Relations between occupational exposure to coal mine dusts, erythrocyte catalase and Cu\textsuperscript{++}/Zn\textsuperscript{++} superoxide dismutase activities, and the severity of coal workers’ pneumoconiosis

Rachel Nadif, Eve Bourgkard, Martine Dusch, Pierre Bernadac, Jean-Pierre Bertrand, Jean-Marie Mur, Quang-Thieu Pham

Abstract

Objectives—To better understand the relations between occupational exposure, blood antioxidant enzyme activities, total plasma antioxidant concentration, and the severity of coal workers’ pneumoconiosis (CWP).

Methods—Blood samples were obtained from miners without CWP exposed to low dust concentrations for \textgreater=4 years at the time of the study (n = 105), or exposed to high dust concentrations for \textgreater=14 years at the time of the study (n=58), and from retired miners with CWP (n=19). Miners without CWP were classified into three subgroups according to their estimated cumulative exposure to dust. Chest x ray films were obtained for each miner. Miners were classified in five subgroups according to their International Labour Organisation (ILO) profusion grades. Univariate tests were completed by multiple linear regression analyses.

Results—The estimated cumulative exposure to dust was strongly positively related to erythrocyte catalase activity and strongly negatively related to Cu\textsuperscript{++}/Zn\textsuperscript{++} SOD activity only in miners exposed to high dust concentrations for \textgreater=14 years at the time of the study (F tests p=0.006 and p=0.004 respectively). Moreover, catalase activity was strongly related to the severity of CWP expressed as five subgroups of ILO profusion grades (F test p=0.003); the greatest difference in the mean values was found between the group of 1/1 to 1/2 ILO profusion grades and the group of 2/1 to 3/3 ILO profusion grades.

Conclusion—These results are in good agreement with the hypothesis that production of reactive oxygen species may be an important event in the exposure to coal mine dusts and the severity of CWP. Erythrocyte catalase and Cu\textsuperscript{++}/Zn\textsuperscript{++} SOD activities are more closely related to recent exposure to high dust concentrations than to cumulative exposure, and could be considered as biological markers of exposure rather than as markers of early adverse biological effect.

Keywords: antioxidant enzymes; coal workers’ pneumoconiosis; dust exposure; reactive oxygen species

The biological pathways of the development of coal workers’ pneumoconiosis (CWP) remains partly unknown. Reactive oxygen species could well play an essential part in this process.\textsuperscript{1} Reactive oxygen species are released from alveolar macrophages and polymorphonuclear leucocytes during the phagocytosis of inhaled dust particles.\textsuperscript{2,3} Moreover, reactive oxygen species may be generated from dust particles themselves.\textsuperscript{4} The continuous production of reactive oxygen species may overwhelm antioxidant defences, and may result in an oxidative stress defined as an imbalance between antioxidants and oxidants, in favour of the oxidants, leading to lung cellular damage.\textsuperscript{5}

Erythrocytes are circulating antioxidant carriers that can penetrate lung capillaries and have been shown to play a significant part in protection against lung damage due to reactive oxygen species.\textsuperscript{6,7-9} Erythrocyte Cu\textsuperscript{++}/Zn\textsuperscript{++} superoxide dismutase (Cu\textsuperscript{++}/Zn\textsuperscript{++} SOD), catalase, and a selenoenzyme glutathione peroxidase provide protection against reactive oxygen species.\textsuperscript{7-9} The Cu\textsuperscript{++}/Zn\textsuperscript{++} SOD converts O\textsubscript{2}\textsuperscript{=} to H\textsubscript{2}O\textsubscript{3}, and catalase and glutathione peroxidase convert H\textsubscript{2}O\textsubscript{3} to oxygen and water. To a lesser extent, plasma physiological antioxidants and plasma glutathione peroxidase also provide protection against reactive oxygen species.\textsuperscript{10}

The measurement of blood antioxidants and antioxidant enzyme activities is a way of investigating the role of oxidative stress in the severity of CWP in miners chronically exposed to coal mine dust particles. Recently, red blood cell antioxidant parameters were shown to decrease in some stages of pneumoconiosis.\textsuperscript{11,12} Erythrocyte glutathione S-transferase activity and glutathione concentration were decreased in the early stages of pneumoconiosis.\textsuperscript{11} More recently, we reported increased antioxidant enzyme activities in miners exposed to coal dust particles and in miners with pneumoconiosis.\textsuperscript{14}

In all the epidemiological studies already mentioned, and as it is traditionally done in health surveillance, clinical diagnosis, and in disability determinations, CWP is diagnosed from chest radiography according to the classification of the International Labour Office (ILO).\textsuperscript{15} However, chest radiography seems to be insensitive at early or moderate stages of pneumoconioses.\textsuperscript{10} Recently, advanced medical imaging have provided new techniques for better viewing of lung and chest wall structures. Thus, computed tomography (CT) is more
often applied in the clinical evaluation of pneumoconioses, and gives rise to increased sensitivity in radiological identification at early stages of pneumoconioses.\textsuperscript{17–20}

The purpose of the present study is to better understand the relations between occupational exposure defined as exposure to low or high dust concentrations at the time of the study and also as individual estimated cumulative exposure to dust, blood antioxidant enzyme activities, and total plasma antioxidant concentration and the severity of CWP diagnosed from chest radiography and CT.

**Materials and methods**

**STUDY SAMPLE**

This study is part of a larger project in which coal miners of a French coalmine, Houillères du Bassin de Lorraine, (north-east France) have been selected according to a detailed protocol previously described.\textsuperscript{21–24} The sample consisted of 240 coal miners aged 34–50 years in 1990, including 80 subjects heavily exposed to underground coal dusts (≥10 years at coal face) with pulmonary radiographs classified by four independent physicians as 0/1 or 0/0 according to the ILO classification of pneumoconioses, 80 subjects exposed to underground coal dusts with normal radiographs, and 80 subjects slightly exposed with normal radiographs. These coal miners have been examined in 1990 and in 1994.

Our study sample was composed of miners examined at the second survey. Miners who had developed CWP (ILO profusion grades ≥1/1) and miners in whom exposure to dust concentrations had been changed between 1990 and 1994 were excluded from the study. One group consisted of 105 miners exposed to low dust concentrations and one group consisted of 58 miners exposed to high dust concentrations. One group of 19 retired miners with CWP was added: ILO profusion grades ≥1/1 in 1994 and an evolution of two stages of profusion grade within the past 10 years.

The examination included a questionnaire on job and medical history, medication, dietary habits, and the questionnaire of the European Community for Coal and Steel on respiratory symptoms\textsuperscript{25} and smoking habits. The subjects were classified as miners who had never smoked, and as ex-smokers if they had never smoked and had stopped smoking for <1 year, as non-smokers if they had never smoked, and as ex-smokers if they had smoked in the past and had stopped smoking for ≥1 year. Cigarette smoking was recorded as the current number of cigarettes smoked a day. For some statistical analyses, non-smokers and ex-smokers were combined.

Occupational exposure was defined as exposure to low or high underground concentrations of dust at the time of the study and also as individual estimated cumulative exposure to dust. The subjects were classified as miners exposed to low dust concentrations if they worked at ventilation maintenance, pumping, haulage, shaft, stock equipment, safety, or were retired for ≥4 years in 1994, and as miners exposed to high dust concentrations if they worked at the coal face, mining, stope, or drift advance for ≥14 years in 1994. Cumulative personal exposure to dust was estimated from each person’s job history and from dust measurements at various sites of the coal mine according to the calculation previously described.\textsuperscript{26} The estimates were the summations of each dust measurement (mg/m\textsuperscript{3}) for the respective time spent in each job. Estimated cumulative exposures to dust were calculated until June 1994 and expressed as mg/m\textsuperscript{3}.y.

Chest x-ray films, taken in the yearly medical examinations, were interpreted according to the ILO\textsuperscript{15} classification of radiographs of pneumoconiosis by three independent and experienced physicians. The films were presented in a random order, without any information about the professional and medical history of the subjects. Subjects with a profusion grade of ≤1/0 were considered as not having pneumoconiosis. For statistical analyses, the 12 point ILO profusion grades were compressed to five points: 0/- and 0/0=1, 0/1=2, 1/0=3, 1/1 and 1/2=4, ≥2/1=5. This classification was used not only to label qualitatively the five subgroups but also to assess the severity of ILO profusion grades in the quantitative regression analysis. Computed tomography was carried out according to Bernadac \textit{et al.}\textsuperscript{27} All scans were obtained in each miner without pneumoconiosis. Two thin slices (1 mm) and one thick slice (8 mm) were obtained at upper, middle, and lower zones of the lungs. Computed tomography was interpreted by an experienced radiologist blinded to individual exposure data and to chest radiographic interpretation. The interpretation of subpleural, pleural, and parenchymal nodular abnormalities was based on published descriptions.\textsuperscript{28,29} The extent of the lesion was coded according to a profusion scale ranging from 0 (absence) to 3 (abundant). The micronodular score, ranging from 0 to 18, was the sum of the greatest profusions found in each third of the lungs.

This study was approved by the Research National Committee of Charbonnage de France. Written informed consent was collected from each subject.

**BLOOD SAMPLES**

Blood samples were collected into 5 ml Vacut\textsuperscript{35}iner tubes containing lithium heparinate as an anticoagulant (Becton Dickinson, USA). On the same day, corresponding plasma and haemolysates were prepared as described previously.\textsuperscript{30} Plasma and ghost free haemolysees were stored at −35°C.

Two hundred microlitres of haemolysate were diluted by addition of 1800 µl NaCl (0.9%). Cu\textsuperscript{2+}/Zn\textsuperscript{2+} SOD was extracted from 0.5 ml of this haemolysate by addition of 0.5 ml chloroform/ethanol (15/25, v/v). The solution was vortexed and centrifuged at 10 500 g for five minutes. The upper aqueous phase was assayed for Cu\textsuperscript{2+}/Zn\textsuperscript{2+} SOD activity.

**ENZYMATIC ASSAYS**

Total plasma antioxidant concentration includes ascorbate, protein thiols, bilirubin, urate, and α-tocopherol. Total plasma antioxidant concentration was measured at 37°C in
Table 1 Characteristics of miners reported by group

<table>
<thead>
<tr>
<th>Variables</th>
<th>Miners without CWP</th>
<th>Miners exposed to high dust concentrations for &gt;14 y at the time of the study (n=58)</th>
<th>Retired miners with CWP (n=19)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=105)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (y, mean (range))</td>
<td>46.5 (39–53)</td>
<td>45.6 (39–50)</td>
<td>54.7 (50–61)*</td>
</tr>
<tr>
<td>Estimated cumulative exposure to dust (mg/m³.y, mean (range))</td>
<td>35.3 (4–180)</td>
<td>74.0 (25–180)</td>
<td>123.0 (48–272)$\dagger$</td>
</tr>
<tr>
<td>Cigarette smoking (cigarettes/day, mean (range))</td>
<td>17.7 (2–50)</td>
<td>13.3 (2–30)</td>
<td>11.3 [6–20]‡</td>
</tr>
<tr>
<td>Smoking habits (%):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-smokers</td>
<td>20.0</td>
<td></td>
<td>47.4</td>
</tr>
<tr>
<td>Ex-smokers</td>
<td>32.4</td>
<td></td>
<td>36.8</td>
</tr>
<tr>
<td>Current smokers</td>
<td>47.6</td>
<td></td>
<td>15.8</td>
</tr>
</tbody>
</table>

*p=0.0001 Mann–Whitney U test.  
‡p=0.002 Kruskal–Wallis test; p=0.003 trend test.  
\( \dagger \)=p=0.0001 trend test.

Results are expressed as mean (range) except for erythrocyte enzyme activities (geometric mean (range)).

Table 2 Comparison of antioxidant enzyme activities and total plasma antioxidant concentration in subgroups of estimated cumulative exposure to dust in coal miners without CWP

<table>
<thead>
<tr>
<th>Estimated cumulative exposure to dust</th>
<th>Miners exposed to low dust concentrations for ≥4 y at the time of the study (n=67)</th>
<th>Miners exposed to high dust concentrations for ≥14 y at the time of the study (n=23)</th>
<th>p Value</th>
<th>Miners exposed to high dust concentrations for ≥14 y at the time of the study (n=58)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤26 mg/m³.y</td>
<td>91.8 (35.6–152.3)</td>
<td>96.2 (53.9–302.8)</td>
<td>0.76</td>
<td>89.4 (41.6–144.9)</td>
</tr>
<tr>
<td>26–74 mg/m³.y</td>
<td>90.0 (44.1–254.1)</td>
<td></td>
<td>0.53</td>
<td>147.1 (104.0–234.5)</td>
</tr>
<tr>
<td>&gt;71 mg/m³.y</td>
<td>148.1 (105.0–234.1)</td>
<td>143.8 (102.7–195.1)</td>
<td>0.68</td>
<td>34.1 (14.2–65.8)</td>
</tr>
<tr>
<td></td>
<td>41.7 (28.3–61.1)</td>
<td></td>
<td>0.47</td>
<td>692 (277–1103)</td>
</tr>
<tr>
<td></td>
<td>656 (237–865)</td>
<td></td>
<td>0.42*</td>
<td>689 (329–1004)</td>
</tr>
</tbody>
</table>

In erythrocytes:
- Catalase (k/g Hb): 91.8 (35.6–152.3)
- Cu/Zn SOD (U/g Hb): 140.0 (100–233.2)
- Glutathione peroxidase (U/g Hb): 38.0 (13.3–67.7)

In plasma:
- Catalase (k/g Hb): 96.2 (53.9–302.8)
- Cu/Zn SOD (U/g Hb): 143.8 (102.7–195.1)
- Glutathione peroxidase (U/g Hb): 39.3 (16.8–61.2)
- Total antioxidant concentration (mmol/l): 1.25 (0.90–1.68) / 1.42 (0.92–2.29) / 1.35 (1.07–1.65) / 1.35 (0.88–1.68) / 1.34 (0.93–1.49)

Results are expressed as mean (range) except for erythrocyte enzyme activities (geometric mean (range)).

*p=0.86 without the outlier.

Pyridine nucleotide-linked enzymes such as metmyoglobin, the relatively long lived radical cation ABTS is formed. In the presence of antioxidant reductants and hydrogen donors (5 µl plasma), the absorbance of this radical cation is quenched to an extent related to the antioxidant capacity of the added fluid. An antioxidant ranking was established based on the reactivity relative to a 1.0 mmol/l Trolox standard (the Trolox equivalent antioxidant capacity of plasma). Total plasma antioxidant concentration is defined as the amount of radicals captured non-enzymatically in 1 litre plasma. Results were expressed in mmol/l (Trolox units, rate (%)).

The Cu/Zn SOD activity was measured as previously described, and adapted on a Cobas-Mira S analyser (Hoffman-La Roche, Basle, Switzerland). The concentration of xanthine oxidase in the reaction mixture was 18 U/ml. The volume sample was 4 µl of the upper aqueous phase. Human erythrocyte SOD (Sigma) was used as a standard.

Glutathione peroxidase activity was measured as previously described in the Cobas-Mira S analyser.

Catalase activity was measured at 25°C in a UV 25 Spectrophotometer (Beckman Instruments, France) as previously described.

Catalase activity of 1 k was defined as the rate constant of the first order reaction.

Haemoglobin (Hb) content (g/l) was determined as previously described. Activities were expressed as U/g Hb (Cu/Zn SOD, glutathione peroxidase) or k/g Hb (catalase) in erythrocytes, and U/l (glutathione peroxidase) in plasma.

All samples were analysed in duplicate or triplicate and the precision (coefficient of variation) was <10% for each enzymatic assay. The accuracy was checked by analysing external reference samples together with the test samples.

STATISTICAL METHODS

All variables presented a normal distribution except erythrocyte antioxidant enzyme activities, age, estimated cumulative exposure to dust (mg/m³.y), cigarette smoking (cigarettes/day), and micronodular score. The erythrocyte antioxidant enzyme activities were log transformed before statistical analysis and the normality was checked with the Shapiro–Wilk test. The univariate procedure of SAS software found one very high value of plasma total antioxidant concentration and defined it as an outlier; thus, the statistical analyses were performed with and without this outlier. Miners were classified into three subgroups according to the tertiles of estimated cumulative exposure to dust. In miners exposed to high dust concentrations, only two subgroups of estimated cumulative exposure to dust were presented due to the few subjects in the first tertile.
tertile. Previously, we calculated that four was the discriminating value of the micronodular score which distinguished at best, miners suspected of pneumoconiosis from miners with CWP. Miners were classified into two subgroups according to this value threshold.

The homogeneity $X^2$ test was performed to compare the proportions between the groups. For comparison between means, Student’s $t$ test and analysis of variance (ANOVA) were used except when the data were not distributed normally or when the sizes of the groups were small, in which case Mann-Whitney $U$ test and Kruskal-Wallis test were used. For evaluation of the relations between antioxidant enzyme activities or total plasma antioxidant concentration and qualitative variables classified into more than two groups, a trend test was performed with the GLM procedure. For evaluation of the relations between quantitative variables, Pearson’s and Spearman’s rank correlation ($r$) were used.

To describe the association between antioxidant enzyme activities with occupational exposure and ILO profusion grades, multiple linear regressions were used. The interaction between estimated cumulative exposure to dust and exposure to dust concentrations at the time of the study was investigated in each model by contrasting the regression coefficients of the antioxidant enzyme activities according to exposure to high dust concentrations versus low dust concentrations at the time of the study. Smoking and age influenced antioxidant enzyme activities but the available publications showed no consensus about these relations.

Thus, smoking and age which may confound the associations studied—that is, occupational exposure and antioxidant enzyme activities or antioxidant enzyme activities and the severity of CWP—were forced in the models. Coefficients were expressed as $10^{-3}$ for more easy readings. $F$ Tests were used to check the significance of coefficients in each regression model. The stability of the variance and approximate linearities in the models were checked on residual plots.

Significance was assessed at the 5% two sided level. All these analyses were performed with the SAS statistical software (SAS Institute, Cary, North Carolina).

**Results**

Table 1 shows the characteristics of the miners. Retired miners with CWP were significantly older than miners without CWP. There were significant differences between the three groups for estimated cumulative exposure to dust, cigarette smoking, and smoking habits. Estimated cumulative exposure to dust was positively and cigarette smoking was negatively related to exposure to dust concentration and the presence of CWP.

### Relations between occupational exposure with activities of antioxidant enzymes and total plasma antioxidant concentration in miners without CWP

A comparison of antioxidant enzyme activities and total plasma antioxidant concentration showed several significant differences only in miners exposed to high dust concentrations (table 2). Erythrocyte catalase and glutathione peroxidase activities were higher whereas Cu/ Zn SOD activity was lower in

### Table 3 Multiple linear regression of catalase and Cu/Zn SOD activities on the estimated cumulative exposure to dust according to dust concentrations at the time of the study in miners without CWP

<table>
<thead>
<tr>
<th>Miners exposed to low dust concentrations for ≥ 4 y at the time of the study (n=105)</th>
<th>Miners exposed to high dust concentrations for ≥ 14 y at the time of the study (n=58)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coefficient (95% CI)</td>
<td>p Value</td>
</tr>
<tr>
<td>Catalase (k/g Hb):</td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>5.32 (4.52 to 6.12)</td>
</tr>
<tr>
<td>Estimated cumulative exposure to dust (mg/m$^3$.y):</td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>0.87 (10^{-3}) (−0.94 to 2.69.10^{-3})</td>
</tr>
<tr>
<td>Cu/Zn SOD (U/g Hb):</td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>4.99 (4.93 to 5.05)</td>
</tr>
<tr>
<td>Estimated cumulative exposure to dust (mg/m$^3$.y):</td>
<td></td>
</tr>
<tr>
<td>Cigarette smoking (cigarettes/day)</td>
<td>0.015 (10^{-3}) (−1.21 to 1.18.10^{-3})</td>
</tr>
</tbody>
</table>

### Table 4 Comparison of antioxidant enzyme activities and total plasma antioxidant concentration in subgroups of ILO profusion grades in all miners (n=182)

<table>
<thead>
<tr>
<th>ILO profusion grades</th>
<th>CWFP</th>
<th>Kruskal-Wallis test, p value</th>
<th>Trend test, p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0/0 (n=133)</td>
<td>1/1 or 1/2 (n=12)</td>
<td>2/1 or 2/2 or 3/3 (n=7)</td>
<td>1</td>
</tr>
<tr>
<td>In erythrocytes:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Catalase (k/g Hb)</td>
<td>91.5 (35.5–152.3)</td>
<td>103.1 (65.4–302.8)</td>
<td>97.0 (72.3–161.2)</td>
</tr>
<tr>
<td>Cu/Zn SOD (U/g Hb):</td>
<td>141.0 (100.2–234.5)</td>
<td>149.4 (105.0–224.0)</td>
<td>139.2 (100.7–187.7)</td>
</tr>
<tr>
<td>Glutathione peroxidase (U/g Hb)</td>
<td>38.2 (13.3–67.7)</td>
<td>38.4 (14.1–62.0)</td>
<td>34.9 (16.9–53.1)</td>
</tr>
<tr>
<td>In plasma:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glutathione peroxidase (U/l)</td>
<td>727 (220–1401)</td>
<td>660 (237–898)</td>
<td>631 (378–864)</td>
</tr>
<tr>
<td>Total antioxidant concentration (mmol/l)</td>
<td>1.35 (0.88–2.29)</td>
<td>1.39 (1.22–1.63)</td>
<td>1.36 (0.93–1.82)</td>
</tr>
</tbody>
</table>

Results are expressed as mean (range) except for erythrocyte enzyme activities (geometric mean (range)).

* $p$=0.16 without the outlier; † $p$=0.03 without the outlier.
miners with estimated cumulative exposure to dust $>71$ mg/m$^3$.y. Moreover, catalase and glutathione peroxidase activities were positively and significantly correlated with estimated cumulative exposure to dust ($r=0.35$, $p=0.006$; $r=-0.27$, $p=0.04$) whereas Cu$^{++}$/Zn$^{++}$ SOD activity was negatively and significantly correlated with estimated cumulative exposure to dust ($r=-0.38$, $p=0.003$).

Some relations between antioxidant enzyme activities with age and smoking were found. In miners exposed to low concentrations of dust, erythrocyte catalase activity was lower in miners $>47$ years old (median age, $p=0.05$) and was inversely related to age ($r=-0.21$, $p=0.03$); Cu$^{++}$/Zn$^{++}$ SOD activity was negatively whereas plasma glutathione peroxidase activity was positively related to the number of cigarettes smoked a day ($r=-0.31$, $p=0.03$; $r=0.31$, $p=0.03$). Moreover, a negative association was found between the number of cigarettes smoked a day and the estimated cumulative exposure to dust ($r=-0.26$, $p=0.081$). Moreover, in both groups with different concentrations of dust exposure, miners with estimated cumulative exposure to dust $>71$ mg/m$^3$.y were older than miners with estimated cumulative exposure to dust $\leq 71$ mg/m$^3$.y ($p=0.027$, $p=0.004$). As expected, estimated cumulative exposures to dust were strongly related to the age of the miners in each group ($r=0.37$, $p=0.0001$; $r=0.47$, $p=0.0002$).

Table 3 shows the results of the multiple linear regression and indicates the associations between catalase and Cu$^{++}$/Zn$^{++}$ SOD activities (the dependent variables) and the estimated cumulative exposure to dust in the 163 coal miners without CWP according to their exposure at the time of the study. In miners exposed to low dust concentrations for $\geq 4$ years at the time of the study, no association between the estimated cumulative exposure to dust and catalase activity was found. On the contrary, in miners exposed to high dust concentrations for $\geq 14$ years at the time of the study, catalase activity was strongly and positively related to the estimated cumulative exposure to dust.

Similarly, in miners exposed to low dust concentrations for $\geq 4$ years at the time of the study, no association between the estimated cumulative exposure to dust and Cu$^{++}$/Zn$^{++}$ SOD activity was found. On the other hand, in miners exposed to high dust concentrations for $\geq 14$ years at the time of the study, Cu$^{++}$/Zn$^{++}$ SOD activity was strongly and negatively related to the estimated cumulative exposure to dust.

Plasma glutathione peroxidase activity was not significantly related to the estimated cumulative exposure to dust in miners exposed to low dust concentrations for $\geq 4$ years at the time of the study nor in miners exposed to high dust concentrations for $\geq 14$ years at the time of the study (data not shown).

### RELATIONS BETWEEN ANTIOXIDANT ENZYME ACTIVITIES AND TOTAL PLASMA ANTIOXIDANT CONCENTRATION WITH THE SEVERITY OF CWP

A comparison of antioxidant enzyme activities and total plasma antioxidant concentration between the five subgroups of ILO profusion grades showed that catalase activity was positively and Cu$^{++}$/Zn$^{++}$ SOD activity was negatively associated with the severity of CWP (table 4), the greatest differences being found between the group of miners with 1/1 or 1/2 ILO profusion grades and the group of miners with 2/1, 2/2, or 3/3 ILO profusion grades. Total plasma antioxidant concentration was weakly related to the severity of CWP.

Table 5 shows the results of the multiple linear regression and indicates the associations between catalase and Cu$^{++}$/Zn$^{++}$ SOD activities (the dependent variables) and the estimated cumulative exposure to dust in the 163 coal miners without CWP according to their exposure at the time of the study. In miners exposed to low dust concentrations for $\geq 4$ years at the time of the study, no association between the estimated cumulative exposure to dust and catalase activity was found. On the contrary, in miners exposed to high dust concentrations for $\geq 14$ years at the time of the study, catalase activity was strongly and positively related to the estimated cumulative exposure to dust.

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Plasma glutathione peroxidase activity was not significantly related to the estimated cumulative exposure to dust in miners exposed to low dust concentrations for $\geq 4$ years at the time of the study nor in miners exposed to high dust concentrations for $\geq 14$ years at the time of the study (data not shown).

### RELATIONS BETWEEN ANTIOXIDANT ENZYME ACTIVITIES AND TOTAL PLASMA ANTIOXIDANT CONCENTRATION WITH THE SEVERITY OF CWP

A comparison of antioxidant enzyme activities and total plasma antioxidant concentration between the five subgroups of ILO profusion grades showed that catalase activity was positively and Cu$^{++}$/Zn$^{++}$ SOD activity was negatively associated with the severity of CWP (table 4), the greatest differences being found between the group of miners with 1/1 or 1/2 ILO profusion grades and the group of miners with 2/1, 2/2, or 3/3 ILO profusion grades. Total plasma antioxidant concentration was weakly related to the severity of CWP.

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### RELATIONS BETWEEN ANTIOXIDANT ENZYME ACTIVITIES AND TOTAL PLASMA ANTIOXIDANT CONCENTRATION WITH THE SEVERITY OF CWP

A comparison of antioxidant enzyme activities and total plasma antioxidant concentration between the five subgroups of ILO profusion grades showed that catalase activity was positively and Cu$^{++}$/Zn$^{++}$ SOD activity was negatively associated with the severity of CWP (table 4), the greatest differences being found between the group of miners with 1/1 or 1/2 ILO profusion grades and the group of miners with 2/1, 2/2, or 3/3 ILO profusion grades. Total plasma antioxidant concentration was weakly related to the severity of CWP.

Table 5 shows the results of the multiple linear regression and indicates the associations between catalase and Cu$^{++}$/Zn$^{++}$ SOD activities (the dependent variables) and the estimated cumulative exposure to dust in the 163 coal miners without CWP according to their exposure at the time of the study. In miners exposed to low dust concentrations for $\geq 4$ years at the time of the study, no association between the estimated cumulative exposure to dust and catalase activity was found. On the contrary, in miners exposed to high dust concentrations for $\geq 14$ years at the time of the study, catalase activity was strongly and positively related to the estimated cumulative exposure to dust.

Similarly, in miners exposed to low dust concentrations for $\geq 4$ years at the time of the study, no association between the estimated cumulative exposure to dust and Cu$^{++}$/Zn$^{++}$ SOD activity was found. On the other hand, in miners exposed to high dust concentrations for $\geq 14$ years at the time of the study, Cu$^{++}$/Zn$^{++}$ SOD activity was strongly and negatively related to the estimated cumulative exposure to dust.

Plasma glutathione peroxidase activity was not significantly related to the estimated cumulative exposure to dust in miners exposed to low dust concentrations for $\geq 4$ years at the time of the study nor in miners exposed to high dust concentrations for $\geq 14$ years at the time of the study (data not shown).

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between catalase and Cu"+/Zn" SOD activities (the dependent variables) and the five subgroups of ILO profusion grades in all the 182 coal miners according to their exposure at the time of the study. In miners exposed to no or low dust concentrations for ≥4 years at the time of the study (n=124), catalase activity was strongly and positively associated with the five subgroups of ILO profusion grades whereas there was no association with the estimated cumulative exposure to dust. On the contrary, as expected, there was no association between catalase activity and the five subgroups of ILO profusion grades in miners exposed to high dust concentrations for >14 years at the time of the study: they have ILO profusion grades ≤1/0. However, catalase activity was related to the estimated cumulative exposure to dust.

Cu"+/Zn" SOD activity was not significantly related to the five subgroups of ILO profusion grades in miners exposed to low dust concentrations for ≥4 years at the time of the study nor in miners exposed to high dust concentrations for ≥14 years at the time of the study.

**Discussion**

In this study, we found that erythrocyte catalase and Cu"+/Zn" SOD activities were associated with occupational exposure to coal mine dusts and that catalase activity was related to the severity of CWP.

To our knowledge, this study is the first to describe significant associations of erythrocyte catalase and Cu"+/Zn" SOD activities with the estimated cumulative exposure to dust only in miners exposed to high dust concentrations for >14 years at the time of the study. Recently, we have compared these enzyme activities between miners exposed or not to underground dusts. Unfortunately, their estimated cumulative exposure to dust was unknown, and we found that Cu"+/Zn" SOD activity was higher in miners exposed to underground dusts than in non-exposed miners, whereas we found no differences in catalase activity between these groups. More recently, in another study, we found no differences in catalase and Cu"+/Zn" SOD activities between underground miners and surface workers in three different mines. As our studies are the only ones that have investigated the relations between occupational exposure and blood antioxidant enzyme activities, the available information is perhaps too incomplete to explain these apparently contradictory results.

In this study, we found that a better knowledge of the occupational exposure of each miner, not only the estimated cumulative dust exposure reflecting all the coal mining jobs worked by the miner, but the exposure at the time of the study led us to report significant associations of catalase and Cu"+/Zn" SOD activities with occupational exposure. Thus, miners with the same estimated cumulative exposure to dust could not be expected to have similar antioxidant enzyme activities if they were recently exposed to low or high dust concentrations. The associations of antioxidant enzyme activities with the estimated cumulative exposure to dust are significantly much weaker among coal miners recently exposed to high dust concentrations than among coal miners exposed to low dust concentrations. In this study, erythrocyte catalase and Cu"+/Zn" SOD activities were more closely related to recent exposure to high dust concentration than to cumulative exposure, and could be considered as biological markers of exposure rather than as markers of early adverse biological effect. Moreover, it can be hypothesised that, if miners with high estimated cumulative exposure to dust and occupational exposure to high dust concentrations are transferred to exposure to low dust concentrations, their activities of catalase and Cu"+/Zn" SOD will return to normal.

The positive association found for catalase activity is concomitant with the negative association found for Cu"+/Zn" SOD activity. In healthy lungs, a fine balance exists between generation of reactive oxygen species and antioxidant defences. When this balance is overwhelmed by either an excessive production of reactive oxygen species or a loss of antioxidant defences, an oxidative stress is induced resulting in lung injury. As we have mentioned before, chronic exposure to and inhalation of coal mine dust particles give rise to a high production of reactive oxygen species, specially H2O2. As expected, an increase in catalase activity as a compensatory mechanism in response to the generation of H2O2 was found. The decrease in Cu"+/Zn" SOD activity can be interpreted with the findings of Tsan. As Cu"+/Zn" SOD can be inactivated by its enzymatic reaction product H2O2, we suppose that the high concentration of H2O2 in lungs partially inactivates Cu"+/Zn" SOD. Normal concentrations of Cu"+/Zn" SOD are essential for the pulmonary defence against oxygen toxicity and a decrease in this enzyme activity is related to considerable sensitivity to pulmonary oxygen toxicity.

We found that catalase activity was strongly related to the severity of CWP expressed as five groups of ILO profusion grades; the greatest difference in the mean values being found between the group of miners with 1/1 or 1/2 ILO profusion grades and the group of miners with 2/1 to 3/3 ILO profusion grades. Previously, Borm et al. and Engelen et al. reported no significant differences in catalase activity...
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between one group of miners with 0/0 ILO profusion grades and one group of miners with CWP, nor between miners with CWP classified into three subgroups of ILO profusion grades. More recently, we compared catalase activity in retired miners with and without CWP and found no differences between both groups. Several discrepancies may explain the differences found. The significant relation between catalase activity and the severity of CWP shows good agreement with other studies. Rom et al. found that alveolar macrophages from people with CWP who did not smoke were spontaneously releasing increased amounts of H$_2$O$_2$. Dalal et al. found that the free radical content of the lung tissues of necropsied coal miners correlated with the severity of pneumoconiosis; the more severe the disease, the higher the radical concentration in the lung tissue. These results seem to confirm the suggestion of Agar et al. that the main function of catalase is to protect against high concentrations of exogenous H$_2$O$_2$ (from inflammation and coal dust particles). Moreover, we suggest that the increase in catalase activity can reflect the consequence of the disease, or the individual susceptibility to reactive oxygen species, or both.

In this study, we found no relation between Cu$^{+}/$Zn$^{+}$ SOD and the severity of CWP. This confirms our previous study in which Cu$^{+}/$Zn$^{+}$ SOD was not different between retired miners with and without CWP who did not smoke. The present results could also be consistent with the findings of Borm et al. They found a relation between SOD activity and CWP in miners classified into three subgroups of ILO profusion grades. Unfortunately, no regression analyses were applied taking into account estimated cumulative exposure to dust and the level of exposure at the time of the study. Thus this remains open to debate.

Another interesting result of the present study is that catalase activity and total plasma antioxidant concentration were higher in miners in the CT class of micronodular score ≥4. Previously, during a four year follow up study in coal miners, we have evaluated methods allowing an early diagnosis of CWP. We found that CT was the technique which detected at best CWP four years later than respiratory symptoms and lung function and that this value of 4 was defined as the value which separates at best subjects who develop pneumoconiosis or not four years later among miners suspected of pneumoconiosis (ILO profusion grades of 0/1 or 0/1). Our results accord with those of Schins et al. who have studied the relation between several blood antioxidant parameters measured in coal workers and the development of CWP at five follow up studies. They found that miners with newly developed pneumoconiosis had significantly higher total radical trapping antioxidant parameter concentrations than the control miners who did not develop CWP. Moreover, in these healthy miners who developed pneumoconiosis during the follow up, catalase activity was higher and Cu$^{+}/$Zn$^{+}$ SOD was lower. Furthermore, significant discrimination was found between miners classified according to newly developed CWP, with catalase (positive) in a discriminant model.

In conclusion, our results are in good agreement with the hypothesis that production of reactive oxygen species is related to the exposure to coal mine dust particles and the severity of CWP. Erythrocyte catalase and Cu$^{+}/$Zn$^{+}$ SOD activities are more closely related to recent exposure to high dust concentrations than to cumulative exposure, and could be considered as biological markers of exposure rather than as markers of early adverse biological effects. Moreover, the multiple antioxidant marker approach and complete knowledge of exposure are preferable to single antioxidant measurements and partial knowledge of exposure in occupational health screening of coal miners.

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Relations between occupational exposure to coal mine dusts, erythrocyte catalase and Cu++/Zn++ superoxide dismutase activities, and the severity of coal workers' pneumoconiosis.

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