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# RED BLOOD CELL ANTI-OXIDANT PARAMETERS IN HEALTHY ELDERLY SUBJECTS VERSUS SILICOSIS PATIENTS

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The anti-oxidant phenotype was determined in red blood cell haemolysates of 62 healthy elderly persons (Mean age: 56) and a number of male silicosis patients (Mean age: 65, $n = 19$). Moreover, analysis of watersoluble fluorescent substances in plasma, recently introduced as a new test for *in vivo* lipidperoxidation, was included. Within the control group results were analyzed on the effect of smoking (no effect), use of medication (lowered GSH-content) or gender (no differences apart from haemoglobine content). No simple relationship between any pair of the measured parameters in erythrocytes was present. When comparing the male control persons with the silicosis group a significantly higher red blood cell GSH-level was observed in the latter. Moreover, some factors of the anti-oxidant system are strongly correlated in the diseased, but not in the healthy subjects.

KEY WORDS: Silicosis, anti-oxidant factors, red blood cell.



## INTRODUCTION

A continuously growing body of evidence suggests that the human inflammatory immunologic axis may be etiologic in diseases as pulmonary, joint and endothelial diseases.[1] Many materials secreted by inflammatory cells are cytotoxic, e.g. $H_2O_2$, HOCl, elastase and $O_2^-$ and are capable of initiating and/or prolonging tissue injury.[2,3]

Most of these compounds are also generated during the immune defense reaction against free silica and are released from alveolar macrophages in the lung. Their cytotoxic effect varies from peroxidizing lipids, depolymerizing polysaccharides, altering enzyme activity (e.g. alpha-1-antitrypsin), cleaving DNA to transforming or/and killing whole cells.[3]

Fortunately, cells have an elaborate defense mechanism against released active species. Every respiring cell produces free radicals,[4] the reduction of $O_2$ to $H_2O$ and the cytochrome P-450 enzyme system being the most important sources of radicals. The defense line against released radicals is formed by several antioxidant enzymes

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(superoxide dismutase, SOD, catalase and GSH-peroxidases), low molecular weight sulfhydryls (glutathione, cysteine, cysteinylglycine) and some additional physiological molecules (vitamins C and E, $\beta$-carotene and urate).[5,6]

In normal state the antioxidant system is able to deal with the continuous stream of radicals. A disturbance in the balance on either side, i.e. either by oxidative burst of phagocytes or by deficient or malfunctioning components of the antioxidant system in lung tissue can provoke a devastating response.[2,7]

Our hypothesis is that differences in individual susceptibility towards inhaled silica dust[8–10] might be associated with a certain antioxidant phenotype in the lung. People susceptible to the fibrogenetic effect of silica might be less well equipped to deal with the active species released by alveolar macrophaghes following prolonged exposure to silica dust.

This study is a pilot study to investigate the above hypothesis. The main purpose was to investigate whether differences were present in anti-oxidant parameters using red blood cells and plasma as relatively easy obtainable media.

Therefore we studied in detail some frequently assayed anti-oxidant parameters in a population of healthy (elderly) blood donors and established the effects of smoking, gender and use of medication. The obtained data were used to filter bias and to interprete differences between red blood cell parameters of the control group and the silicosis patients. Like lung tissue cells, red cells are normally exposed to high concentrations of $O_2$ and therefore possess a number of defense mechanisms.[11] Moreover, erythrocytes can act as a circulating antioxidant system due to their deformability and ability to inactivate locally released $H_2O_2$ and $O_2^-$ in the lung.[5] The results of this study could provide a potential means or lead for screening out those whome it would be unwise to employ in settings with high silica containing dust exposure, because they are at higher risk of developing silicosis.

## MATERIALS AND METHODS

### *Chemicals*

Superoxide dismutase (SOD, bovine erythrocytes), xanthine oxidase (Grade I, buttermilk), cytochrome c (Type III, horse heart), haemoglobin (Type I, beef blood), GSSG-reductase (Type III), NADPH and o-phtaldialdehyde were obtained from Sigma. Reduced (GSH) and oxidized (GSSG) glutathione were products of Boehringer (Mannheim, West Germany). Xanthine and N-ethylmaleimide (NEM) were purchased from Merck and Aldrich (Beerse, Belgium), respectively.

All other chemical used were of analytical grade.

### *Sample Collection and Processing*

Blood samples were drawn from healthy blood donors, all from the caucasion race and aged between 50 and 65, and a number of male silicosis patients receiving medical treatment for CARA in the hospital of St. Annadal, Maastricht. Samples were taken between April and September in 1985. All subjects were living in Maastricht or its suburban area, in the south of the Netherlands. Control subjects were asked for their birthdate, present use of medications and smoking behavior. Information on clinical history was available from blood bank data. For silicosis patients the necessary

TABLE I

Frequency distributions of age, smoking, medication and occupational history on the control group and the silicosis patients

| | Control group | | Silicosis patients |
|---|---|---|---|
| | *Female* | *Male* | (*All Male*) |
| Age | | | |
| Mean ± S.D | 56.9 ± 4.4 | 55.5 ± 4.7 | 64.9 ± 6.7 |
| Range | 51–65 | 50–65 | 52–76 |
| Number | 12 | 50 | 20 |
| Smoking | | | |
| Yes | 7 | 23 | 8 |
| No | 5 | 27 | 12 |
| Medication | | | |
| Yes | 3 | 8 | 20 |
| No | 9 | 42 | 0 |
| Type of medication | | | |
| Circulatory[a] | 2 | 2 | 12 |
| Bronchodilating agents[b] | 1 | – | 19 |
| Mucolytics | – | – | 2 |
| Corticosteriods | – | – | 9 |
| Diuretics | – | 2 | 8 |
| Others | – | 4 | 2 |
| Former Occupation[c] | | | |
| Miners | | - | 15 |
| Ceramic Industry | - | – | 4 |
| Foundary Workers | - | – | 1 |
| Years exposed | | | |
| Mean ± S.D | – | – | 24.3 ± 8.7 |
| Range | – | – | 10–38 |

[a] All drugs affecting the circulatory and/or cardiovascular function are included.

[b] Included are: sympathomimetic bronchodilators, theophylline and its derivatives, and bronchospasmolytics.

[c] The control subjects were not interviewed for occupational history.

information was obtained from their medical records. Some data of the investigated groups are given in Table I.

Samples of blood were taken by venapuncture using evacuated tubes and immediately transferred into a glass tube containing 1.0 ml of sodium-citrate (3.8%, pH 7.4). One ml of sodium citrate was mixed with 5 ml blood (1:5, v/v). Isoosmotic phosphate-buffered saline (9 mM $KH_2PO_4$, 34 mM $Na_2HPO_4$, 90 mM NaCl) was used for washing red cells three times. Centrifugation was performed at 4°C (10 min, 3000 rpm). Plasma as well as the washed pellets consisting of red blood cells (RBC) were stored at −80°C until analysis.

At time of the analysis RBC pellets were thawn at room temperature. To 1.0 ml pellet 2.5 ml cold distilled water was added, vortexed, again frozen, at −80°C and thawed. After centrifugation of the haemolysate, the supernatant was divided into three 1.0 ml portions and frozen until analysis (within one week). Plasma was used immediately after thawing.

*Haemoglobin determination*

Immediately after sampling, haemoglobin analysis in whole blood was done in a blood-gas analyzer (V: CO-Oximeter Model 282, Laboratory Instruments); the HbCO amount gave us a mean for checking smoking/non-smoking behaviour.

Total haemoglobin was measured in the red cell lysate by the following procedure: to 1.0 ml haemolysate 1.0 ml of solution 4 mM $K_3Fe(CN)_6$, 30 mM KCN and 1 mM EDTA in 0.1 M phosphatebuffer (pH 7.0) was added and mixed. After 10 minutes, 30 $\mu$l of the above mixture was diluted in 3 ml $H_2O$ and its extinction was measured at 540 nm. A standard solution of haemoglobin was prepared in water (1–200 mg/ml) and subjected to the same procedure.

*GSH-Peroxidase measurement*

The assay of GSH-$P_x$ activity in red cell lysates was performed in combination with the determination of haemoglobin. One ml of haemolysate is mixed with an equal volume of the potassium ferricyanide and KCN containing buffer (pH 7.0) as above.

GSH-$P_x$ was then assayed by the method of Maral *et al.* (1977);[12] 50 $\mu$l of the mixture was added to the incubation mixture containing 1 mM GSH, 200 $\mu$M NADPH and 1 IU/ml GSSG-reductase and the rate of oxidation of NADPH was followed during 3 min at 340 nm in a Beckman Model 24 UV/VIS spectrophotometer. The peroxide used was t-butylhydroperoxide at a final concentration of 1 mM. The reaction was started by addition of the peroxide (substrate) and NADPH consumption was corrected for the nonenzymatic conversion of GSH by t-butylhydroperoxide.

Units of enzyme activity were expressed as nmol NADPH consumed per min per mg haemoglobin using a molar extinction coefficient for NADPH of $6.22 \times 10^3 \text{mM}^{-1}.\text{cm}^{-1}$.

*Superoxide dismutase measurement*

SOD activity was assayed in red cell lysates after removal of haemoglobin. Therefore 1.0 ml of lysate was added to 1.0 ml of a mixture of chloroform/ethanol = 1:2 (v/v). After mixing thoroughly (vortex, 10 seconds) the mixture is centrifuged for 5 min at 3000 rpm (4°C). One hundred $\mu$l of the supernatant is used in the SOD-assay. The activity when in the supernatant was described to be stable at least during 10 days at 4°C.[12]

SOD activity was determined by the method of McCord and Fridovich,[13] based on the inhibition of cyochrome *c* reduction. The reaction was started by the xanthine/xanthine oxidase system. A standard curve was prepared using commercially available SOD (Sigma).

*Determination of reduced glutathione (GSH)*

GSH was assayed according to the method described by Hissin and Hilf[14] using *o*-phtalaldehyde as a fluorescent agent. To 1.0 ml haemolysate 0.266 ml TCA (25%) was added and mixed. After precipitation was complete (15 min, 4°C) 2.0 ml distilled water was added and the samples were centrifuged for 10 min at 3000 rpm. The clear supernatant was used for the assay of GSH (0.3 ml sup). Standard solutions of GSH (0.1–1 mM) were subjected to the same procedure (apart from the addition of 2.0 ml $H_2O$) and used to calculate the glutathione content in RBC-lysates.

Fluorescence was measured at exc 350 nm and em 460 nm, using a Perkin Elmer Model 3000 fluorimeter (Both slitwidths 2.5 nm), equipped with a Sipper system (Hellma cuv-o-mat).

*Determination of watersoluble fluorescent substances (WFS)*

Watersoluble fluorescent substances were measured in the plasma of individuals using a procedure as described by Tsuchida *et al.* (1985).[15] 100 $\mu$l of plasma were pipetted into a 10 ml glass test tube and 4 ml of ethanol/ether (3:1, v/v) were poured into it. The tubes were mixed thoroughly for 15 seconds, allowed to stand 5 min and centrifuged 5 min for 3000 rpm. The supernatant was discarded and another 4 ml of the solvent mixture was added to the pellet, mixed on a vortex (2 seconds) and again centrifuged. The solvent was discarded and the sediment dissolved in 2 ml of distilled water. The fluorescence of the solution was then measured (see above) at an excitation wavelength of 350 nm and emission of 460 nm. Sensitivity dial at 10 and both slit-widths at 2.5 nm.

*Statistical analysis*

Analytical results have been evaluated using the "SPSX-Program" (SPSS-x Inc.) incorporated in a VAX/VMS computer (Digital, version V 4.1). The difference between the means of a variable in two groups has been tested with the Mann-Whitney U-test (Tables II and III). Odds-ratio's (Table III) were calculated using variables dichotomized at the total group mean and 95%-confidence intervals were calculated according to Lilienfeld and Lilienfeld.[16]

Spearman's correlation was used as a test for correlation between pairs of (not normally distributed) parameters.

Subsequently, the intercorrelated parameters were related in a multiple regression analysis, and the significance of the fit was calculated using analysis of variances (ANOVA).

## RESULTS

*Control subjects*

Table II gives the measured values of the assayed parameters in the control group as a total and with respect to gender, smoking and use of medication.

Apart from a lower Hb in red blood cells of the female subjects ($P < 0.005$) as compared with male subjects, we found that neither gender nor smoking had a significant effect on any of the parameters measured in red blood cells or plasma (WFS).

On the other hand, a reduced GSH-content was measured in subjects using medication ($P < 0.03$). The group is too small, however, to indicate which type of medication is (mainly) responsible.

No other parameters appeared to be influenced by current use of medication.

*Silicosis vs. controls*

From Table I it can be seen that the former occupation for the majority of silicosis patients was coalmining. Interestingly, 4 out of 20 patients has been working in the

TABLE II
Values for the anti-oxidant factors determined in plasma (WFS) and red blood cells (rest) of healthy elderly control subjects

| Parameter tested | Total group ($n$ = 62) | Smokers ($n$ = 30) | Non-Smokers ($n$ = 32) | Male ($n$ = 50) | Female ($n$ = 12) | Medication Yes ($n$ = 11) | Medication No ($n$ = 51) |
|---|---|---|---|---|---|---|---|
| $Hb_{total\ blood}$ | 15.1 ± 1.5 | 15.2 ± 1.7 | 15.0 ± 1.3 | 15.4 ± 1.5 | 14.0 ± 1.1[b] | 15.9 ± 1.4 | 14.9 ± 1.5 |
| $Hb_{lysate}$ | 112.0 ± 9.1 | 110.4 ± 10.4 | 113.4 ± 7.5 | 113.5 ± 8.3 | 105.5 ± 9.8[b] | 112.8 ± 14.0 | 111.8 ± 7.8 |
| $GSH_{RBC}$ | 5.43 ± 1.22 | 5.57 ± 1.28 | 5.29 ± 1.16 | 5.45 ± 1.12 | 5.34 ± 1.42 | 4.64 ± 1.39 | 5.60 ± 1.12[a] |
| GSH-$P_x$ | 2.85 ± 0.88 | 2.72 ± 0.75 | 2.97 ± 0.98 | 2.84 ± 0.92 | 2.90 ± 0.71 | 3.01 ± 0.54 | 2.80 ± 0.93 |
| SOD | 6.27 ± 0.43 | 6.25 ± 0.41 | 6.29 ± 0.46 | 6.28 ± 0.39 | 6.22 ± 0.60 | 6.25 ± 0.55 | 6.27 ± 0.41 |
| WFS | 11.9 ± 2.7 | 12.1 ± 3.0 | 11.8 ± 2.5 | 12.0 ± 2.7 | 11.5 ± 2.8 | 12.0 ± 3.2 | 11.9 ± 2.7 |

Listed values are mean ± S.D of the indicated number ($n$) of subjects.
$Hb_{total}$ is expressed as gram Hb/100 ml blood, $Hb_{lysate}$ as mg Hb/ml lysate, $GSH_{RBC}$ as μmol/g Hb.
GSH-$P_x$ in μmoles NADPH. $min^{-1}.gHb^{-1}$, SOD in U/ml haemolysate, WFS is in relative fluorescence units × 10.
Significance of the difference with the reference group was tested using a non-parametric test for unequal samples: Mann-Whitney U-test. [a]$P < 0.01$
[b]$P < 0.001$

TABLE III
Comparison of blood Haemoglobin (Hb) and antioxidant factors in male control subjects and silicosis patients

| Parameter[a] | Total group ($n$ = 67) | Controls ($n$ = 48) | Silicosis patients ($n$ = 19) | Odds-ratio (95% confidence interval) |
|---|---|---|---|---|
| Hb (g/100 ml) | 15.4 ± 1.4 | 15.3 ± 1.5 | 15.4 ± 1.3 (NS)[b] | 0.81 (0.3–2.3) |
| $Hb_{lysate}$ (mg/ml lysate) | 114.3 ± 8.3[c] | 113.7 ± 8.3 | 115.8 ± 8.5 (NS) | 0.89 (0.3–2.5) |
| GSH ($\mu$mol/g Hb) | 5.78 ± 1.16 | 5.40 ± 1.10 | 6.68 ± 1.40 ($P < 0.001$) | 3.20 (1.1–9.4) |
| GSH-$P_x$ $\mu$moles NADPH · $min^{-1}$ . g $Hb^{-1}$ | 2.74 ± 0.85 | 2.86 ± 0.93 | 2.44 ± 0.52 ($P < 0.1$) | 2.53 (0.84–7.6) |
| SOD units/ml lysate | 6.23 ± 0.45 | 6.28 ± 0.39 | 6.10 ± 0.56 ($P < 0.08$) | 2.58 (0.9–7.5) |
| WFS | 12.0 ± 3.7 | 12.0 ±2.7 | 11.9 ±5.6 (NS) | 1.83 (0.6–5.4) |

All listed values are mean ± S.D

[b] Testing for significance was done by the non-parametric Mann-Whitney U-test.

[a] Neither smoking, nor use of medication (Table I) was associated with a change in any parameter. Only GSH was significantly increased ($P < 0.03$) in control subjects receiving medication (Table II); when tested within the total group (42 vs. 26) this effect was on the border of significance ($P < 0.064$).

[c] Conforms to 18.7 ± 0.2 Hb/100 ml when recalculated with Ht: 0.47 and dilution 3.5.

ceramic industry. Moreover, 1 person working for 18 years in the mines and 10 years in a ceramic industry was scored in the mining category.

Table III shows the mean values ± S.D of the assayed parameters in the male control group and silicosis patients. Apart from reduced glutathione (GSH) no differences in Hb or ani-oxidant factors were observed between male control subjects and silicosis patients ($P < 0.1$). Both GSH-$P_x$ and SOD are slightly lowered in the patient group; these decreases, however, are not statistically significant.

GSH, is increased ($P < 0.001$, Mann Whitney-U test) in patients having silicosis. As already mentioned GSH is *decreased* in control subjects receiving medication ($P < 0.03$). However, when tested within the total group this effect was on the border of significance ($P < 0.068$).

Odds-ratio's were calculated as a quantitative measure of risk of having silicosis in the presence of a certain factor. The mathematical mean of the total group was used as a cut-off point. For example, if someone's GSH in erythrocytes is 5.78 μmol GSH/g Hb or more, he has a 3.2 fold risk of being silicosis patient (Table III). Confidence intervals (95%), however, are wide due to the relatively small number of tested individuals.

No significant correlation between any of two measured anti-oxidant parameters was observed in the control group. This pattern is changed remarkably within the group of silicosis patients.

While in male control subjects only Hb in lysate was found to be slightly correlated with SOD-activity in red blood cells ($r = 0.300$, $n = 47$) Spearman's correlation resulted in one pair of correlated parameters within the silicosis group:

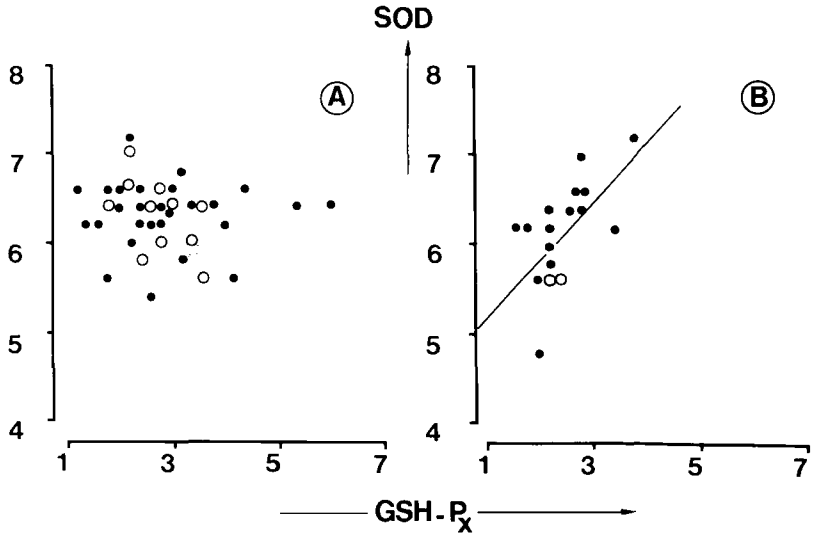


FIGURE 1 Scatterdiagrams of superoxide dismutase (SOD) activity (Units .$ml^{-1}$lysate) versus glutathione peroxidase (GSH-$P_x$) activity (μmoles NADPH. $min^{-1}$.g $Hb^{-1}$). Enzyme activities were determined in red blood cell lysates of control subjects (panel A, $n = 48$) and silicosis patients (panel B, $n = 19$). The values of individual subjects are represented by the symbol, ●; if two subjects coincided the symbol ○ is used. Linear regression resulted in correlation coefficients of $r = -0.138$ ($n = 48$, NS) in the control group and $r = 0.607$ ($n = 19$, $P < 0.004$) in silicosis patients.

– SOD with GSH-$P_x$: $r = 0.593$ ($n = 19$), $P < 0.004$

When investigating this relationship in detail using multiple linear regression, the variance in SOD-activity can be well explained by the variance in the other five parameters ($r = 0.74$; $P < 0.05$ tested with ANOVA); this, again, in silicosis patients only.

Finally, Fig. 1 gives a graphical presentation of this relationship between SOD and GSH-$P_x$ in control subjects and silicosis patients.

## DISCUSSION

Many theories to explain the fibrinogenesis in different types of pneumoconioses have been elaborated in the past. The silicotic process appears to be *initiated* by the cytotoxic effect of free silica on alveolar macrophages and the release of fibrogenic factor(s) from these cells. As a result, a number of lysosomal enzyme activities are increased in serum of silicosis patients.[17-19] This study was designed to explore differences in individual susceptibility towards inhaled silica dust.[8-10]

We suggest that people susceptible to the fibrogenetic effect of silica might be less well equiped to deal with the active species ($O_2^{\cdot}$, $H_2O_2$, HOCl) released by alveolar macrophages after exposure to silica. Several anti-oxidant parameters in red blood cells and serum may reflect a toxic risk to lung tissue. Several reasons to adopt this assumption can be mentioned. Firstly, a relation was observed between red blood cells and lung tissue levels of anti-oxidant enzymes.[20,21] Secondly, it was demonstrated in rabbits[22] that chronic inflammation in the lung results in changes in the anti-oxidant system and in lipidperoxidation products in lung tissue and red blood cells.

Thirdly, erythrocytes may act as a circulating anti-oxidant system.[23,24]

When comparing the mean values of the assayed parameters (Table III) no striking differences are observed between silicosis patients and the control group. Only reduced glutathoine, GSH, is significantly *increased* in patients. This finding is strengthened by our observation that control subjects receiving medication had a significantly lower red blood cell GSH-content as compared to subjects receiving no medication ($P < 0.03$). Medication groups (see also Table I), however, were too small to indicate which type of medication was (mainly) responsible for this decrease in GSH.

A preliminary conclusion would be that medication is not the cause of the increased GSH content in silicosis patients. Still, the rise in the patients' GSH could be explained by the *different type* of medication (Table I). Most patients are treated by a therapy including: $\beta_2$-sympathicomimetics, theophyllin, anticholinergic agents and corticosteriods. An interference particularly with N-acetylcysteine (2 out of 19 patients) and/or prednisolon (8/19) of glutathione synthesis cannot be excluded.[25]

Secondly, it is possible that the higher GSH-content is the indirect result of the lowered GSH-peroxidase activity. However, no significant (negative) correlation was observed between GSH and GSH-$P_x$ in the Spearman's correlation test.

Thirdly, it could be that the increased GSH is not changed due to the disease itself but that high GSH is (genetic) a determinant for developing silicosis when exposed to dust. This possibility seems to be very unlikely considering the overall protective role of glutathione in biological systems. It was, however, demonstrated[26] that in the presence of $H_2O_2$ and $Fe^{2+}$, GSH can produce $OH^{\cdot}$ radicals.

The most likely explanation for the higher GSH-level in red blood cells of silicosis patients is an induced protection level against the continuous stream of radicals and lipidhydroperoxides from lung tissue due to chronic inflammation. Most *in vitro* studies report that GSH-concentrations are decreased during induced peroxidation.[27] On the other hand, *in vivo* induction of various antioxidant parameters in (human) red cells due to chronic ingestion of ethanol[28] and (life) exposures to air pollution[29] is a well-known process. It has however to be remarked that none of the studied patients is actively exposed to dust at present.

Probably, the most intersting fact for future studies on individual susceptibility towards silicosis is observed when studying the relation between GSH-$P_x$ and SOD in red blood cell-lysates. A small (not statistically significant) negative correlation is observed between both enzymes in the control subjects. In the silicosis group, however, GSH-$P_x$ and SOD were *positively correlated* ($r = 0.6$). Neither GSH-$P_x$ nor SOD activity were found to be confounded by smoking or medication. Moreover, in neither of these a statistically significant difference was observed between cases and controls (Table III) though values were somewhat lower in silicosis patients. Due to the transversal character of the study two explanations are possible. First, we have to assume that RBC levels of GSH-$P_x$ and SOD are proportionally related to lung tissue levels.[20] If a subject has a lowered activity of these enzymes he would be less well equipped to deal with oxidative stress. As a result, more oxidative damage will occur on cellular components during excessive formation of free radicals by the inflammatory reaction induced by silica dust. Moreover, it was shown[30] that the cellular defense system against free radicals is damaged during oxidative stress. They showed that the impairment of the defense system was due to partial inactivation of SOD and Se-dependent GSH-$P_x$. Summarizing, the increased susceptibility of lung tissue to damaging effects of radicals is accompanied by an increased vulnerability due to partial impairment of the cellular defence system.

Still, it is possible we are just measuring an effect on erythrocytes *per se*. As stated above GSH-$P_x$ and SOD are vulnerable targets for (lipid) peroxidation production. The erythrocytes are able to penetrate in the smallest blood vessels and therefore can reach the site of inflamation. Diffusion of released toxic products from the inflamed tissue into the erythrocyte could result in a direct damage cellular components. Erythrocytes in this way act as a circulating anti-oxidant carrier[23,24] and SOD/GSH-$P_x$ as the vulnerable constituents.

Further detailed investigations relating lung tissue anti-oxidant levels with RBC-anti-oxidant levels are necessary to prove the use of this anti-oxidant phenotype assay in predicting individual susceptibility towards the potential hazards of inhaled silica dust.

## *References*

1. Lynn, W.S. In Inflammatory cells and lung disease, ed. Lynn WS (CRC Press Inc., Boca Raton, Florida, 1983), Chapter 4.
2. Riley, D.J. and Kerr, J.S. *Lung*, **163**, 1, (1985).
3. Halliwell, B. and Gutteridge, J.M.C. *Free radicals in biology and medicine* (Clarendon Press, Oxford England, 1985).
4. Halliwell, B. and Gutteride, J.M.C. *Biochem. J.*, **219**, 1, (1984).
5. White, C.W. and Repine, J.E. *Exp. Lung Res.*, **8**, 1, (1985).
6. Hornsby, P.J. and Crivello, J.F. *Mol. Cell. Endocrinol.*, **30**, 1, (1983).
7. Minchin, R.F. and Boyd, M.R. *Ann. Rev. Pharmacol. Toxicol.*, **23**, 217, (1983).

8. Hendricks, Ch.A. and Mey, A.V. In Arbeids- en Bedrijfsgeneeskunde, ed. G.C. Burger (Stenfert Kroese, Leiden, The Netherlands, 1971), p. 304.
9. Lidell, D. and Miller, K. *Scand. J. Work. Env. Hlth.*, **9**, 1, (1983).
10. Noweir, H.H., Moselhi, M. and Amine, E.K. *Brit. J. Ind. Med.*, **37**, 399, (1980).
11. Beutler, E. In Toxicology of the Blood and Bone Marrow, ed R.D. Irons (Raven Press, New York, 1985), p. 39.
12. Maral, J., Puget, K. and Michelson, A.M. *BBRC*, **77**, 1525, (1977).
13. McCord, J.M. and Fridovich, I. *J. Biol. Chem.*, **244**, 6049, (1969).
14. Hissin, P.J. and Hilf, R. *Anal. Biochem.*, **74**, 214, (1976).
15. Tsuchida, M., Miura, T., Mizutani, K. and Aibara, K. *Biochim. Biophys. Acta*, **834**, 196, (1985).
16. Lilienfeld, A.M. and Lilienfeld, D.E. *Foundations of Epidemiology*, 2nd ed. (Oxford University Press, Oxford, 1980), p. 323.
17. Koskinen, H., Jävisalo, J., Huuskonen, M.S., Koivula, T., Mutanen, P. and Pitkänen, E. *Eur. J. Resp. Dis.*, **64**, 182, (1983).
18. Koskinen, H., Nordman, H. and Fröseth, B. *Eur. J. Resp. Dis.*, **65**, 481, (1984).
19. Bucca, C., Veglio, F., Rolla, G., Cacciabue, M., Cicconi, C., Ossola, M., Nuzzi, A., Aviolo, G. and Angeli, A. *Eur. J. Resp. Dis.*, **65**, 477, (1984).
20. Lipecka, K., Domanski, T., Daniszewska, K., Grabowska, B., Pietrowicz, D., Lindner, P., Cisowska, B. and Górski, H. *Studia Biophys.*, **89**, 57, (1982).
21. Minami, M., Koshi, K., Homma, K. and Suzuki, Y. *Arch. Toxicol.*, **49**, 215, (1982).
22. Yarosz, A.M. *Bull. Eksp. Biol. Med.*, **97**, 486, (1984).
23. Agar, N.S., Sadrzadeh, S.M.H., Hallaway, P.E. and Eaton, J.W. *J. Clin. Invest.*, **77**, 319, (1986).
24. van Asbeck, B.S., Hoidal, J., Vercellotti, G.M., Schwarts, B.A., Moldow, C.F. and Jacob, H.S. *Science.*, **277**, 756, (1984).
25. Patterson, C.E., Butler, J.A., Byrne, F.D. and Rhodes, M.L. *Lung*, **163**, 23, (1985).
26. Rowly, D.A. and Halliwell, B. *FEBS Lett.*, **138**, 33, (1982).
27. Bast, A. and Haenen, G.R.M.M. *Trends in Biochem. Sci.*, **9**, 510, (1984).
28. Clemens, M.R., Einsele, H., Remmer, H. and Waller, H.D. *Clin. Chim. Acta*, **145**, 283, (1985).
29. Medeiros, M.H.G., Bechara, E.J.H., Naoum, P.C. and Mouráo, C.A. *Arch. Env. Hlth.*, **38**, 11, (1983).
30. Julicher, R.H.M., Tijburg, L.B.M., Sterrenberg, L., Bast, A., Kooman, J.M. and Noordhoek, J. *Life Sci.*, **35**, 1281, (1984).

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