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Blood Anti-Oxidant Parameters At Different Stages of Pneumoconiosis in Coal Workers

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The pneumoconioses are associated with chronic inflammatory processes during which increased amounts of reactive oxygen species are formed in the lower respiratory tract. To characterize the effect(s) of these processes on the defense system against free radicals, we studied 91 individuals with long-term occupational exposure to coal mine dust. Thirty-one subjects were classified with radiological evidence to be pneumoconiotics, while 58 control miners had no pulmonary disorders. We measured antioxidant parameters in red blood cells, considering the latter to reflect the oxidative stress in the lung. Glutathione levels were significantly decreased ($p = 0.04$) in red blood cells of miners with coal workers' pneumoconiosis with radiograph classification 0/1 to 2/1, while in miners with classification 3/2 to 3/3, the plasma iron concentrations were significantly decreased ($p = 0.04$). Moreover, some factors of the anti-oxidant system (superoxide dismutase, catalase, glutathione peroxidase) were correlated in the diseased but not in the control miners. Taken together, all data support the role of the erythrocyte as a circulating anti-oxidant carrier and also that changes in red blood cell anti-oxidant factors reflect the oxidative stress imposed by the pneumoconiotic (inflammatory) processes in the lung.

Introduction

Lung fibrosis related to the inhalation of dusts containing toxic particles such as silica or asbestos is an important occupational hazard, and the investigation of pneumoconiosis has led to a better understanding of the mechanisms of cell injury and repair in the lung. It is well known that during the immune defense reaction against dust particles, reactive oxygen species (ROS) are secreted by various types of inflammatory cells with the alveolar macrophages playing a key role in this process (*1*). These reactive species include the $OH^{\bullet}$ radical, H_2O_2, HOCL and their common precursor $O_2^{\bullet -}$. In addition, secreted proteolytic enzymes and elastases, in concert with ROS, denature proteins, destroy carbohydrates, peroxidize lipids, and consequently cause connective tissue alterations like fibrosis (*2*).

Fortunately, the lung possesses an elaborate defense system against released ROS. This defense system consists of several anti-oxidant enzymes like superoxide dismutase (SOD), glutathione peroxidases (GSHpx) and catalase, low molecular weight sulfhydryls (glutathione, cysteine, cysteinylglycine), and some additional factors (vitamins C and E, urate, ceruloplasmine, ferritin, and β-carotene). These factors are located in three compartments: lung tissue, interstitial fluid, and (circulating) erythrocytes.

In the normal state the anti-oxidant system is able to deal with the continuous stream of radicals produced endogeneously—mainly by the reduction of $O_2^{\bullet -}$ to H_2O. A disturbance on either side, i.e., by oxidative burst of phagocytes or by deficient or malfunctioning components of the antioxidant system (AOS) in lung tissue, can provoke a devastating response (*3,4*).

Our original hypothesis was that differences in individual susceptibility toward inhaled silica (containing) dust (*5,6*) might be associated with antioxidant defenses in the lung and/or red blood cells. Previously, we reported higher red blood cell reduced glutathione (GSH) levels in silicotic patients as compared to healthy elderly controls (*7*). Moreover, some factors of the AOS, i.e., SOD and GSHpx, were strongly correlated in the diseased, but not in healthy subjects. However, the data were confounded by the use of medication by the patients, the stage of the disease, and the selection of the control group (*8*).

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This paper presents the results of a similar study performed among active coal miners with and without simple coal workers' pneumoconiosis (CWP). They constituted a homogeneous group in which all individuals were exposed to similar dust levels and composition.

Materials and Methods

Chemicals

Superoxide dismutase (SOD, bovine erythrocytes), xanthine oxidase (cow milk), glutathione reductase (yeast), NADPH and reduced (GSH) and oxidized (GSSG) glutathione were products of Boehringer (Mannheim, West Germany). Xanthine, cytochrome-C, hemoglobin, sodium azide, and α-tocopherol were obtained from Merck (Darmstadt, West Germany). *N*-Ethylmaleimide and *o*-phthaldialdehyde were purchased from Aldrich (Steinheim, West Germany). All other chemicals used were of analytical grade.

Sample Collection and Processing

Blood samples were taken from 91 coal miners, working in four different Belgian coal mining industry pits (Kempische Steenkolen Mijnen, KS). All blood donors were between 32 and 59 years of age and were Caucasians. Blood samples were taken between February and April 1987. The study population consisted of 33 men with pneumoconiosis and 58 controls, matched for age and years of underground work in the coal mine. They were interviewed on their present and past smoking habits, previous work history, medication, and family history of pneumoconiosis.

Three samples (5 mL per sample) of blood were taken from each donor after he completed an 8-hr work shift. All samples were taken by venapuncture with evacuated tubes containing 7.5 mg Na_2EDTA. Sample tubes were transferred to our laboratory within 2 hr where red cells were washed three times using isoosmotic phosphate-buffered saline (9 mM KH_2PO_4, 34 mM Na_2HPO_4, 90 mM NaCl).

Centrifugation was performed at 4°C (10 min, 3000 rpm). Plasma and the washed pellets, consisting of red blood cells (RBC), were stored at −70°C until analysis. One milliliter of the RBC suspension was used immediately for the lipid peroxidation measurement. At the time of analyzing anti-oxidant factors, RBC pellets were thawed at room temperature. To a 1-mL pellet, 2.5 mL cold distilled water was added, vortexed, again frozen at −70°C, and thawed. After centrifuging the hemolysate, the supernatant was used for analysis (within 1 day).

Radiograph Classification

All radiographs were read to the current UICC/ILO classification (*9*). The most recent chest radiographs (not more than 1 year) of 91 volunteers with or without coal workers' pneumoconiosis were independently judged in one session by three experienced, occupational Kempische Steenkolen physicians to reach a final consensus. They distinguished 33 cases of pneumoconiosis and 58 healthy volunteers.

All 91 men had been heavily exposed to coal dust underground at the coal face for at least 12 years. The mean duration of exposure was similar for both groups. The mean age of the group ranged between 42 years (control group) and 47 years (pneumoconiosis group). In our study the persons with classification 0/1, 1/0, 1/1, and 1/2 were gathered in group 1; the persons with classification 2/1, 2/2, and 2/3 in group 2; and 3/2 and 3/3 in group 3.

Assays in Red Blood Cells

Hemoglobin Determination. One milliliter of whole blood was frozen and thawed twice. Hemoglobin (Hb) was measured in whole blood and in red cell lysates by the following procedure: to 1 mL of whole blood or lysate, a 1-mL solution of 4 mM $K_3Fe(CN)_6$, 30 mM KCN, and 1 mM EDTA in 0.1 M phosphate buffer (pH 7.0) was added and mixed. After 10 min 30 μL of the above mixture was diluted in 3 mL H_2O, and its extinction was measured at 340 nm. A standard solution of hemoglobin was prepared in water (1–200 mg/mL) and subjected to the same procedure.

Assays of Anti-Oxidant Factors. The assay of glutathione peroxidase activity in red cell lysates was performed in combination with the determination of hemoglobin. One milliliter of lysate was mixed with an equal volume of the $K_3Fe(CN)_6$ and KCN containing buffer (pH 7.0) as above. The lysates were adjusted to 50 mg Hb/mL, and GSHpx was then assayed, as described previously (*8*), at 25°C using an LKB Ultraspec 2 UV/VIS spectrophotometer. Substrates used were 0.33 mM H_2O_2 for measurement of Se-dependent GSHpx (GSHpx-Se) and 1 mM cumene hydroperoxide for quantitation of total GSHpx (GSHpx-To) activity. Units of enzyme activity were expressed as nmole NADPH consumed/min/g hemoglobin using a molar extinction coefficient for NADPH of 6.22×10^{-3} mM^{-1} cm^{-1}.

SOD activity was determined by the method of McCord and Fridovich (*10*) described in detail previously (*8*). We used an Aminco Bowman DW 2A UV/VIS spectrophotometer. A standard curve was prepared using commercially available SOD (Boehringer). The enzyme activity at 25°C was expressed in units enzyme per gram hemoglobin.

GSSG and GSH were assayed according to the method described by Hissin and Hilf (*11*) using *o*-phthaldialdehyde as a fluorescent agent described previously (*8*). Standard solutions of GSH (0.1–1 mM) and GSSG (0.01–0.1 mM) were subjected to the same procedure and used to calculate the glutathione content in the RBC lysates. Fluorescence was measured at extinction 350 nm and emission 460 nm, using an

SLM/AMINCO SPF-500 C spectrophotometer (both slit widths 2 nm).

Catalase activity was measured by the method of Aebi (*12*). Prior to the catalase measurement, the hemoglobin concentration was determined, and the lysates were diluted to 50 mg Hb/mL. At 25°C 4 μL lysate and 1 mL H_2O_2 (30 mM) were added to 2 mL PBS (pH 7.0). The rapid decomposition of H_2O_2 was followed during 15 sec by the decrease in absorbance at 240 nm. Enzyme activity was expressed as gram H_2O_2 per minute per gram Hb using a molar extinction coefficient for H_2O_2 of 0.0394 mM^{-1} cm^{-1} (*12*).

Red Blood Cell Resistance to Induced Oxidative Stress. In a 2.5-mL red blood cell suspension (5% partial cell volume), lipid peroxidation was initiated by the addition of 2.5 mL H_2O_2 (10 mM) in the presence of sodium azide (1 mM). After a given time of incubation at 37°C in a shaking water bath, the thiobarbituric acid reactive material formed was measured as described by Stocks and Dormandy (*13*). The malondialdehyde (MDA) lag time was estimated by the extrapolation of MDA production at 10, 20, and 30 min to the time point where MDA production starts (lag-time). Extrapolation was done by linear regression through the three time points measured.

Assays in Plasma

Iron Measurement. For determining total iron in plasma we used the Iron Ferro-Zine test of Roche Diagnostica (*14*). The iron bound to transferrin in the blood is split off by guanidine hydrochloride and reduced to Fe^{2-} by ascorbic acid. With Ferro-Zine, a red-colored chelate is produced; the color intensity is directly proportional to the iron concentration and is measured spectrophotometrically.

Vitamin A and Vitamin E Measurement. Vitamins A and E were simultaneously measured with high performance liquid chromatography according to the method described by Cuesta Sanz et al. (*15*). A stock solution of retinol (0.5 mg/mL), of *dl*-α-tocopherol (7 mg/mL), and a stock solution of internal standard, retinyl acetate (1.2 mg/mL), was prepared in ethanol. The reference solutions were stored at −70°C, and on the day of analysis they were diluted 1:100 with ethanol. A Kratos liquid chromatograph equipped with a reverse-phase Ligochrome RP-18 column and a KRATOS UV detector were used.

Statistical Evaluation

Analytical results have been evaluated using SPSX (SPSS-x Inc.) incorporated in a VAX/VMS computer (Digital, version V4.1). The difference between the means of a variable in two groups was tested with the Mann-Whitney *U*-test. Significance was set at 5% level ($p < 0.05$). Spearman's correlation was used as a test for correlation between the pairs of (not normally distributed) parameters.

Results

Mean age and the years of exposure to coal dust are slightly higher in the pneumoconiosis group as compared to the control miners (Table 1). However, these differences are not statistically significant. No difference is observed between the two groups with respect to the age of starting work in the coal mines.

Table 2 shows the mean values ± SE of the parameters assayed in the blood of the control and the pneumoconiosis group. Apart from a negative effect of smoking on red blood cell catalase activity ($p = 0.025$), neither the effect from smoking nor the use of medication and (starting) age on any parameter was observed. No statistically significant differences in hemoglobin, plasma iron, and antioxidant factors could be distinguished between the controls and the miners with pneumoconiosis, with the latter considered as a homogeneous group.

However, in Table 3 we see that in the cases with beginning CWP (group 1), GSH, and GSSG are decreased ($p = 0.04$), while the total GSHpx activity is increased ($p = 0.04$) when compared to the controls. In group 3 the plasma iron value is significantly decreased ($p = 0.03$) when compared to the controls. Moreover, the MDA-lag time, considered to be a parameter for the resistance against oxidative stress (*13*), is decreased ($p = 0.08$) in miners of group 3. This finding (though on the border of significance) indicates that red blood cells of these miners are more sensitive to lipid-peroxidative damage induced by H_2O_2.

No significant correlation between any of two measured parameters was observed in the control group. Within the pneumoconiosis groups this pattern changed remarkably. Spearman's correlation resulted in three pairs of correlated parameters within group 1. Both erythrocyte catalase and GSHpx-Se are correlated to plasma vitamin A levels ($r = 0.48$, $n = 19$, $p = 0.02$ resp. $r = -0.45$, $n = 19$, $p = 0.032$). Also, within this group the SOD and GSH levels in erythrocytes are positively correlated ($r = 0.44$, $n = 19$, $p = 0.032$). The strongest correlations, however, were observed in group 3. In this group red blood cell SOD was strongly related to GSHpx-Se ($r = 0.62$, $n = 14$, $p = 0.009$) and catalase ($r = 0.42$, $n = 14$, $p = 0.067$). In controls there is only a weak and negative correlation of SOD with GSHpx-Se ($r = -0.33$, $n = 58$, $p = 0.007$) and no correlation at all between red blood cell SOD and catalase ($r = 0.01$, $n = 58$, $p = 0.46$) (Fig. 1).

Discussion

It is generally accepted that the interaction between silica and pulmonary alveolar macrophages leads to the release of fibrogenic factors from these cells and induces pulmonary fibrosis (*1,16,17*). Moreover, *in vivo* and *in vitro* exposure of alveolar macrophages to inorganic dusts containing silica leads to an

Table 1. Frequency distribution of age, occupational history, smoking habits, medication, and family history regarding pneumoconiotic diseases in the control group and the miners with coal workers' pneumoconiosis (CWP).

	Control group (n = 38)	CWP group (n = 33)
Age		
Mean ± SE	42± 0.6	47± 0.8
Range	32− 2	38− 59
Occupational history		
Starting age	17.7	17.5
Range	14− 25	14− 24
Years exposed ± SE	21± 0.5	25± 0.8
Range	12− 32	14− 32
Smoking		
Yes	30	22
No	28	11
Medication		
Yes[a]	6	7
No	52	26
Blood relatives with pneumoconiosis		
Yes	23	11
No	35	22

[a]None of these subjects received sympathomimetic bronchodilators, theophylline or its derivates, bronchospasmolytics, mucolytics, or corticosteroids.

Table 2. Comparison of blood hemoglobin, plasma iron, and anti-oxidant factors in red blood cells and plasma of miners with and without coal workers' pneumoconiosis (CWP). Values are mean ± SE.

Parameter, unit[a]	Controls (n = 58)	CWP[b] (n = 33)
Red blood cells		
HB, mg Hb/mL	14.8 ± 0.25	15.0 ± 0.24
SOD, U/g Hb	1945 ± 64	1996 ± 87
Catalase, μmole H_2O_2/min/g Hb	0.58 ± 0.01	0.58 ± 0.01
GSHpx-To, μmole NADPH/min/g Hb	9.58 ± 0.08	9.80 ± 0.05
GSHpx-Se, μmole NADPH/min/g Hb	9.17 ± 0.09	9.40 ± 0.09
GSH, μmole/g Hb	3.97 ± 0.08	3.78 ± 0.11
GSSG, μmole/g Hb	0.036 ± 0.001	0.039 ± 0.0003
MDA-lag, min	3.9 ± 0.22	4.1 ± 0.37
Plasma		
Fe μmole/mL plasma	19.0 ± 0.81	17.7 ± 1.4
Vitamin A, μg/mL plasma	9.2 ± 0.4	8.8 ± 0.5
Vitamin E, μg/mL plasma	14.1 ± 0.7	15.1 ± 1.3

[a]Abbreviations: Hb, hemoglobin; SOD, superoxide dismutase; GSHpx-To, total glutathione peroxidase; GSHpx-Se, selenium dependent glutathione peroxidase; GSH, reduced glutathione; GSSG, oxidized glutathione; Fe, total plasma iron; MDA-lag, lag-time for induction of lipid peroxidation as measured by one of its products: malondialdehyde (MDA).
[b]No significant differences were found between healthy miners and those classified as having CWP (0/1 through 3/3).

increased superoxide anion production via the reduction of oxygen by NADPH oxidase (*18,19*). This macrophage-derived superoxide can readily dismutate to form hydrogen peroxide; subsequently, hydroxyl radicals and hypochloric acid can be generated. All these reactive oxygen species have been demonstrated to be capable of injuring lung tissue *in vitro* and *in vivo* (*20,21*).

Our previous studies (*7,8*) were designed to explore what role antioxidants play in the development of coal workers' pneumoconiosis. We suggested that people who develop coal workers' pneumoconiosis might be less equipped to deal with the reactive oxygen species that are released by the alveolar macrophages after exposure to silica dust. There are several reasons to adopt the assumption that anti-oxidant parameters in red blood cells and serum may reflect toxic risks to lung tissue. First, a relationship was observed between red blood cell and lung tissue levels of anti-oxidant enzymes (*22,23*). Second, chronic inflammation in the lung results in concomitant changes in the antioxidant system and in lipid-peroxidative products in lung tissue and red blood cells (*24*). Finally, it has been demonstrated that erythrocytes may act as a circulating anti-oxidant system

Table 3. Comparison of Hb, plasma iron, and anti-oxidant factors in red blood cells and plasma of the three coal workers' pneumoconiosis (CWP) subgroups of miners with CWP. Values are mean ± SE.

Parameter[a]	Group 1[b] (n = 19)	Group 2 (n = 10)	Group 3 (n = 4)
Red blood cells			
Hb	15.1 ± 0.35	15.0 ± 0.41	15.0 ± 0.9
SOD	2026 ± 112	1981 ± 189	1890 ± 166
Catalase	0.58 ± 0.02	0.59 ± 0.02	0.56 ± 0.02
GSHpx-To	9.88 ± 0.08[c]	9.73 ± 0.15	9.79 ± 0.09
GSHpx-Se	9.44 ± 0.13	9.40 ± 0.20	9.45 ± 0.21
GSH	3.62 ± 0.14[c]	3.98 ± 0.20	3.99 ± 0.44
GSSG	0.031 ± 0.001[c]	0.038 ± 0.001	0.039 ± 0.001
MDA-lag	4.33 ± 0.53	4.4 ± 0.4	2.2 ± 0.7[d]
Plasma			
Fe	19.2 ± 2.2	16.8 ± 1.5	12.8 ± 1.3[c]
Vitamin A	8.5 ± 0.7	9.2 ± 0.7	8.9 ± 2.9
Vitamin E	13.9 ± 1.5	16.0 ± 2.5	18.4 ± 4.3

[a]For a list of abbreviations, see Table 2, footnote *a*.
[b]Group 1: ILO classification 0/1, 1/0, 1/1 and 1/2; group 2: ILO classification 2/1, 2/2 and 2/3; group 3: ILO classification 3/2 and 3/3.
[c]Significantly different from value in control group, $p < 0.04$ Mann-Whitney U-test.
[d]Significantly different from value in control group, $p = 0.08$ Mann-Whitney U-test.

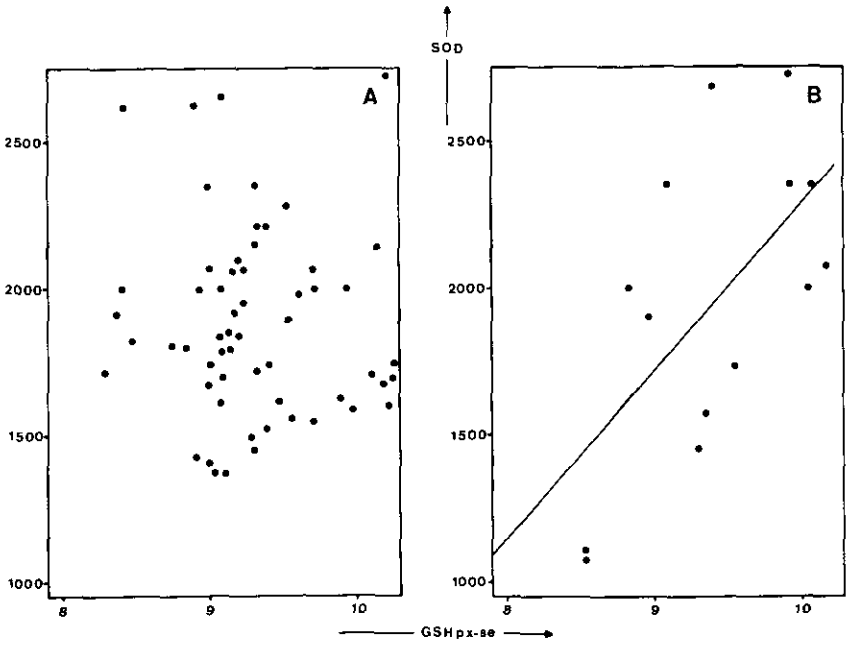

FIGURE 1. Scatterdiagrams of superoxide dismutase SOD activity (U/g Hb) versus glutathione peroxidase (GSHpx) activity (μmole NADPH/min/g Hb). Enzyme activities were determined in red blood cell lysates of control miners (*A*, $n = 58$) and miners with CWP (*B*, $n = 14$) with classification between 2/1 and 3/3. The values of the individual subjects are represented by the symbol O. Linear regression resulted in correlation coefficients of $r = -0.33$ ($n = 58$, $p = 0.007$) in the control group and $r = 0.62$ ($n = 14$, $p = 0.009$) in groups 2 and 3 of the miners with CWP.

(*25–27*) and protect endothelial cells *in vitro* from H_2O_2 (*28*).

Interestingly, Toth et al. (*28*) found that red blood cells of cigarette smokers contained more GSH and catalase than red blood cells from nonsmokers. Neither in this study nor in our previous studies (*7,8*) did we find an effect of smoking on red blood cell GSH content. Perhaps this is due to methodological differences, but the effect of exposure to coal mine dust should also be considered. All individuals in this study have been exposed to mine dust for at least 12 years and are still active in mining. Possibly the (inducing) effect of smoking on red blood cell GSH (*28*) is masked by an increase (or decrease) caused by oxidants released in response to inhaled dust particles.

The present study demonstrates that erythrocyte GSH levels are decreased in the subjects at an early stage of CWP (group 1) when compared to the controls, while at a progression of CWP (group 2 and 3) erythrocyte GSH levels were no longer different from control miners' erythrocytes. We believe that the decreased GSH content of the blood is caused by the excessive release of reactive oxygen species by the alveolar macrophages and neutrophils in the lung tissue. Reactive oxygen production and blood GSH decrease *in vivo* in relation to silica dust exposure is never signaled in men.

There are several reasons to suggest that this decrease in GSH content of the red blood cells is not a cause of the disease but an effect. First, the release of oxidants by bronchoalveolar cells from patients with interstitial lung disease was shown to be increased (*19,29*). More specifically, it appeared that macrophages of persons with CWP release far more $O_2^{\cdot -}$ and H_2O_2 than macrophages of controls (*19*). As it was shown that reactive oxygen species can consume GSH directly (*30*) or indirectly via lipid-peroxidation (*31*), our findings can be explained by above mechanisms causing excessive radical production in the lung tissue. Second, a high recruitment of macrophages in miners with pneumoconiosis is observed, although the number of alveolar macrophages in the lower respiratory tract expands considerably in all persons chronically exposed to high concentrations of silica dust (*32*). Third, not only the alveolar macrophages but also the neutrophils are present in greater number. Neutrophils are also able to secrete substantial amounts of reactive oxygen species at the command of macrophage- and lymphocyte-derived cytokines as interleukin-1 (*33*) and tumor necrosis factor (*34*). Another argument for the GSH involvement in the protection against oxidants produced by the action of silica in the lung is the observation (*35,36*) that glutamic acid (a GSH precursor) has an antisilicotic effect when it is administered in the drinking water of rats.

We suppose that the restoration of erythrocyte GSH content in group 2 and 3 to control levels is caused by a hepatic efflux of GSH. Such a mechanism was proposed by Sies and co-workers (*37,38*) who stated that generation of reactive oxygen species in a peripheral site (for example an inflammatory response or tissue injury) leads to a transportable signal of hormonal nature. This stimulation of hepatic GSH release then would counteract the loss of

thiol groups in the periphery using the red blood cell as carrier. Also our earlier studies (*7,8*) reporting an increased GSH content in the red blood cells of patients with progressive massive fibrosis (PMF) are consistent with an inflammatory response driven efflux of hepatic GSH. A graphical presentation of the fluctuation in red blood cell GSH during progressive fibrosis is shown in Figure 2. All data are consistent with the earlier statement that red blood cell GSH is a (biological effect) parameter determined by an excessive radical formation in the lung on which the liver GSH efflux is superimposed. Dependent on the stage of the disease, GSH is decreased (group 1), normal, or increased (PMF).

Apart from effects on GSH, some other interesting effects on anti-oxidant enzymes were noted. In the initial stage of CWP, miners with CWP have higher total GSHpx activity than controls and as CWP progresses plasma iron drops significantly (Table 3). The latter finding corresponds with the results of Chan and coworkers (*39*) who found that serum iron was decreased during complicated pneumoconiosis. The biological significance of these observations can be understood, knowing the role of transition metals (such as iron and copper) in the conversion of H_2O_2 into more reactive hydroxyl radicals:

$$Fe^{2-} + H_2O_2 \rightarrow Fe^{3-} + OH^- + OH^{\cdot}$$

This reaction is also known as the Fenton Reaction (*40*). Initially, GSHpx is induced to degrade the increased amount of H_2O_2 (and lipid-hydroperoxides) formed by interaction of alveolar macrophages and polymorphonuclear leukocytes with dust in the alveoli. This reaction consumes GSH which is, accordingly, decreased in stage 1.

Due to the increasing dust load in developing CWP (local) inflammation proceeds. A following measure to prevent formation of hydroxyl radicals is to withhold iron from the inflammatory site. A decreasing effect on serum iron is seen during progression of CWP, becoming statistically significant in group 3. Similar phenomena were described previously (*40,41*) for chronic inflammation, where iron is lost from the blood (e.g., the percentage iron saturation of circulating transferrin drops), and iron accumulates in the liver, spleen, and bone marrow.

In addition to the alterations in red blood cell antioxidant levels that were explained as a measure to protect the lung from further oxidant damage, we found that some antioxidant enzymes are significantly correlated during CWP, but not in controls. Previously, we reported a correlation between red blood cell SOD and GSHpx in patients with PMF (*8*). The same observation is now made in CWP patients beyond classification 2/1, while these enzyme-levels are not correlated in controls. Still, we do not have a valid explanation for this phenomenon, nor do we know its biological significance. We suggest, however, that we are looking at an effect of chronically increased oxidant production that manifests itself in red blood cell enzymes. One reason to do so is the (new) observation that also SOD and catalase are correlated in CWP. Unfortunately, we did not measure catalase in our previous study (*8*).

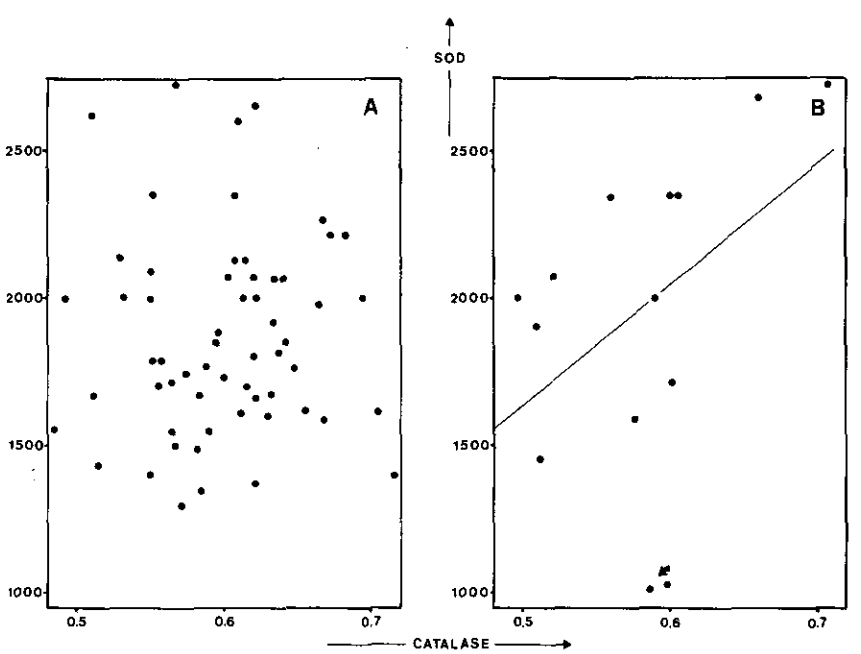

FIGURE 2. Scatterdiagrams of SOD activity (U/g Hb) versus catalase activity (μmole H_2O_2/min/g Hb). Enzyme activities were determined in red blood cell lysates of control miners (*A*, $n = 58$) and miners with CWP (*B*, $n = 14$) with classification between 2/1 and 3/3. The values of the individual subjects are represented by the symbol O. Linear regression resulted in correlation coefficients of $r = 0.01$ ($n = 58$, $p = 0.46$) in the control group and $r = 0.42$ ($n = 14$, $p = 0.067$) in the groups 2 and 3 of the miners with CWP. Removal of the two data points nearest to the x axis in *B* (indicated by the arrow) upgrades the correlation on coefficient to $r = 0.68$.

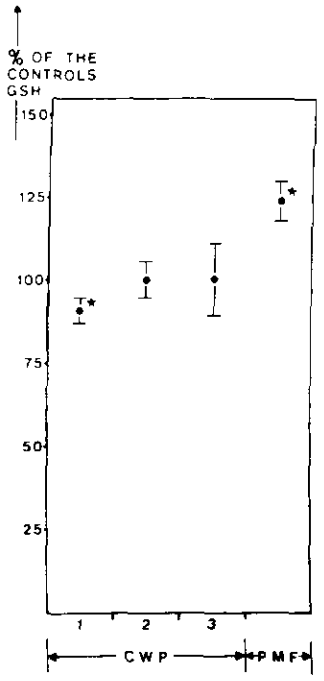

FIGURE 3. Graphical presentation of red blood glutathione in different stages of CWP and in progressive massive fibrosis (PMF). The mean values ± SE of group 1 (0/1, 1/0, 1/1, 1/2; $n = 19$), group 2 (2/1, 2/2, 2/3; $n = 10$), and group 3 (2/3, 3/3; $n = 4$) are presented as the percentage of the control GSH content ($n = 58$). The data from patients with PMF ($n = 19$) were taken from previous work (*8*). The asterisk (*) indicates a red blood cell GSH content that is significantly different from controls.

The positive relation between SOD and catalase (Fig. 3) in red blood cells of miners with CWP (group 2 and 3, $n = 14$) can be interpreted using the findings of Kono and Fridovich (*42*). These investigators showed that at low concentrations of SOD, $O_2^{\cdot -}$ radicals inhibited catalase *in vitro* by its conversion into the relatively inactive compounds 2 (Fe^{4+}) and 3 (Fe^{2+}). Since SOD can be inactivated by H_2O_2 (*44*), the basis for a reverse synergism in which catalase prevents the inactivation of SOD is also present. Catalase and SOD therefore constitute a mutually protective set of enzymes. We suppose that an oxidative stress on SOD, caused by the release of $O_2^{\cdot -}$ from alveolar macrophages and neutrophils (predominant in CWP), partially inactivates catalase; this in turn causes an inactivation of SOD by H_2O_2. The observed correlation in CWP is supposed to be the mathematical effect of a systematic decrease in both enzyme levels resulting in a smearing of the original scatterplot towards the zero coordinates. The biological significance remains, however, somewhat ambiguous. While all other effects (GSH, GSHpx and Fe) indicate protective measures to prevent oxidative damage, the concomitant decrease of anti-oxidant enzymes in later stages of CWP would imply less defense against released oxidants unless defense by other anti-oxidant levels (GSH, Fe) is sufficient. The increased susceptibility of red blood cells to H_2O_2 (shorter lag-time) in group 3 supposes that the RBC anti-oxidant defense is affected. However, this might be a "red herring" as this concerns a toxic effect on RBC itself and has nothing to do with its protective action of lung tissue cells (*26,28*).

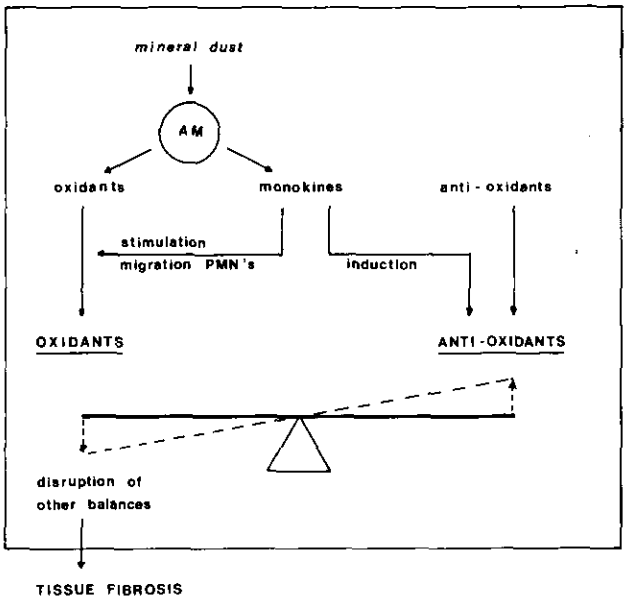

FIGURE 4. Schematical presentation of the suggested role of oxidants, anti-oxidants and monokines (interleukin-1, tumor necrosis factor) in mineral dust-induced pulmonary fibrosis.

Present knowledge demands readers to consider pulmonary fibrosis as the end result of a process in which the balance of normal injury/repair-protection mechanisms is disrupted. There is clearly no single fibrogenic event, but rather a number of levels where disruption of different balances may begin. We consider the oxidant/anti-oxidant balance (Fig. 4) being just one balance in the total mechanism leading to lung fibrosis. The changes in anti-oxidant levels (and their interrelationship) in red blood cells reflect the exaggerated production of reactive oxygen species in the lung in individuals with coal workers' pneumoconiosis. These changes are most probably the result from feedback mechanisms trying to prevent tipping over the oxidant/anti-oxidant balance in favor of the oxidants. Probably, these changes can be used as a tool to study (early) health effects in the lung or provide a new way of intervention with therapeutic agents.

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