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EFFECTS OF DECREASED GLUTATHIONE PEROXIDASE ACTIVITY ON THE PENTOSE PHOSPHATE CYCLE IN MOUSE LUNG

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SUMMARY: The effects of reducing glutathione peroxidase activity in the lung by changing dietary selenium intake has been investigated. In animals that were exposed to room air, selenium effects were confined to glutathione peroxidase activity, whereas under conditions of oxidant stress (ozone) the decrease in glutathione peroxidase activity prevented the stimulation of the pentose phosphate cycle (assayed by measuring glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activities) which has been reported to increase in response to oxidant stress. The suppression of glutathione peroxidase activity was found to depend on dietary selenium concentration. The physiological significance of this observation may be related to the process of injury and repair in the lung.

Glutathione peroxidase (GP) (EC.1.11.1.9) is a tetrameric enzyme containing four atoms of selenium (Se) (1). Selenium deficiency reduces GP activity in a variety of organs in different animal species (2,3,4). If Se deficiency is severe enough to limit GP activity, the cellular resistance to peroxidative damage may be impaired. The GP activity has been reported to participate in the reducing of cellular peroxides. The oxidized glutathione (GSSG) thus formed is then reduced to the original state (GSH) utilizing NADPH furnished by the pentose phosphate cycle (PPC). The major enzymes of the PPC are glucose-6-phosphate dehydrogenase (G6PD) (EC.1.1.1.49) and 6-phosphogluconate dehydrogenase (6PGD) (EC.1.1.1.44). The activities of these enzymes were reported to increase significantly in the lung after exposure to O_3 (8,9,10), NO_2 (10,11), hyperoxia (12,13,14) or paraquat (15). The correlation between sulfhydryl metabolism and glucose oxidation via the PPC was made using *in vitro* studies on isolated cells with sulfhydryl inhibitors (17,18) or by varying the amount of GSSG used in cell incubations (16,19). In this study we report that decreased pulmonary GP activity *in vivo*, caused by a selenium

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deficient diet, did not increase the PPC activities under oxidant stress, presumably because GSH was not available to be oxidized by the peroxides, and therefore the stimulus for increased PPC activity was not present.

MATERIAL AND METHODS: Weanling strain A/St mice, weighing 17-21 g., were fed a test diet (Tecklad #170698, Tecklad test diets, Madison, Wisc.) containing either 0 ppm Se (zero-Se), 0.15 ppm Se (low-Se), or 1.0 ppm Se (high-Se) for 11 weeks. All diets contained the same amount of vitamin E (55 ppm). Torula yeast containing organically bound Se, donated by Nutrition 21 (LaJolla, Ca.) was analyzed in our laboratory for Se content before mixing with the diet. Mice from each dietary group were exposed to 0.8 $\pm$ 0.05 ppm O_3 continuously for five days in stainless steel inhalation chambers (20). Ozone was generated by passing 100% O_2 (0.5 liter/min.) through an ozonizer (Sander, W. Germany). The O_3 level inside the chamber was continuously monitored using a Dasibi ozone monitor (Model 1003-PC, Environmental Corp., Glendale, Ca.). A matched number of mice were placed in identical chambers and breathed filtered room air. Animals in both chambers had free access to food and water. After O_3 exposure, both exposed and unexposed mice were anesthetized with an intraperitoneal injection of pentobarbital (50 mg/kg body weight), and sacrificed by exsanguination. The lungs were removed from each mouse, dissected free from connective tissue, rinsed in cold saline, blotted gently on filter paper and weighed. The tissue was homogenized in a Potter-Elvehjem glass-Teflon homogenizer using an ice cold medium containing 0.15 M sucrose, 0.15 M mannitol and 1 mM tris-HCl at pH 7.5. The homogenate was filtered through 2 layers of gauze and adjusted to a final volume of 4 mls. An aliquot of the homogenate was taken for DNA determination according to the method of Schneider (21) and the remainder was centrifuged at 40,000 x g at 4^{0}C for 30 minutes to obtain the cytosol (supernatant layer). The activity of GP was determined using cumene hydroperoxide as a substrate (11). Glutathione reductase (GR)(EC.1.6.4.2) activity was determined by following the rate of NADPH oxidation at 340 nm (22). The activities of G6PD and 6PGD were determined by following the rate of $NADP^+$ reduction at 340 nm (23). The enzyme activities, expressed as umoles/min.mg DNA, were reported as mean $\pm$ standard deviation of air or O_3-exposed mice (n = 4-9) in each dietary level.

RESULTS: Air-exposed mice on a Se-free diet (zero-Se group) had a 70% decrease in GP activity, compared to either high or low Se groups (Fig. 1A). However, the activities of the other enzymes examined (GR, G6PD, and 6PGD) remained essentially the same for all three dietary groups. After O_3 exposure, GP activity remained virtually unchanged when compared to their corresponding air-exposed mice. In contrast, the responses of GR, G6PD, and 6PGD activities to O_3 exposure were proportional to the dietary Se level. For example, GR activity in O_3-exposed lungs showed a significant drop (30%) in the zero-Se group relative to corresponding air-exposed lungs. There was essentially no change in low-Se lungs, and an increase (21%) in high-Se lungs (Fig. 1B). Similarly, the PPC assayed by the activities of G6PD and 6PGD increased progressively as a function of dietary Se levels. The activities of G6PD and

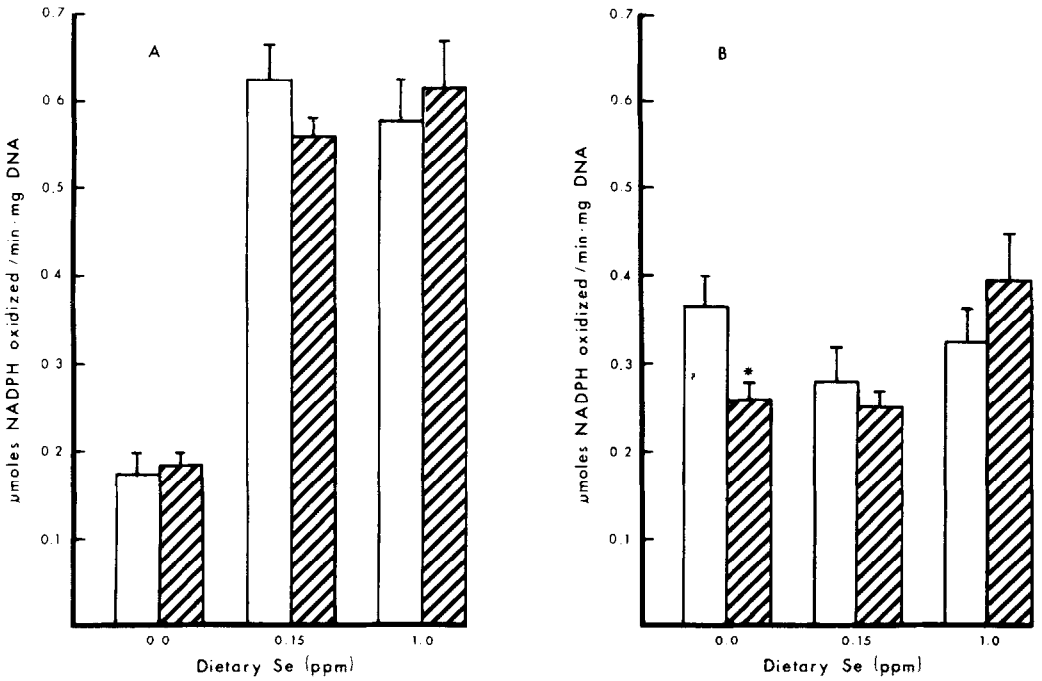


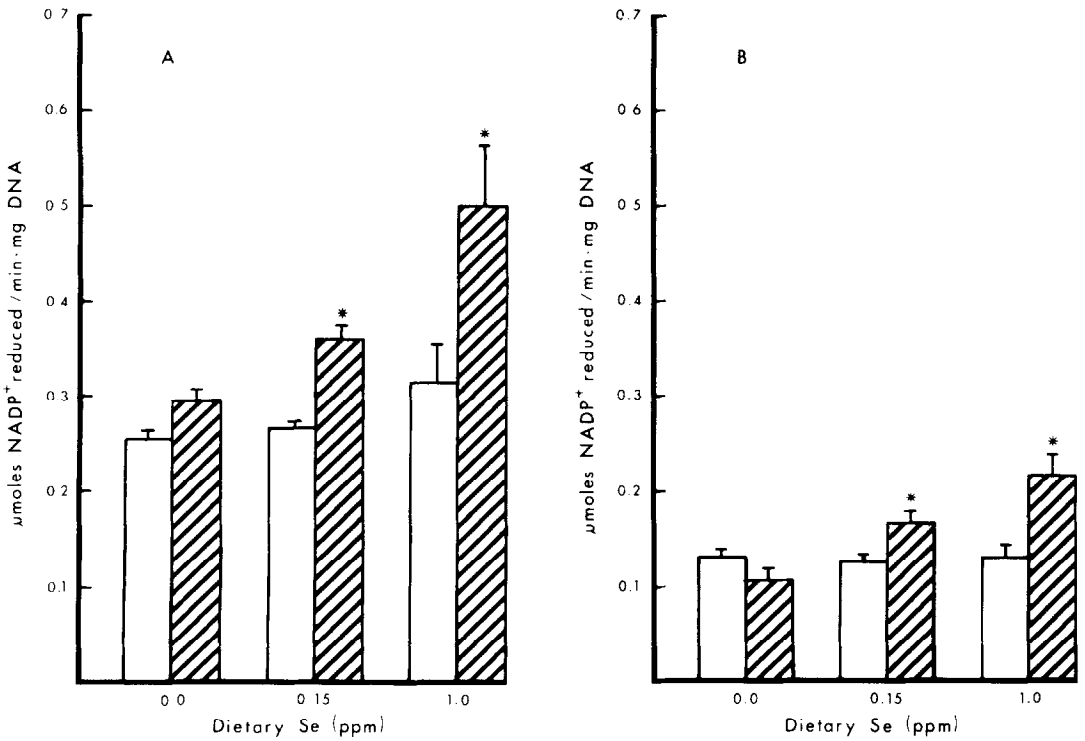
Figure 1. Effects of dietary selenium and ozone exposure on glutathione peroxidase activity (A) and glutathione reductase activity (B).

□ air-exposed, ▨ ozone-exposed

* statistically significant ($p < 0.05$)

6PGD in the zero-Se group remained essentially unchanged after O_3 exposure, but in the low-Se group the trend was reversed significantly and the two enzymes showed a 34% and a 32% increase ($p<0.05$) in their activities. The increases were even greater in high-Se, 59% for G6PD and 69% for 6PGD ($p<0.05$), relative to air-exposed lungs (Fig. 2).

DISCUSSION: Sulfhydryl oxidation-reduction and glucose oxidation via PPC generating NADPH are important cellular defense mechanisms by which the cell can withstand the deleterious effects of oxidant stress, whether the stress is exogenous such as inhaled O_3, NO_2, or high levels of O_2, or endogenous, such as hydrogen peroxide or other peroxides formed in the membrane. In the early experiments of Jacob and Jandel (1) inhibition of GSH by means of blockers such as N-ethylmaleimide (NEM) was shown to prevent the stimulation of PPC



Fioure 2. Effects of dietary selenium and ozone exposure on glucose-6-phosphate dehydrogenase activity (A) and 6-phosphogluconate dehydrogenase activity (B).

☐ air-exposed, ▨ ozone-exposed

* statistically significant ($p < 0.05$)

metabolism, and they suggested that blocking GSH alters the ratio of GSH/GSSG in favor of GSSG,

$$X_2O_2 + 2\ GSH \xrightarrow{GP} GSSG + 2\ XOH \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

$$GSSG + 2\ NADPH \xrightarrow{GR} 2\ GSH + 2\ NADP^+ \quad \ldots\ldots\ldots\ldots\ldots (2)$$

and they concluded that the sulfhydryls regulate PPC metabolism. Further studies by Eggleston and Krebs (16) suggested that the sulfhydryls exert a "coarse" control on PPC and that a "fine control is accomplished by NADPH. The physiological concentrations of free NADPH inhibit G6PD activity, the rate-limiting step of the oxidative pentose phosphate cycle, and this inhibition is competitive with $NADP^+$. Therefore, NADPH utilization (eq. 2) results in "de-inhibition" of the PPC.

$$G6P + NADP^+ \xrightarrow{G6PD} 6PG + NADPH \quad \ldots\ldots\ldots\ldots\ldots (3)$$

$$6PG + NADP^+ \xrightarrow{6PGD} R5P + NADPH \quad \ldots\ldots\ldots\ldots\ldots (4)$$

Recently, May (3) pointed out that using high concentrations of NEM might be too harsh a treatment which may not only block GSH, but also inhibit cellular PPC metabolism. Therefore, he studied PPC activity and found that increasing GSSG/GSH in the medium triggers PPC activity.

In this study we observed that under conditions of ozone exposure, reduced GP activity was accompanied by a significant drop in GR activity (Fig. 1B). A possible explanation for this drop is that the decreased GP activity reduced the rate of GSH oxidation (eq. 1) which in turn reduced the demand for NADPH utilized in GSH regeneration (eq. 2). This resulted in the NADPH/$NADP^+$ ratio remaining high enough to inhibit PPC activity (eq. 3,4). This observation suggests that the relationship between glutathione and PPC was also enzyme dependent and could be affected by dietary Se under conditions of oxidant stress. The physiological significance of these findings are: i) Decreased GP activity may affect the cell's capacity to destroy the peroxides formed under oxidant stress by decreasing its reductive detoxification capacity. ii) Since PPC has been reported to contribute to the biosynthetic processes in cells under oxidant stress where cell injury does occur (12), it follows that suppression of this important pathway may interfere with the capacity of injured cells to regenerate new cell components, i.e., by the repair process. iii) Although 0.1-0.2 ppm Se in the diet is considered adequate, under conditions of oxidant stress, supplementing this level may be beneficial.

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REFERENCES

1 - Wendel, A., Kerner, B. and Graupe, K. (1978) Functions of glutathione in liver and Kidney, pp. 107-113, Springer-Verlag, Berlin.

2 - Hafeman, D.G., Sunde, R.A. and Hoekstra, W.G. (1974) J. Nutr. 104, 580-587.

3 - Chow, C.K. and Tappel, A.L. (1974) J. Nutr. 104, 444-451.

4 - Omaye, S.T. and Tappel, A.L. (1974) J. Nutr. 104, 747-753.

5 - Cohen, G., and Hochstein, P. (1963) Biochemistry 2, 1420-

6 - Christopherson, B.O. (1969) Biochem. Biophys. Acta 176, 463-470.

7 - Little, C. and O'Brien, P.J. (1968) Biochem. Biophys. Res. Comm. 31, 145-150.

8 - Chow, C.K. and Tappel, A.L. (1972) Lipids 7, 517-524.

9 - Chow, C.K. and Tappel, A.L. (1973) Arch. Environ. Hlth. 26, 205-208.

10- Mustafa, M.G. and Tierney, D.F. (1978) Am. Rev. Respir. Dis. 118, 1061-1090.

11- Mustafa, M.G., Elsayed, N., Lim, J.S.T., Postlethwait, E., and Lee, S.K. (1979) Nitrogenous Air Pollutant, pp. 165-178, Ann Arbor Science, Ann Arbor Mich.

12- Tierney, D., Ayres, L., Herzog, S. and Yang, J. (1973) Am. Rev. Respir. Dis. 108, 1348-1351.

13- Tierney, D.F., Ayers, L. and Kasuyama, R.S. (1977) Am. Rev. Respir. Dis. 115, 59-65.

14- Nishiki, K., Jamison, D., Oshino, N. and Chance, B. (1976) Biochem. J. 160, 343-355.

15- Burk, R.F., Niskiki, K., Lawrence, R.A. and Chance, B. (1978) J. Biol. Chem. 253, 43-46.

16- Eggleston, L.V. and Krebs, H.A. (1974) Biochem. J. 138, 425-435.

17- Jacob, H.S. and Jandel, J.H. (1966) J. Biol. Chem. 241, 4243-4250.

18- Reed, P.W. (1969) J. Biol. Chem. 244, 2459-2464.

19- May, J.M. (1981) Arch. Biochem. Biophys. 207, 117-217.

20- Hinners, R.G., Burkart, J.K. and Punte, C.L. (1968) Arch. Environ. Hlth. 16, 194-206.

21- Schneider, W.C. (1956) Methods of Enzymology 3, 680-684.

22- Horn, H.D. (1965) Methods of Enzymatic Analysis, pp. 876-879 Academic Press, N.Y.

23- Lohr, B.W. and Waller, H.D. (1965) Methods of Enzymatic Analysis, pp. 744-751, Academic Press, N.Y.