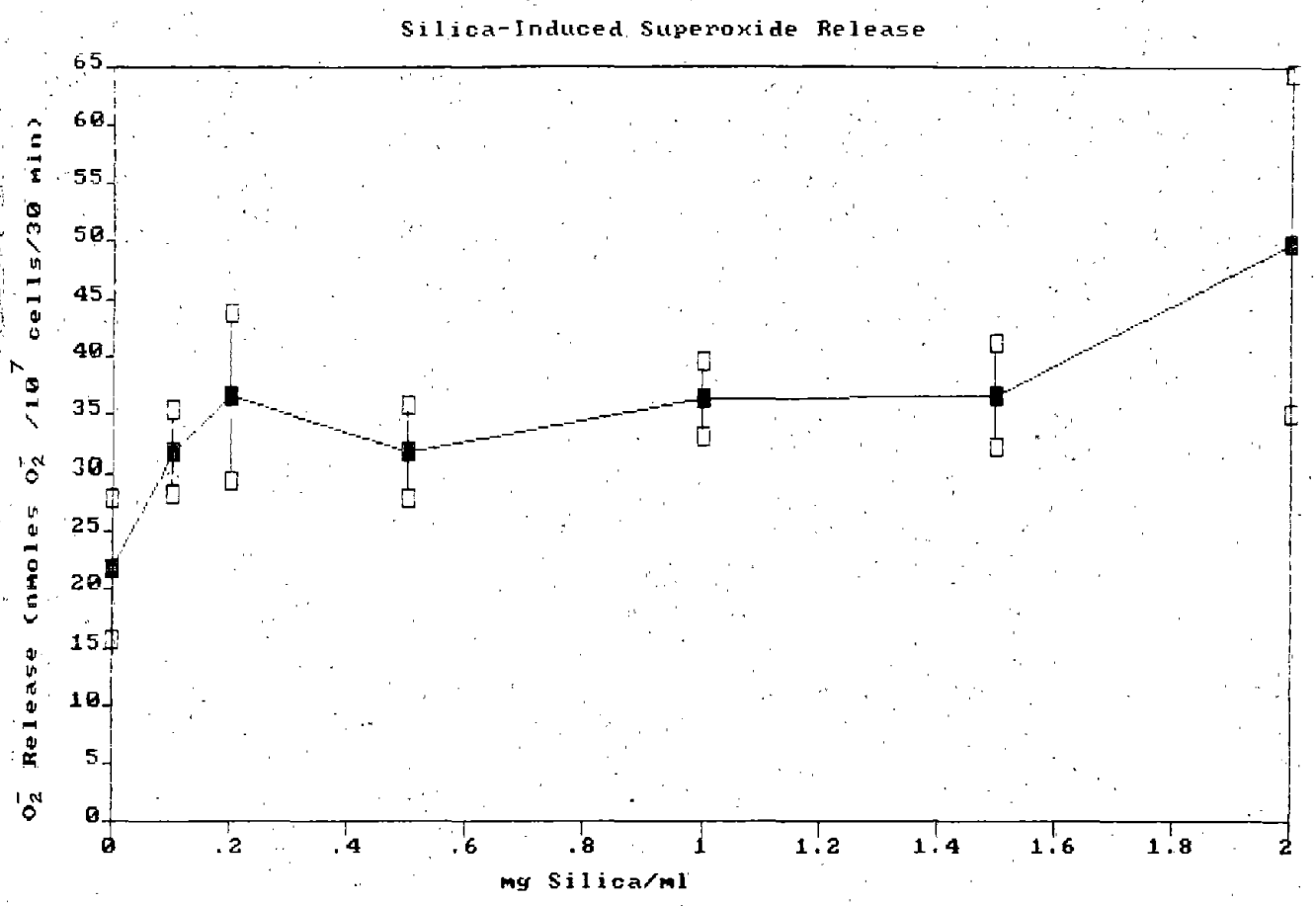

Silica-Induced Superoxide Release
O_2^- Release (nMoles O_2^- /10^7 cells/30 min)
65
60
55
50
45
40
35
30
25
20
15
10
5
0
0
.2
.4
.6
.8
1
1.2
1.4
1.6
1.8
2
mg Silica/ml

Fig 3

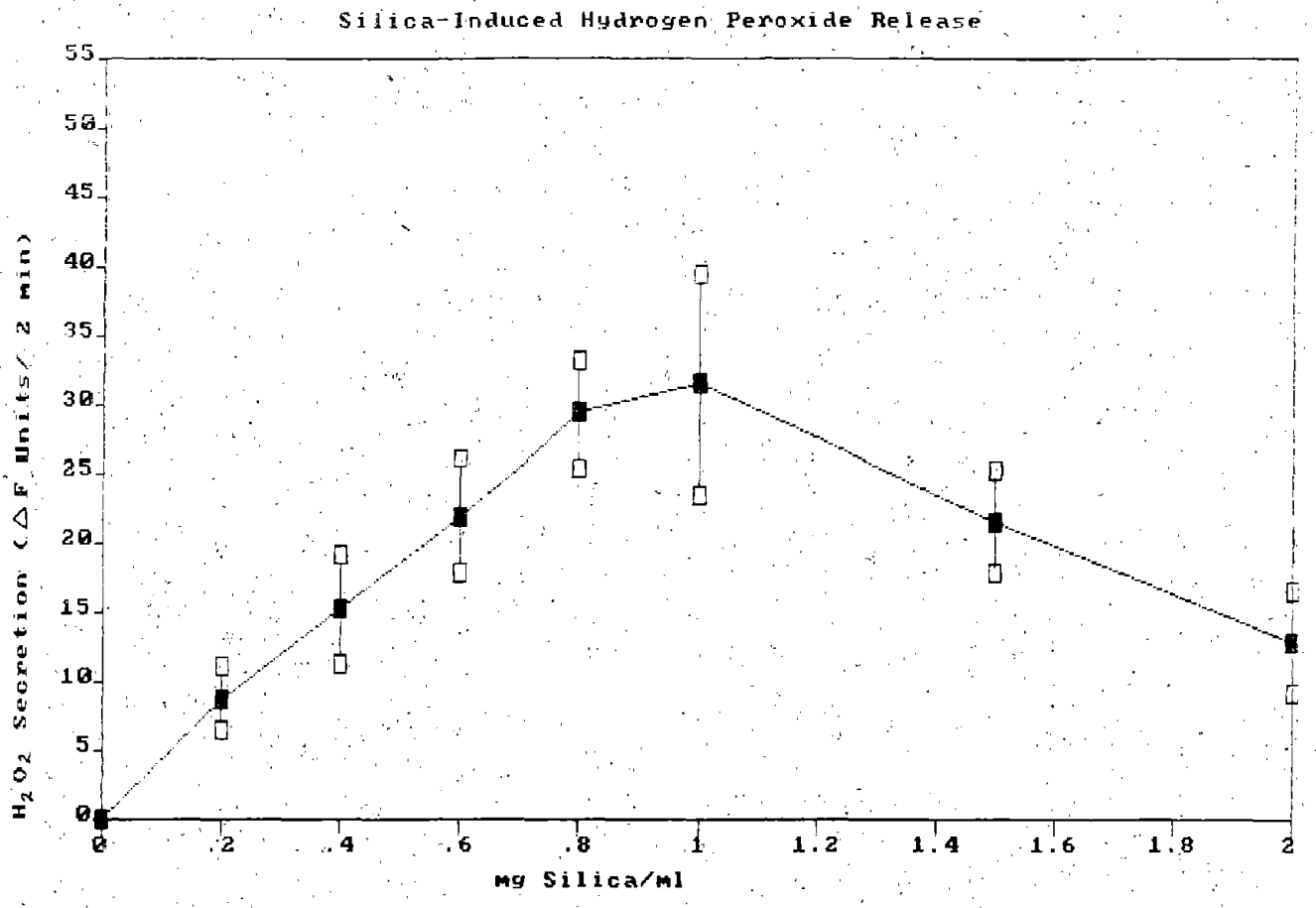

Silica-Induced Hydrogen Peroxide Release
H_2O_2 Secretion (ΔF Units/ 2 min)
55
50
45
40
35
30
25
20
15
10
5
0
0
.2
.4
.6
.8
1
1.2
1.4
1.6
1.8
2
mg Silica/ml

Fig 4

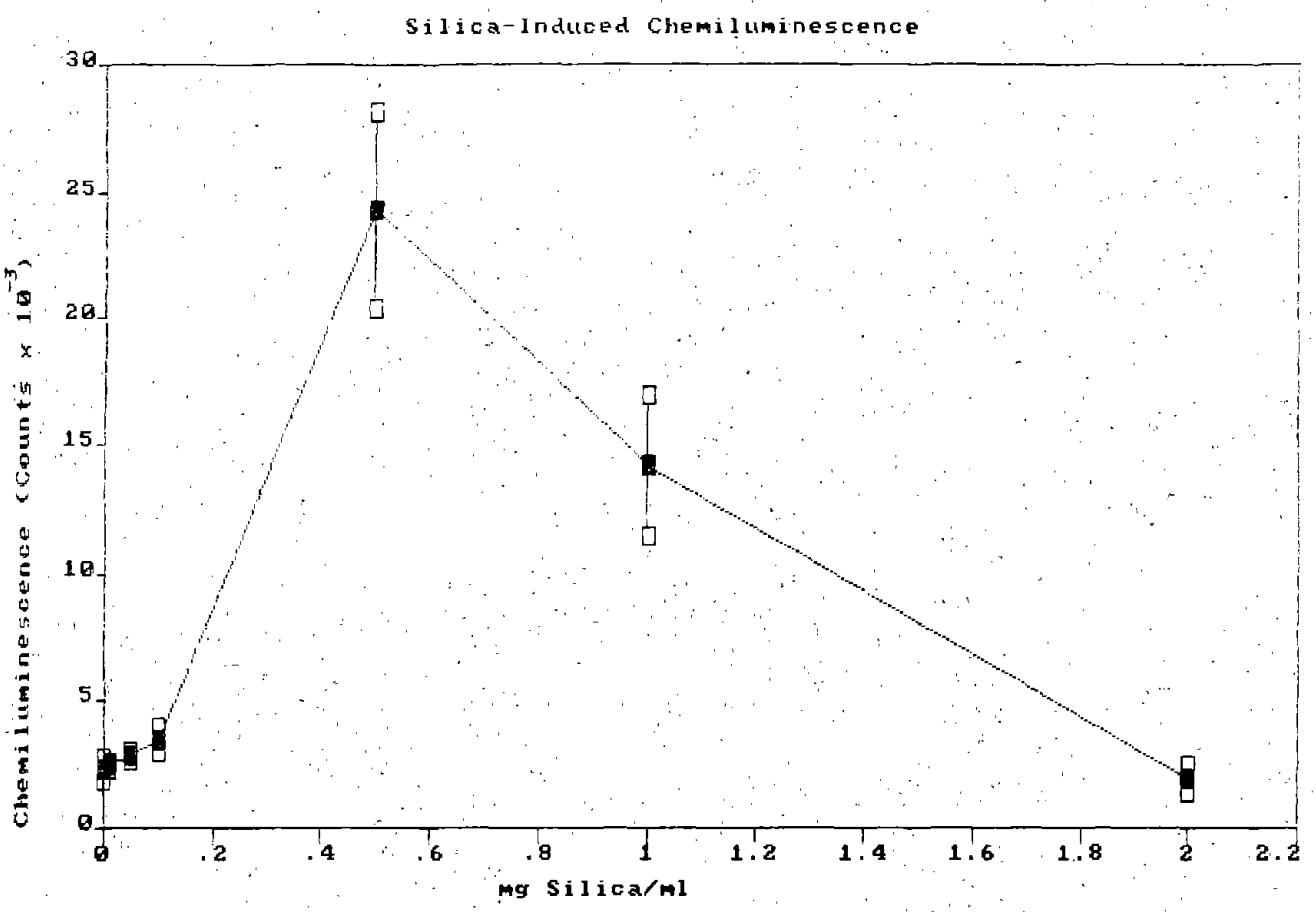

Silica-Induced Chemiluminescence
Chemiluminescence (Counts x 10^{-3})
30
25
20
15
10
5
0
0
.2
.4
.6
.8
1
1.2
1.4
1.6
1.8
2
2.2
mg Silica/ml

Fig 5

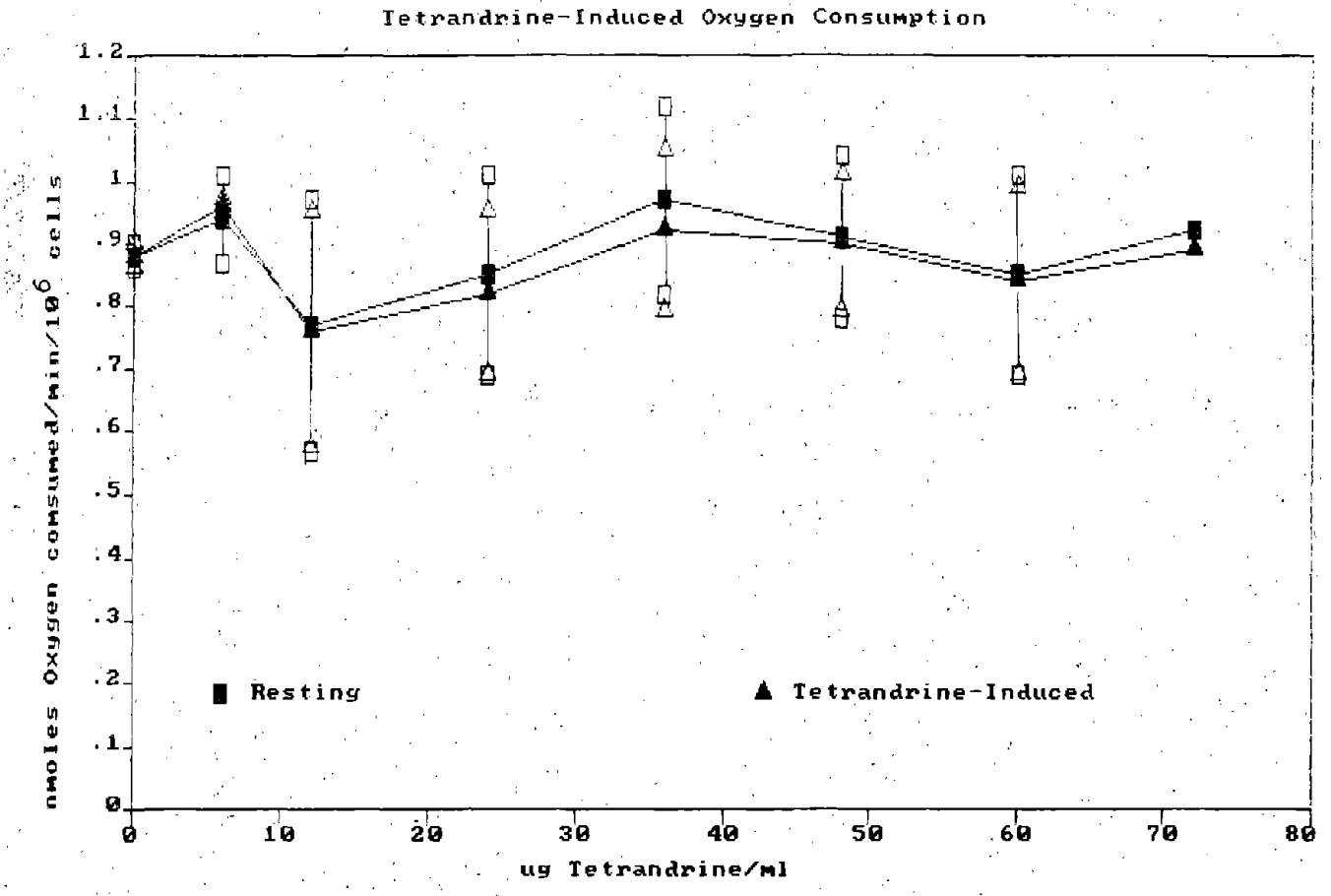

Tetrandrine-Induced Oxygen Consumption
nmoles Oxygen comsumed/min/10^6 cells
1.2
1.1
1
.9
.8
.7
.6
.5
.4
.3
.2
.1
0
Resting
Tetrandrine-Induced
0
10
20
30
40
50
60
70
80
ug Tetrandrine/ml

Fig 6

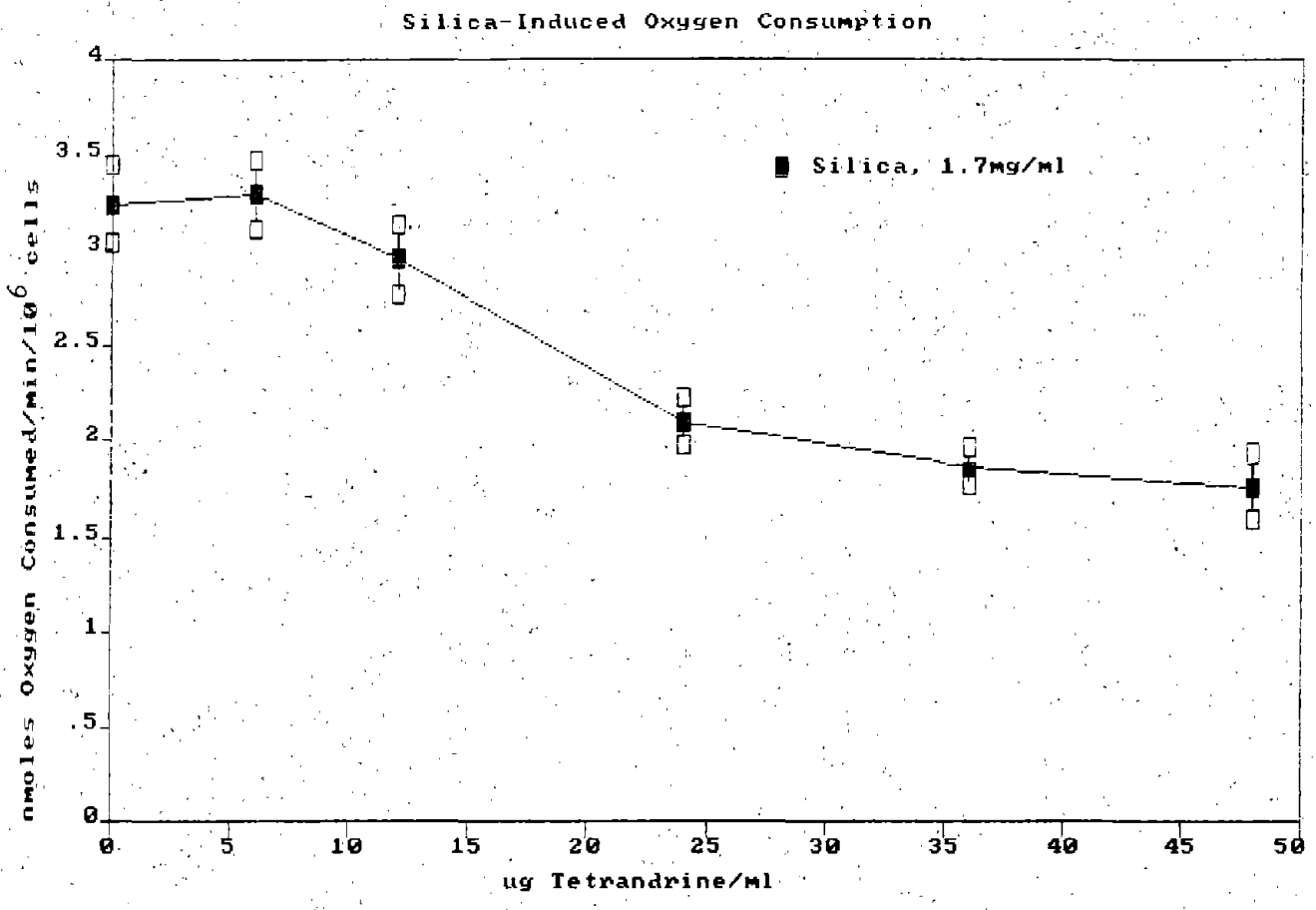

Silica-Induced Oxygen Consumption
Silica, 1.7mg/ml
nmoles Oxygen Consumed/min/10^6 cells
ug Tetrandrine/ml
0
.5
1
1.5
2
2.5
3
3.5
4
0
5
10
15
20
25
30
35
40
45
50

Fig 7

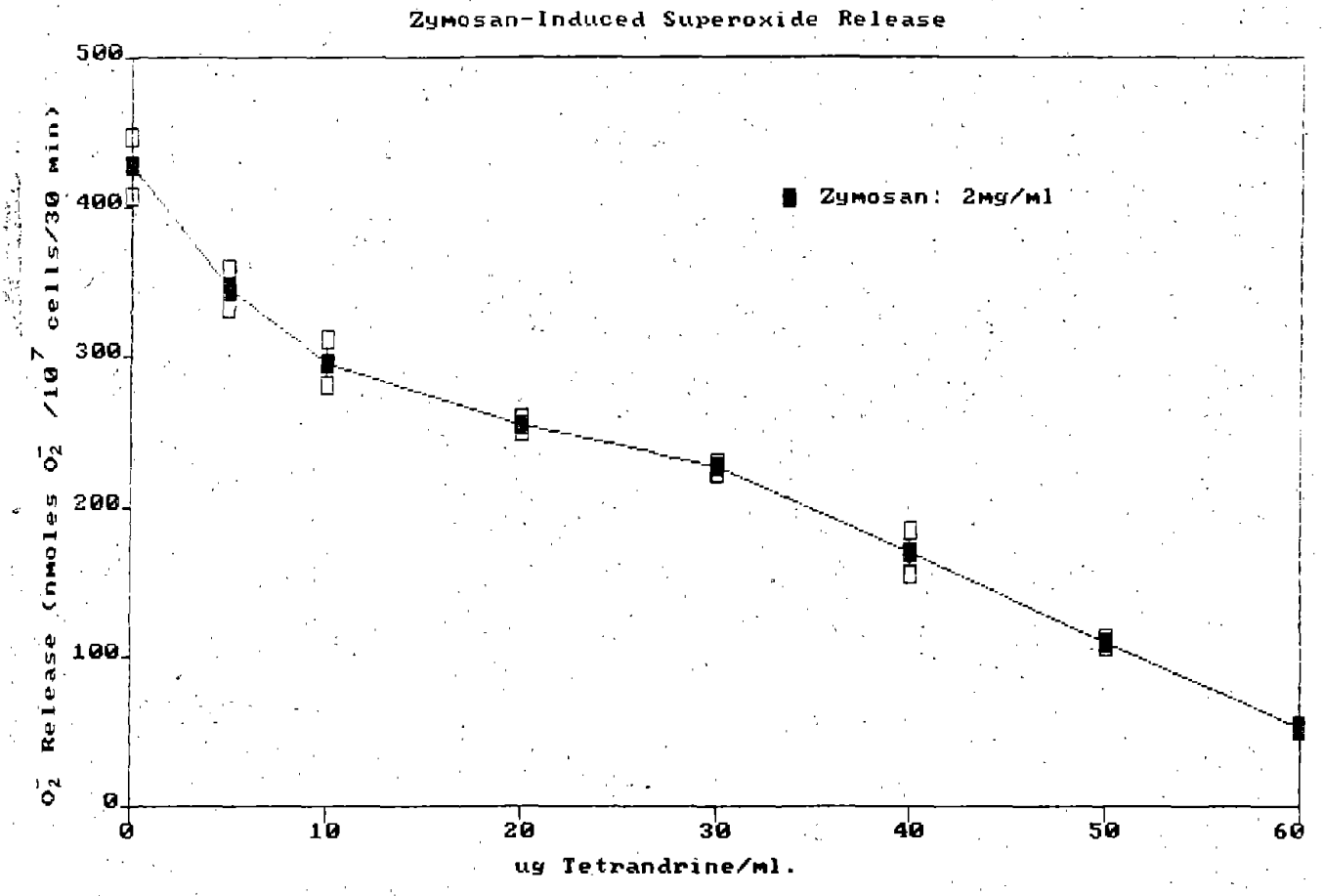

Zymosan-Induced Superoxide Release
Zymosan: 2mg/ml
O_2^- Release (nmoles O_2^- /10^7 cells/30 min)
500
400
300
200
100
0
0
10
20
30
40
50
60
ug Tetrandrine/ml.

Fig 8

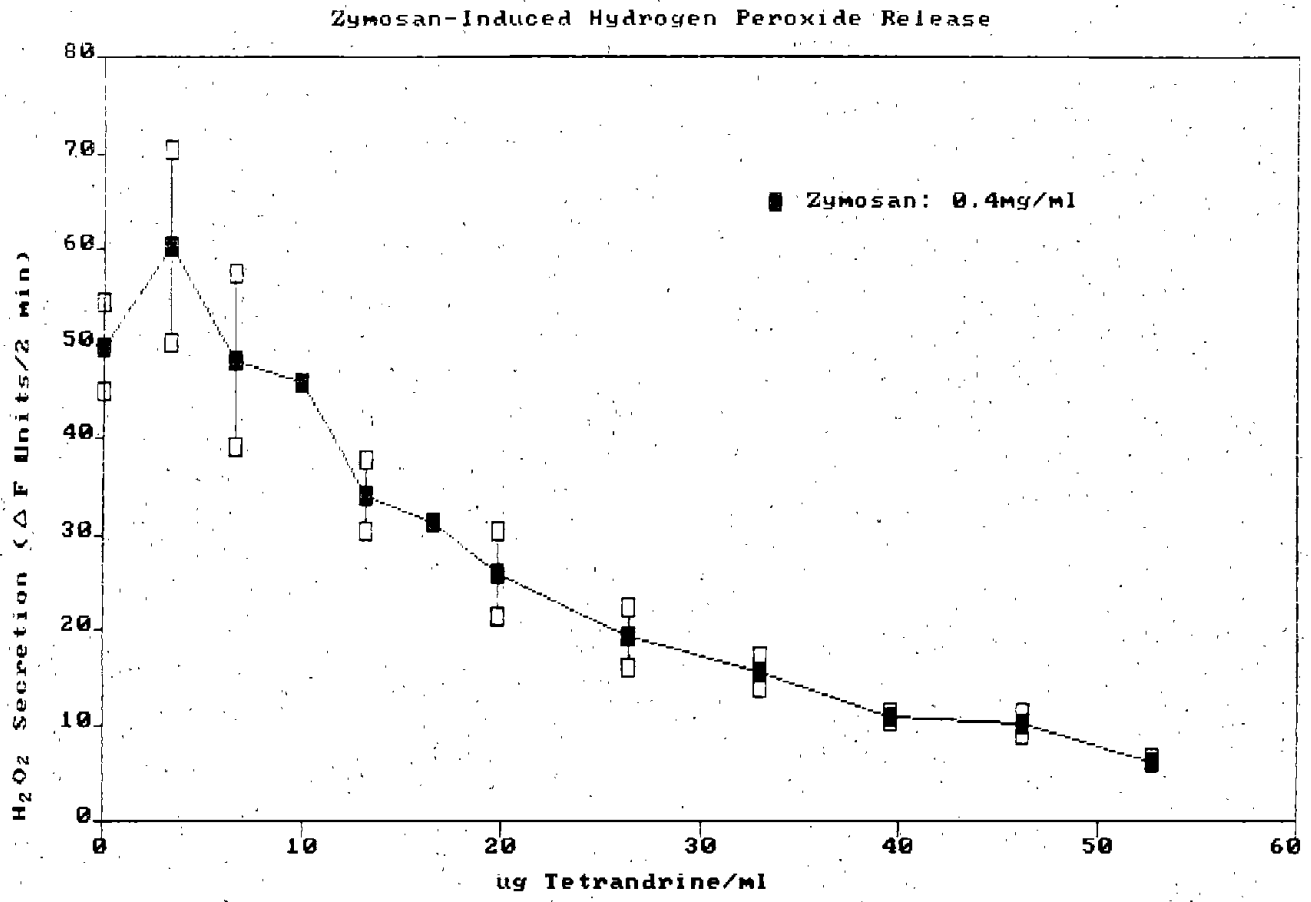
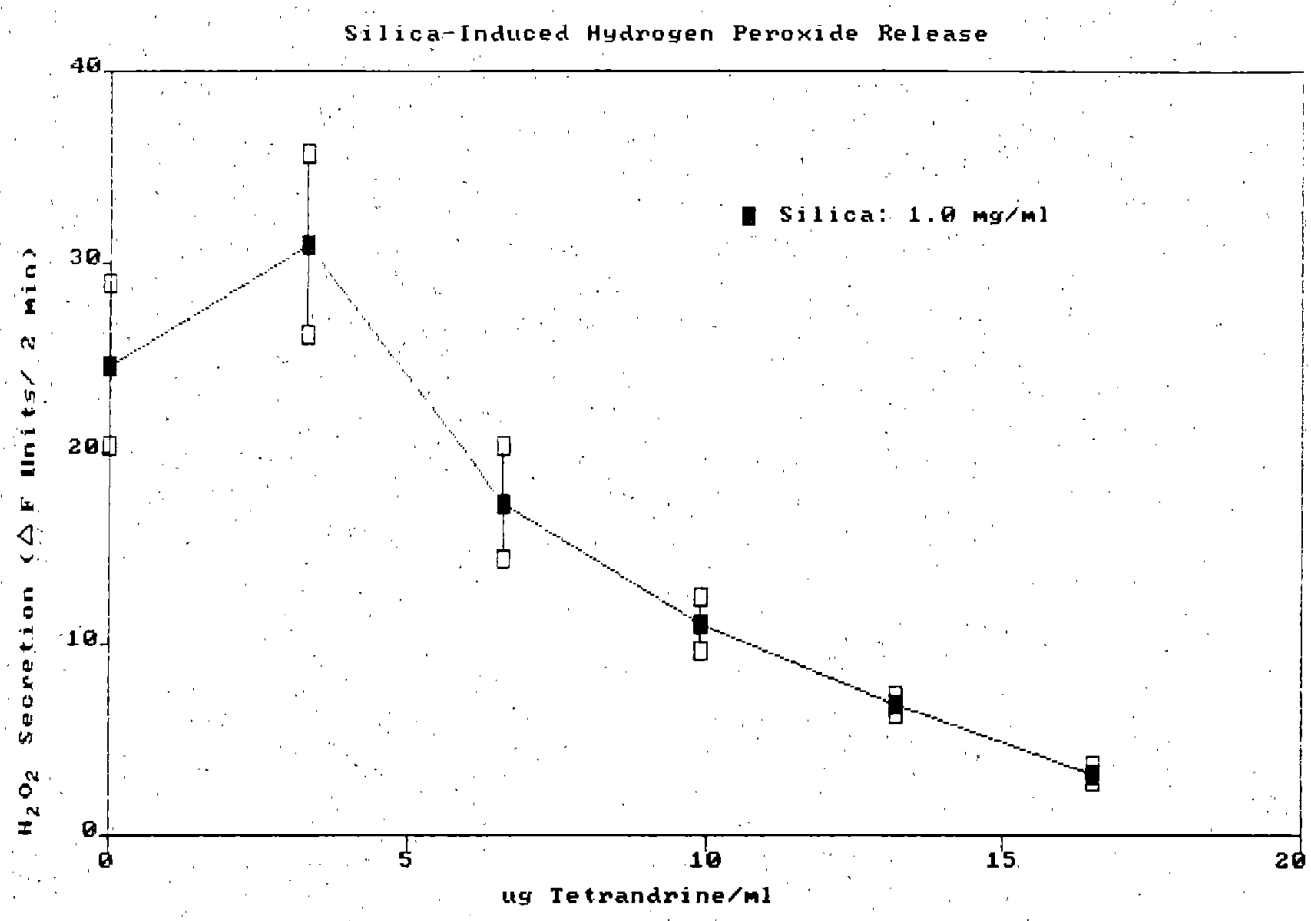

Zymosan-Induced Hydrogen Peroxide Release
Zymosan: 0.4mg/ml
H_2O_2 Secretion (ΔF Units/2 min)
ug Tetrandrine/ml
0
10
20
30
40
50
60
70
80

Fig 9


Silica-Induced Hydrogen Peroxide Release
Silica: 1.0 mg/ml
H_2O_2 Secretion (ΔF Units/ 2 min)
ug Tetrandrine/ml
0
10
20
30
40
0
5
10
15
20