EFFECTS OF ANTIOXIDANTS ON EXPERIMENTAL SILICOSIS

SILVIA GABOR • Mariana Botoc

Institute of Hygiene and Public Health, Cluj-Napoca, Romania

Our previous studies,[15,16,17,18] documented also by other authors,[4,5,9,21,23,34] provided support for the assumption that lipid peroxidation may be one of quartz dust cytotoxic mechanisms in cells and lung tissue. Also, a continuously growing body of evidence indicate that antioxidants as selenium, zinc, vitamins A, E, and C are either incorporated in the biological membranes or/and influence their stability through the antioxidative systems, and, thus can provide line of defense against peroxidative damage.[6,7,11,24,25,30,33] The involvement of lipid peroxidation in lung tissue and macrophage damaging processes promoted by quartz dust justify the use of various antioxidants and free radical scavengers.[2,19,20,22,27]

The present study was conducted to gain some insight into the protective effects of antioxidant agents on experimentally induced by quartz lung changes.

MATERIAL AND METHODS

Male rats with body weights about 180–200 g were used in three experimental series.

Experiment 1. Rats were divided into the following six groups: 1) Control, intratracheally instilled with 1 ml saline; 2) Silicotic rats (DQ12), intratracheally instilled with a single dose of 30 mg DQ12 standard quartz dust, particle size 5 μ (kindly supplied by Prof. K. Robock, Bergbau-Forschung, GmbH in Essen, West Germany); 3) Selenium-supplemented (Se) 1 ppm; 4) Selenium-supplemented (Se) 4 ppm; 5) Se 1 ppm + DQ12; 6) Se 4 ppm + DQ12.

Experiment 2. Designed to investigate the effects of adding Vitamin A (20 mg/kg b.w.), or Vitamin E (40 mg/kg b.w.) to 1 ppm selenium, was performed on the following animal groups: 1) Control; 2) DQ12; 3) Se + Vitamin A; 4) Se + Vitamin A + DQ12; 5) Se + Vitamin E; 6) Se + Vitamin E + DQ12.

Experiment 3. Aimed at evaluating the effectiveness of zinc supplementation (18.5 ppm), and of the concurrent administration of zinc and selenium (1 ppm), used also six animal groups; 1) Control; 2) DQ12; 3) Zn; 4) Zn + Se; 5) Zn + DQ12; 6) Zn + Se + DQ12. The antioxidants were given orally in the drinking water. Rats were maintained on antioxidant supplement for 1 month prior to the dust instillation and 2 months before sacrifice. By the 2 months all the animal groups were killed. The lungs and the tracheal lymph nodes were removed and weighed. The lungs were examined for fibrogenesis development and for peroxidative damage (experiments 2 and 3). In order to evaluate the severity of fibrogenesis the following biochemical parameters were used: lipid,[13,28] phospholipid,[31] and hydroxyproline[29] content of the lungs. The degree of lung peroxidative damage was evaluated by measurements of malondialdehyde formation, as an index of lipid peroxidation release, with thiobarbituric acid (TBA)-test,[32] glutathione peroxidase, GSH-Px,[14] and glucoso-6-phosphate dehydrogenase, G6P-DH,[3] activities.

The data are presented as percent of control and of DQ12-instilled rats. Statistical intergroup significance were performed by using Student's t-test.

RESULTS AND DISCUSSION

Experiment 1. Figure 1 shows that under 1 ppm and 4 ppm selenium treatment no additional benefit was found in the increased by 2 months final lung and lymph node weights of silicotic rats. No significant differences were observed between the DQ12-instilled and supplemented with selenium, and the non-supplemented silicotic groups, except for 1 ppm selenium which diminished lymph node weights, but only at marginal statistical significance ($p < 0.05$).

In contrast, both selenium doses markedly reduced ($p < 0.001$) lung lipids and phospholipids induced by quartz (Figure 2). Selenium supplements exerted the same decrease rate ($p < 0.001$) of lung hydroxyproline in silicotic rats (Figure 3).

Experiment 2. Feeding selenium 1 ppm in combination with vitamin A and E did not modify the increased lung weights induced by quartz, but significantly reduced ($p < 0.01$) the lymph node weights compared to non-supplemented silicotic rats (Figure 4).

Co-administration of selenium with both vitamins was equally effective in decreasing biochemical parameters of lung fibrosis,—lipid, phospholipid and hydroxyproline content (Figures 5 and 6). However, none of the used antioxidant supplements returned the observed changes to control values.

The TBA levels reported in Figure 7 show a significant higher lipid peroxidation in the DQ12-instilled rats. Supplemental to selenium vitamin A and E tended to reduce lipid peroxide release, but the decrease was not significant as against the silicotic rats.

GSH-Px activity depicted in the Figure 8 was found to be increased in silicotic rat lungs, but no significant response was observed compared to control group. The enhanced activity suggests an adaptative reaction and may indicate an

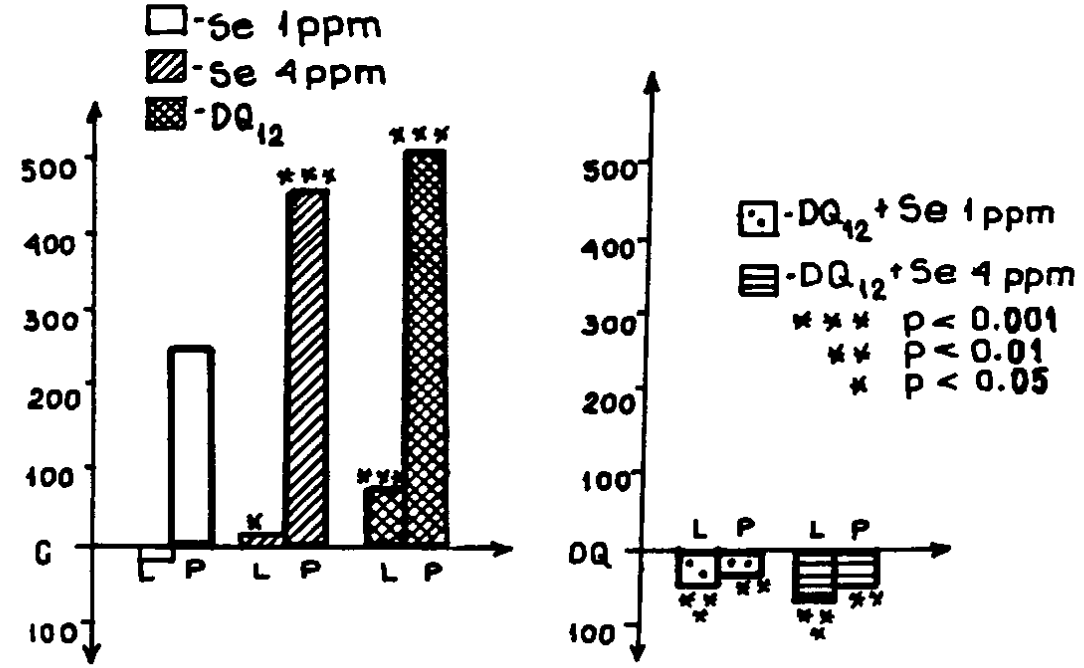


Figure 1. Lung and tracheal lymph node weights of quartz-treated and Se-supplemented silicotic rats.

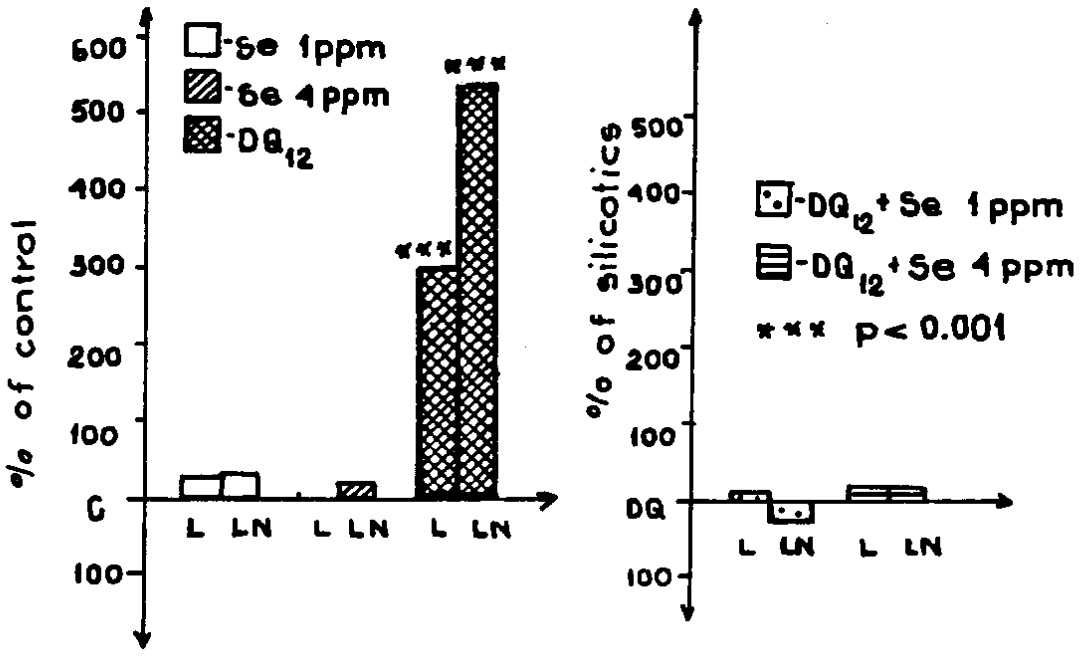


Figure 2. Lung lipids and phospholipids of quartz-treated and Se-supplemented silicotic rats.

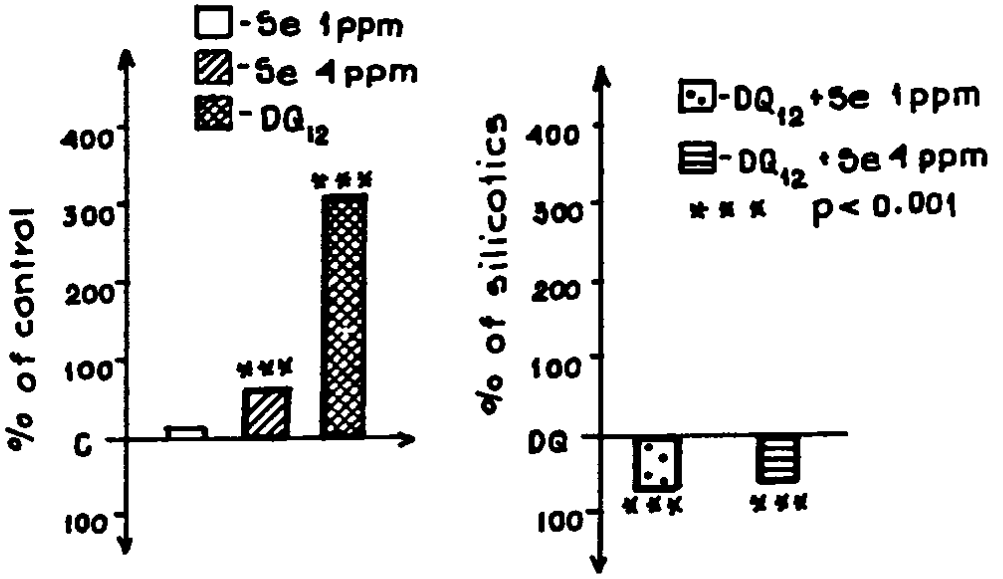


Figure 3. Lung HYPRO of quartz-treated and Se-supplemented silicotic rats.

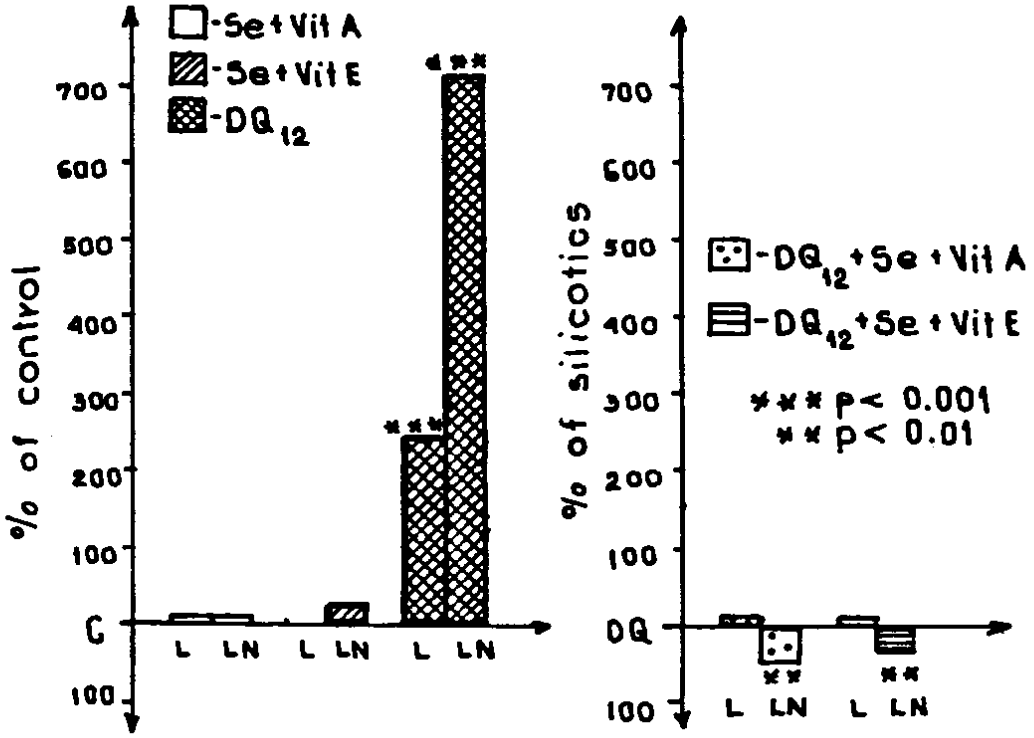


Figure 4. Lung and tracheal lymph node weights of quartz-treated, Se+vitamin A, and Se+vitamin E-supplemented silicotic rats.

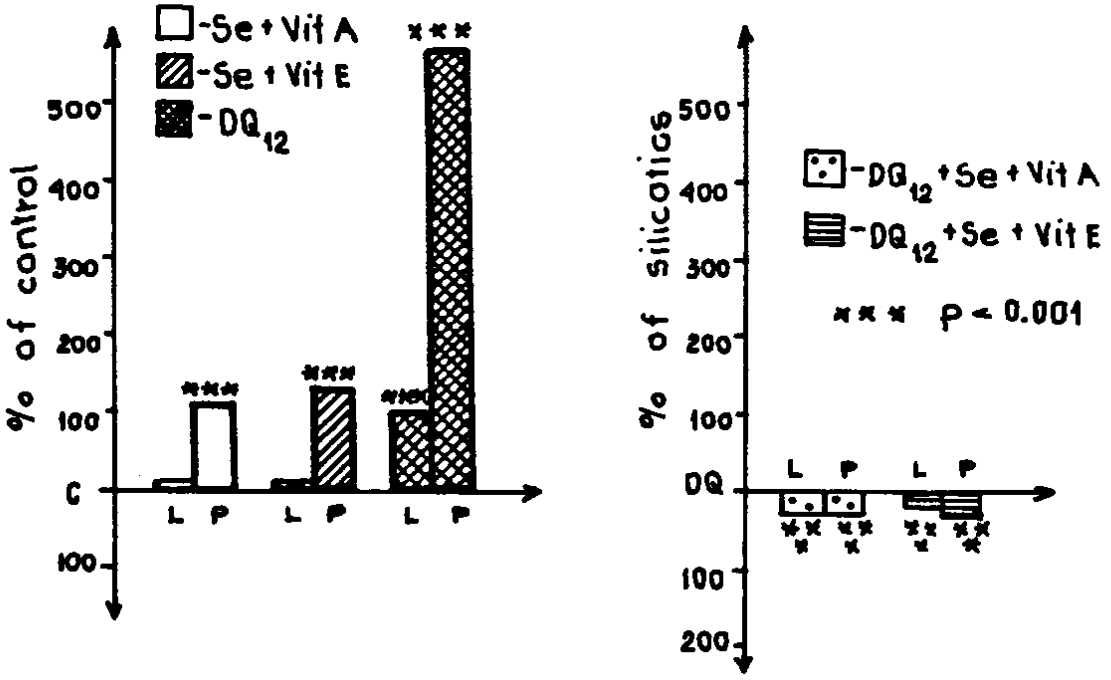


Figure 5. Lung lipids and phospholipids of quartz-treated, Se+vitamin A, and Se+vitamin E-supplemented silicotic rats.

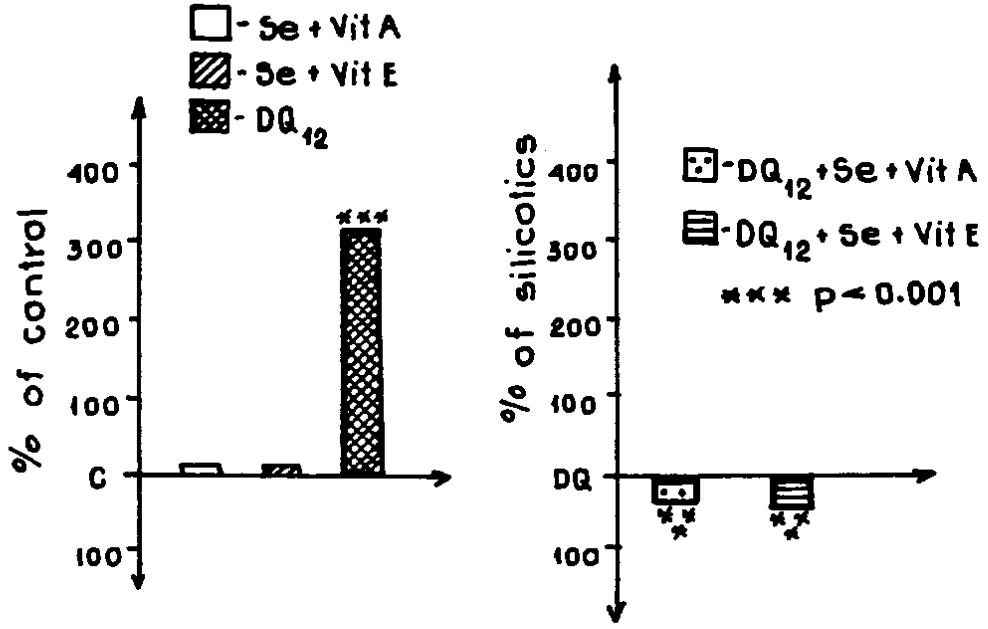


Figure 6. Lung HYPRO of quartz-treated, Se+vitamin A and Se+vitamin E-supplemented silicotic rats.

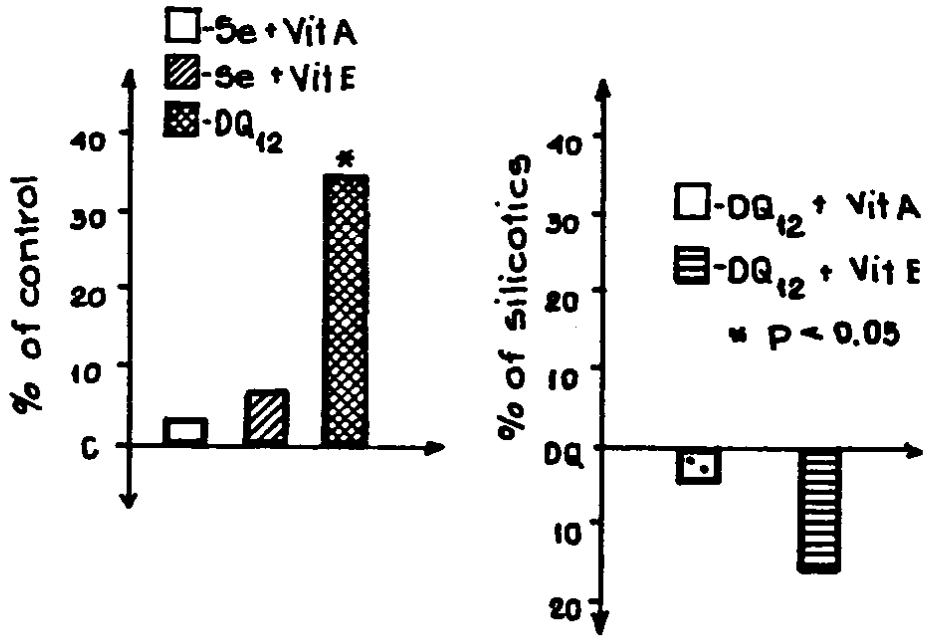


Figure 7. Lung LIPID PEROXIDES of quartz-treated, Se+vitamin A, and Se+vitamin E-supplemented silicotic rats.

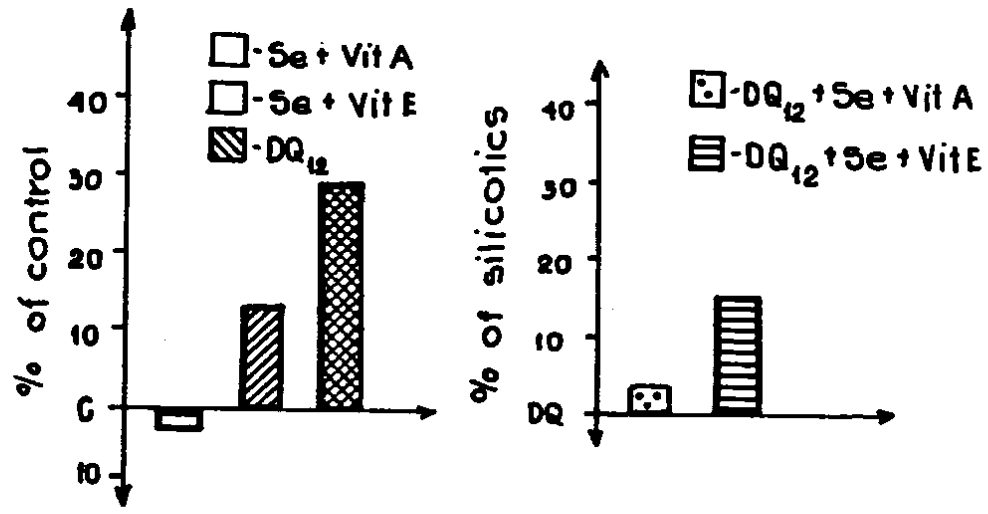


Figure 8. Lung GSH-Px of quartz-treated, Se+vitamin A, and Se+vitamin E-supplemented silicotic rats.

increased demand of the enzymatic activity to cope with tissue peroxidative damage. An inverse relationship between lung GSH-Px activity and lipid peroxidation in selenium + vitamin A and E-supplemented silicotic rats was observed. However, this effect was not significant compared to DQ12-instilled rats. G6P-DH exhibited a significantly higher ($p < 0.001$) activity in the silicotic rat lungs versus of the control group. Addition of both vitamin combinations significantly lowered the enzyme activity by the 2 months when compared to non-supplemented silicotic rats (Figure 9). The reason of the increased enzyme activity in silicosis might be due to the stimulation of the pentoso-phosphate pathway by supplying NADPH for "de novo" lipid biosynthesis and for glutathione redox cycle. Antioxidants exerted a beneficial effect on this metabolic point as demonstrated by the previous Figure 5 showing a reduced rate of lung lipids in antioxidant supplemented silicotic rats.

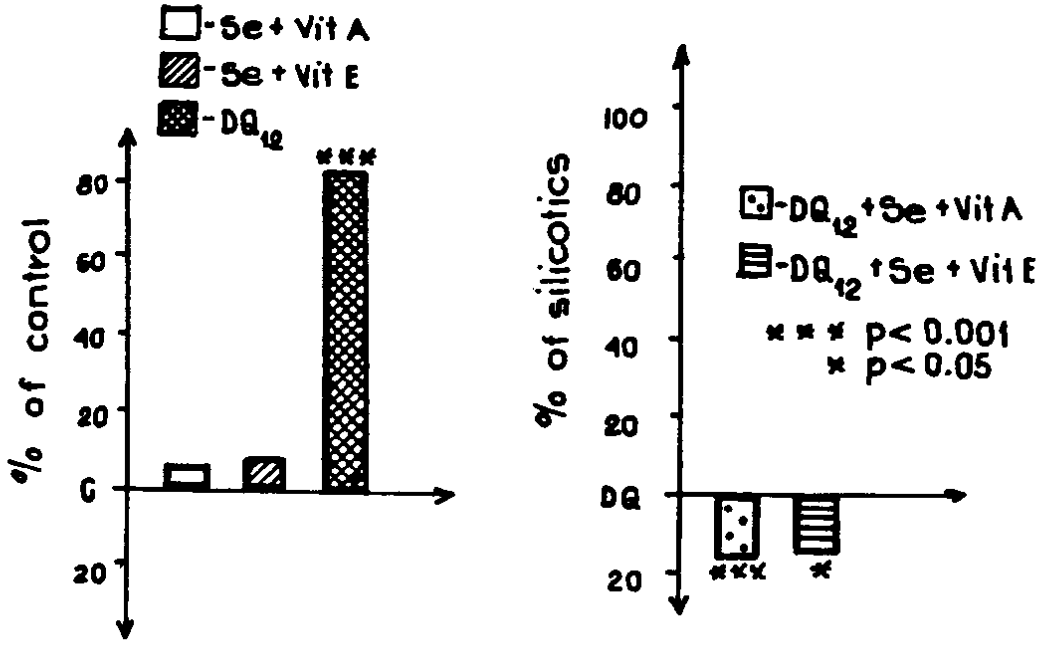


Figure 9. Lung G6P-DH of quartz-treated, Se+vitamin A, and Se+vitamin E-supplemented silicotic rats.

Experiment 3. No response in case of treatment with zinc and zinc + selenium was noted in lung weights of silicotic rats; however, the lymph node weights were found to be increased (Figure 10). In terms of biochemical lesions, zinc supplementation resulted in a significant decrease of lung lipid, phospholipid and hydroxyproline content (Figure 11 and 12). Concurrent administration of zinc and selenium showed the same pattern, except for lung lipids whose values did not differ from those of silicotic non-supplemented group.

Lung lipid peroxides of silicotics were significantly increased ($p < 0.01$) when zinc was supplemented, while zinc + selenium combination alleviated this effect. The failure of zinc to decrease lipid peroxidation induced by quartz dust might be explained by the reported bimodal response of this element in vivo: low doses inhibit, but higher doses enhance lipid peroxidation.[8,26] Most probably, the applied dose of 18.5 ppm zinc, though is considered to be physiological and non-toxic, under our experimental conditions was high enough to increase lipid peroxidation compared to control and silicotic rats.

Lung GSH-Px activity of silicotic rats kept on zinc showed a slight non-significant increment running in parallel with the observed lipid peroxide excess. Adding selenium to zinc resulted in an unexpected decline of this selenium-dependent enzyme at the borderline significance ($p < 0.05$).

Enhanced G6P-DH activity observed in silicotic rats was decreased by zinc supplementation to values significantly lower ($p < 0.001$) when compared to non-treated DQ12-instilled animals. Co-administered selenium to zinc failed to exhibit synergistic effect, the enzyme activity being reduced with a lower significance rate ($p < 0.01$). It is worth mentioning that the less pronounced effect of selenium in the presence of zinc supports the opinion that the biological role of the former might be diminished by its direct binding to the ionized zinc.[12] Consequently, a decreased availability of selenium to antioxidant enzyme systems occurs.

The design of our experiments does not allow a detailed discussion on the mechanisms of the antioxidant effects on silicosis. We can only speculate that selenium, vitamin A and vitamin E as well as their combinations act mainly on the course of inflammatory events preceding fibrosis, by trapping the formed free radicals, and, thus, preventing lipid peroxidation. Zinc, element with a broad range of biologic activity, though is a co-factor of the scavenging free radical metallo-enzyme superoxide dismutase, impairs mainly collagen synthesis and processing.

Our results confirm the reported previously protective effect of zinc with respect to induced by quartz lung collagen accumulation. The beneficial effect of zinc was explained by the interference with macrophage functions, having no direct effect on collagen deposition.[10] Recent evidence, however, indicates that zinc has a direct and selective preventive effect on rat lung collagen accumulation by inhibiting procollagen hydroxylation.[1]

In conclusion, our results give support to the hypothesis that the peroxidative damage plays an important additional role in the fibrotic action of quartz dust. One of the most significant findings in this study is that under antioxidant treatment silicotic fibrosis was diminished. Therefore, given correct concentrations, these antioxidants appear to be beneficial, as prophylactic and therapeutic agents in silicosis. Since the

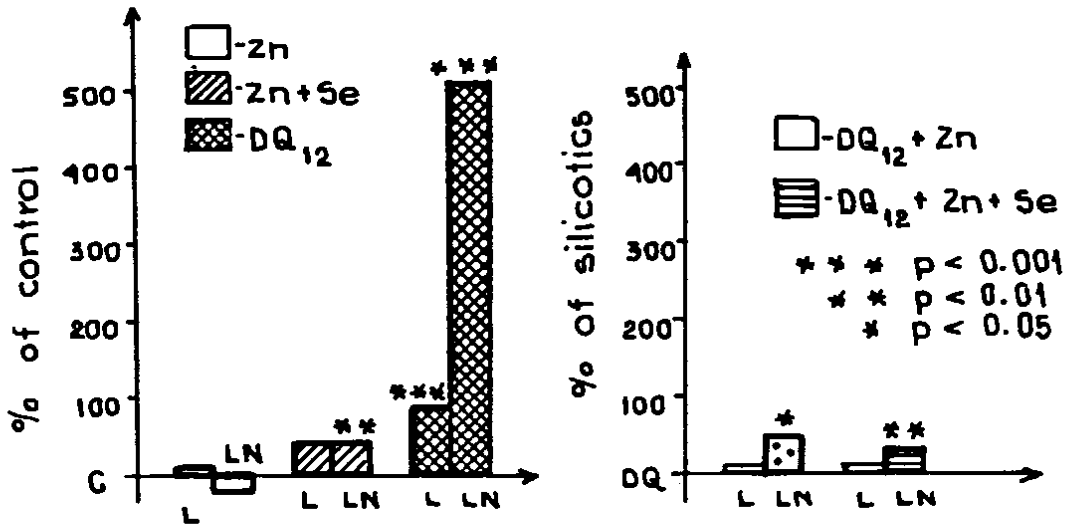


Figure 10. Lung and tracheal lymph node weights of quartz-treated, Zn and Zn+Se-supplemented silicotic rats.

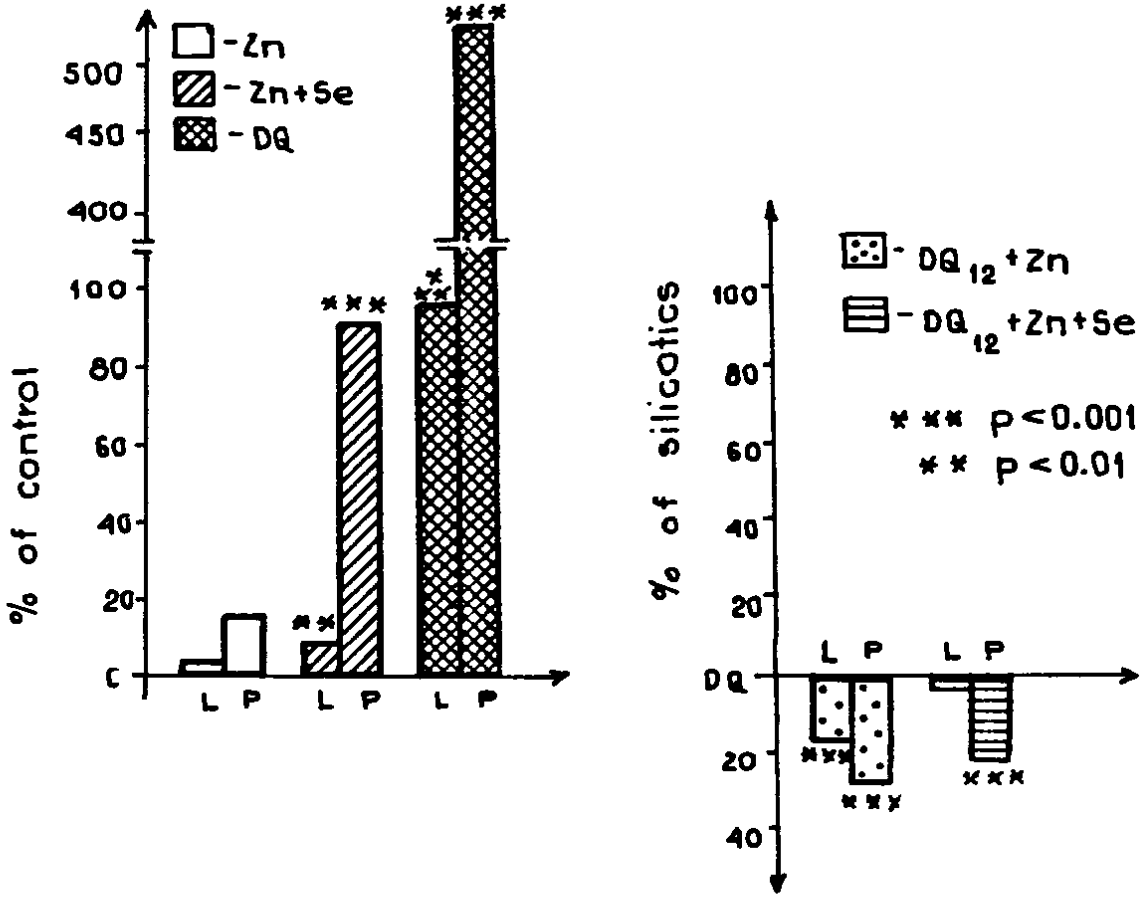


Figure 11. Lung LIPIDS and PHOSPHOLIPIDS of quartz-treated, Zn and Zn+Se-supplemented silicotic rats.

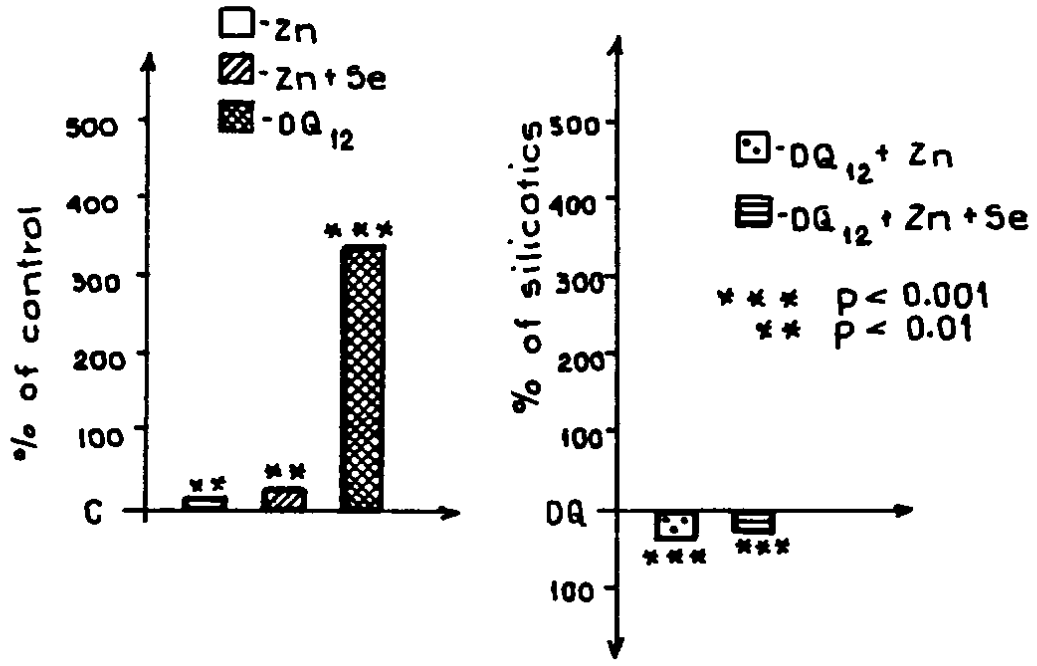


Figure 12. Lung HYPRO of quartz-treated, Zn and Zn+Se-supplemented silicotic rats.

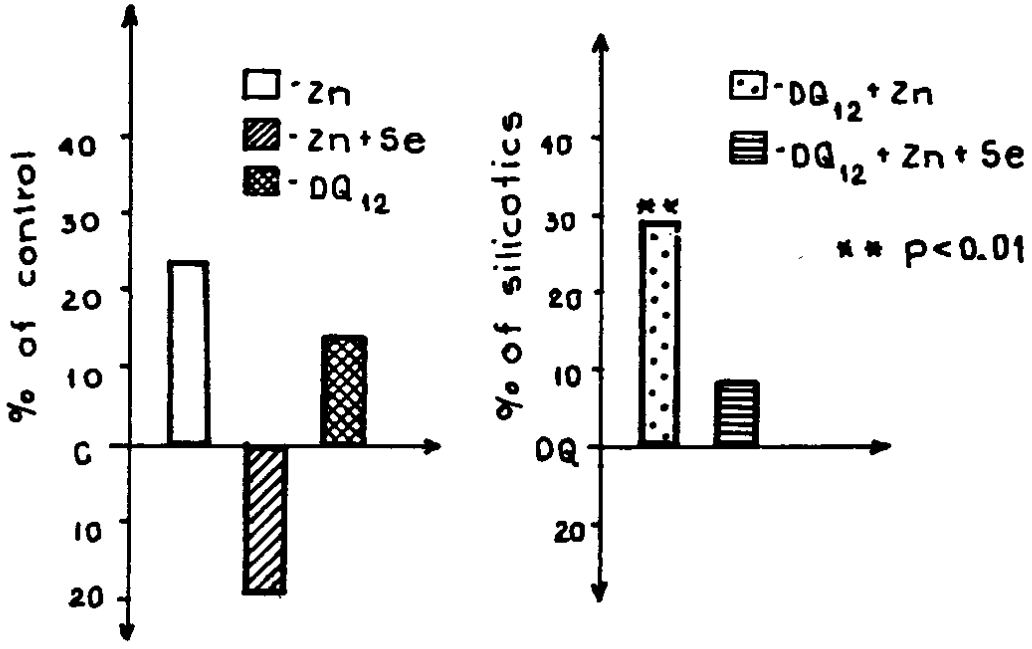


Figure 13. Lung LIPID PEROXIDES of quartz-treated, Zn and Zn+Se-supplemented silicotic rats.

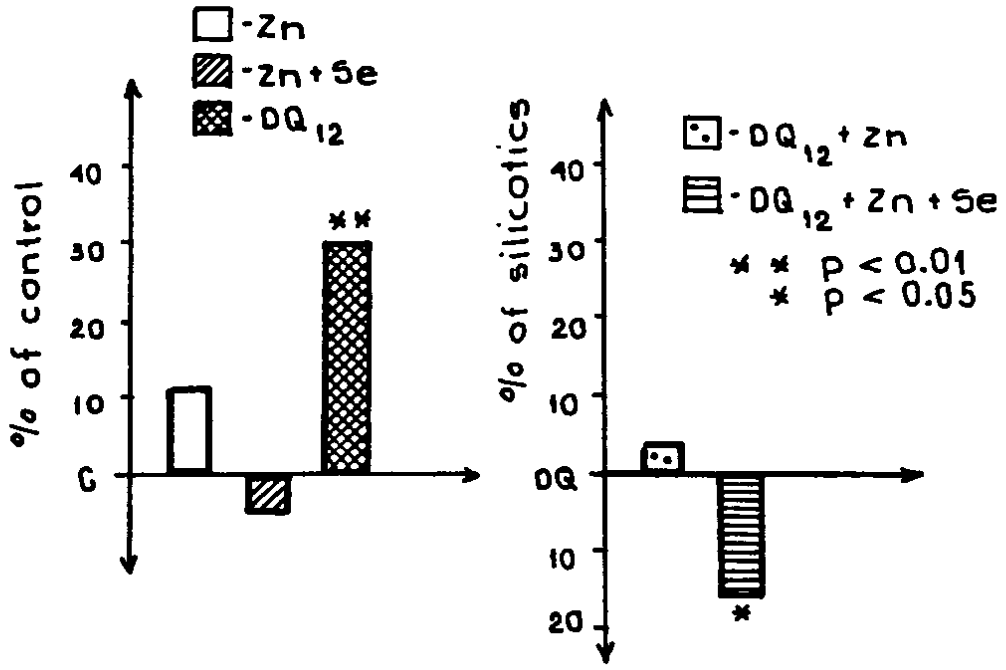


Figure 14. Lung GSH-Px of quartz-treated, Zn and Zn+Se-supplemented silicotic rats.

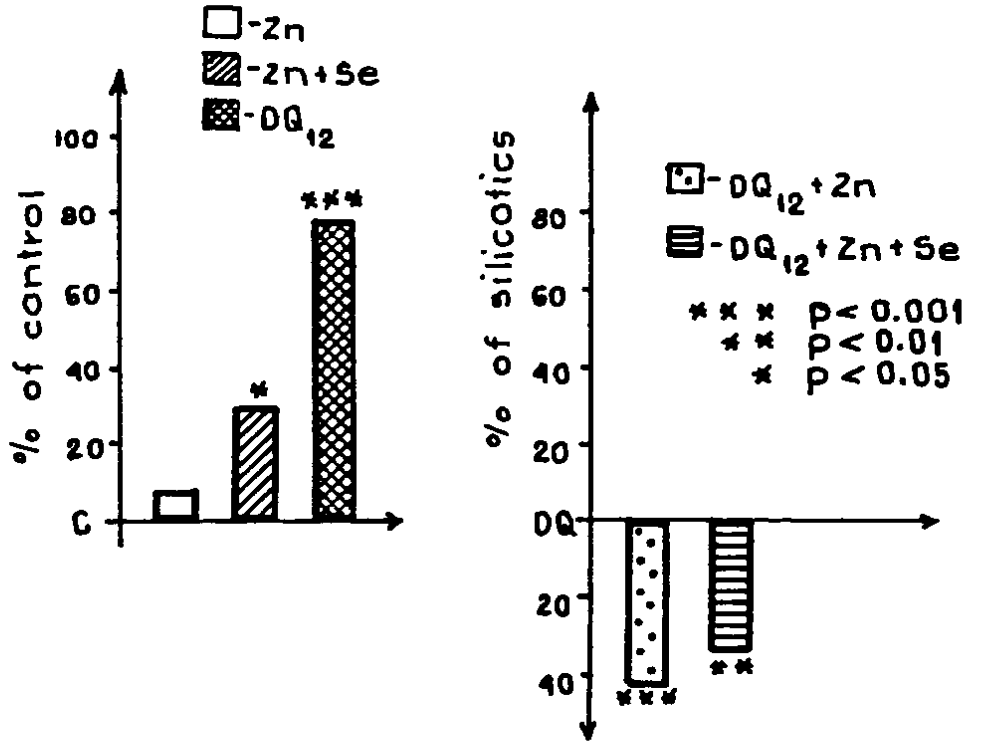


Figure 15. Lung G6P-DH of quartz-treated, Zn and Zn+Se-supplemented silicotic rats.

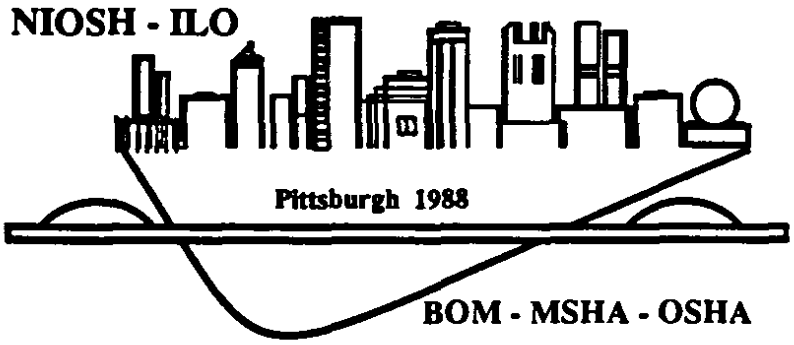
applicability of our findings to humans can only be speculated upon at this time, it is suggested that a clinical trial with antioxidant supplements to silicotic patients will have a similar effect.

REFERENCES

1. Anttinen, H., Oikarinen, A., Puistola, U., Pääkö, P., Ryhänen, L.: Prevention by Zinc of Rat Lung Collagen Accumulation in Carbon Tetrachloride Injury. *Am. Rev. Respir. Dis.*, 132:536-540 (1985).
2. Babushkina, L.G., Katsnelson, B.A., Kislitsina, N.S.: Effect of Antioxidants on Impairment of Lipid Metabolism and on Fibrogenesis in Lung Tissue under Conditions of Experimental Silicosis. *Biul. Exp. Biol. Med.*, 2:466-469 (1981).
3. Bergmeyer, H.U., Gasvethn, K., Grassl, M.: *Methods of Enzymatic Analysis*, Vol. 1, pp. 425-522, Bergmeyer, H.U. ed. Academic Press, New York (1974).
4. Bezrukavnikova, L.M., Arkhipova, O.G., Voznesenskaya, T.V.: Variations in Free Radical Reactions of Lipid Peroxidation under the Effect of Industrial Aerosols of Different Fibrogenicity. *Ghig. San.*, 3:80-81 (1987).
5. Borm, P.J.A., Bast, A., Wouters, E.F.M., Slangen, J.J., Swaen, G.M.H., deBorder, T.J.: Red Blood Cell-Antioxidant Parameters in Silicosis. *Int. Arch. Occup. Environ. Health*, 58:235-244 (1986).
6. Burk, R.F.: Biological Activity of Selenium. *Ann. Rev. Nutr.*, 3:53-70 (1983).
7. Burton, G.W., Ingold, K.U.: Mechanisms of Antioxidant Action. Studies on Vitamin E and Related Antioxidants in Biological Systems. *Protective Agents in Cancer*, pp. 1-92, Academic Press. London (1983).
8. Chvapil, M., Ryan, J.N., Zukoski, Ch.F.: Effect of Zinc on Lipid Peroxidation in Liver Microsomes and Mitochondria. *Proc. Soc. Exp. Biol. Med.*, 141:150-153 (1972).
9. Chvapil, M., Stankova, S., Malshet, V.: Lipid Peroxidation as one of the Mechanisms of Silica Fibrogenicity. *Environ. Res.*, 11:78-88 (1976).
10. Chvapil, M., Stankova, L., Weldy P.: The role of zinc in Function of some Inflammatory Cells. *Zinc Metabolism: Current Aspects in Health and Disease*, pp. 103-122. Brewer, G.J., Prasad, A.S. eds., Alan R. Liss Inc., New York (1977).
11. Diplock, A.T.: The Biological Function of Vitamin E and the Nature of Interaction of Vitamin with Selenium. *Wld. Rev. Nutr. Diet*, 31:178-183 (1978).
12. Eybl, V., Sykora, J., Mertl, F.: In Vivo Interaction of Selenium with Zinc. *Acta Pharmacol. Toxicol.* Suppl. VII, 59:547-548 (1986).
13. Folch, J., Lees, M., Sloane, Stanley G.H.A.: A Simple Method for the Isolation and Purification of Total Lipids from Animal Tissue. *J. Biol. Chem.*, 226:497-509 (1957).
14. Fukuzawa, K., Tomura, G.: Glutathione Peroxidase Activity in Tissue of Vitamin E-Deficient Mice. *J. Nutr. Sci. Vitaminol.*, 22:405-407 (1976).
15. Gabor, S., Zugravu, E., Frits, T., Anca, Z.: Taux des Lipoperoxides dans le poumons et le myocarde droit dans la silicose experimentale chez le rat. *Arch. Mal. Prof.* 32:553-558 (1971).
16. Gabor, S., Anca, Z.: Effect of Silica on Lipid Peroxidation in Red Cells. *Int. Arch. Arbeitsmed.* 32:327-332 (1974).
17. Gabor, S., Anca, Z., Zugravu, E.: In Vitro Action of Quartz on Alveolar Macrophage Lipid Peroxidation. *Arch. Environ. Health*, 30:499-501 (1975).
18. Gabor, S., Anca, Z., Zugravu, E., Ciugudeanu, M.: In Vitro and In Vivo Quartz-Induced Lipid Peroxidation. *The In Vitro Effects of Mineral Dusts*. pp. 131-137, R. C. Brown, M. Chamberlain, R. Davies, I.P. Gormley eds. Academic Press. London (1980).
19. Gabor, S., Ciugudeanu, M., Surcel, D.: Antioxidants in Relation to Quartz-Induced Peroxidative Damage in Macrophages. *VIth International Pneumoconiosis Conference*, Vol. 1, pp. 503-512. International Labour Organization (ILO), Geneva (1983).
20. Gabor, S., Ciugudeanu, M., Botoc, M., Surcel, D.: Selenium Effects on the Cytotoxicity and Lung Fibrosis Induced by Silica. *Acta Pharmacol. Toxicol.* Suppl. VII 59:191-194 (1986).
21. Gulumian, M., van Wyk, A.: Free Radical Scavenging Properties of Polyvinylpyridin N-Oxide: A Possible Mechanism for its Action in Pneumoconiosis. *Med. Lav.*, 78:124-128 (1987).
22. Gulumian, M., Kilroe-Smith, T.A.: Crocidolite-Induced Lipid Peroxidation II. Role of Antioxidants. *Environ. Res.*, 44:254-259 (1987).
23. Gupta, G.S., Kaw, J.L.: Formation of Lipid Peroxides in the Subcellular Fractions of Silicotic Lungs in Rats. *Eur. J. Respir Dis.* 63:183-187 (1982).
24. Hoekstra, W.G.: Biochemical Functions of Selenium and its Relation to Vitamin E. *Fed. Proc.*, 34:2083-2089 (1975).
25. Kappus, H.: Lipid Peroxidation: Mechanisms, Analysis, Enzymology and Biological Relevance. *Oxidative Stress* pp. 273-310, H. Sies ed. Academic Press Inc., London (1985).
26. Karl, L., Chvapil, M., Zukoski, Ch. F.: Effect of Zinc on the Viability and Phagocytic Capacity of Peritoneal Macrophages. *Proc. Soc. Exp. Biol. Med.*, 172:1123-1127 (1973).
27. Kubow, S., Bray, T.M., Bettger, W.J.: Effects of Dietary Zinc and Copper on Free Radical Production in Rat Lung and Liver. *Can. J. Physiol. Pharmacol.*, 64:1281-1285 (1986).
28. Marsh, J.B., Weinstein, D.B.: Simple Charring Method for Determination of Lipids. *J. Lipid Res.*, 7:574-576 (1966).
29. Neumann, R.E., Logan, M.A.: The Determination of Collagen and Elastin in Tissues. *J. Biol. Chem.*, 186:549-551 (1950).
30. Niki, E.: Antioxidants in Relation to Lipid Peroxidation. *Chem. Phys. Lipids*, 44:227-253 (1987).
31. Parker, F.R., Petersen, N.: Quantitative Analysis of Phospholipids and Phospholipid Fatty Acids from Silica Thin-Layer Chromatogramms. *J. Lipid Res.*, 6:455-462 (1965).
32. Serafini-Cessi, F., Cessi, F.: Thiobarbituric Acid Test as an Index of Phospholipid Peroxidation. *Sperimentale*, 5:371-378 (1968).
33. Schwartz, K.: Essentiality and Metabolic Functions of Selenium. *Med. Clin. North Am.*, 4:745-758 (1976).
34. Zsoldos, T., Tigyi, A., Montsko, T., Puppi, A.: Lipid peroxidation in the Membrane Damaging Effect of Silica-Containing Dust on Rat Lungs. *Exp. Pathol.*, 23:73-77 (1983).

Proceedings of the VIIth International Pneumoconioses Conference | *Part*
Transactions de la VIIe Conférence Internationale sur les Pneumoconioses | *Tome*
Transaciones de la VIIa Conferencia Internacional sobre las Neumoconiosis | *Parte*

I



Pittsburgh, Pennsylvania, USA—August 23–26, 1988
Pittsburgh, Pennsylvanie, Etats-Unis—23–26 aout 1988
Pittsburgh, Pennsylvania EE. UU—23–26 de agosto de 1988

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Centers for Disease Control
National Institute for Occupational Safety and Health

CDC
CENTERS FOR DISEASE CONTROL

Sponsors

International Labour Office (ILO)
National Institute for Occupational Safety and Health (NIOSH)
Mine Safety and Health Administration (MSHA)
Occupational Safety and Health Administration (OSHA)
Bureau of Mines (BOM)

September 1990

DISCLAIMER

Sponsorship of this conference and these proceedings by the sponsoring organizations does not constitute endorsement of the views expressed or recommendation for the use of any commercial product, commodity, or service mentioned.

The opinions and conclusions expressed herein are those of the authors and not the sponsoring organizations.

DHHS (NIOSH) Publication No. 90-108 Part I