Relations between occupational exposure to coal mine dusts, erythrocyte catalase and $\text{Cu}^{++}/\text{Zn}^{++}$ 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 ≥ 4 years at the time of the study ($n = 105$), or exposed to high dust concentrations for ≥ 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 $\text{Cu}^{++}/\text{Zn}^{++}$ SOD activity only in miners exposed to high dust concentrations for ≥ 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 $\text{Cu}^{++}/\text{Zn}^{++}$ 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 effect.

(Occup Environ Med 1998;55:533-540)

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.¹ Reactive oxygen species are released from alveolar macrophages and polymorphonuclear leucocytes during the phagocytosis of inhaled dust particles.^{2,3} Moreover, reactive oxygen species may be generated from dust particles themselves.^{4,5} 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.⁶

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.⁷⁻⁹ Erythrocyte $\text{Cu}^{++}/\text{Zn}^{++}$ superoxide dismutase ($\text{Cu}^{++}/\text{Zn}^{++}$ SOD), catalase, and a selenoenzyme glutathione peroxidase provide protection against reactive oxygen species.¹⁰ The $\text{Cu}^{++}/\text{Zn}^{++}$ SOD converts O_2^- to H_2O_2 , and catalase and glutathione peroxidase convert H_2O_2 to oxygen and water. To a lesser extent, plasma physiological antioxidants and plasma glutathione peroxidase also provide protection against reactive oxygen species.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.^{11,12} Erythrocyte glutathione *S*-transferase activity and glutathione concentration were decreased in the early stages of pneumoconiosis.¹³ More recently, we reported increased antioxidant enzyme activities in miners exposed to coal dust particles and in miners with pneumoconiosis.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).¹⁵ However, chest radiography seems to be insensitive at early or moderate stages of pneumoconiosis.¹⁶ Recently, advanced medical imaging have provided new techniques for better viewing of lung and chest wall structures. Thus, computed tomography (CT) is more

INSERM U 420,
Faculté de Médecine,
BP 184, F-54505
Vandœuvre-lès-Nancy
Cedex, France
R Nadif
E Bourgkard
M Dusch
J-M Mur
Q-T Pham

INRS, Avenue de
Bourgogne, BP 27,
F-54501
Vandœuvre-lès-Nancy
Cedex, France
R Nadif
M Dusch
J-M Mur

Service de Radiologie,
Hôpital Central, Case
Officielle n°34, F-54035
Nancy Cedex, France
P Bernadac

Médecine du Travail,
Houillères du Bassin
de Lorraine, 11 rue du
Casino, F-57800
Freyming-Merlebach,
France
J-P Bertrand

Correspondence to:
Dr Rachel Nadif, INSERM
U 420, Epidémiologie Santé
Travail, Faculté de
Médecine, BP 184, 54505
Vandœuvre-lès-Nancy
Cedex, France. Telephone
0033 3 83 59 26 44; fax
0033 3 83 59 25 96.

Accepted 24 March 1998

often applied in the clinical evaluation of pneumoconioses, and gives rise to increased sensitivity in radiological identification at early stages of pneumoconioses.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.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 1/0 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 $\geq 1/1$) 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 $\geq 1/1$ 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 symptoms²⁵ and smoking habits. The subjects were classified as current smokers if they smoked at the time of the study or 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.²⁶ The estimates were the summations of each dust measurement (mg/m^3) for the respective time spent in each job. Estimated cumulative exposures to dust were calculated until June 1994 and expressed as $\text{mg}/\text{m}^3\cdot\text{y}$.

Chest x ray films, taken in the yearly medical examinations, were interpreted according to the ILO¹⁵ 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 $\leq 1/0$ 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, $\geq 2/1$ =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 *et al.*²⁷ All scans were obtained for 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.^{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 Vacutainer tubes containing lithium heparinate as an anticoagulant (Becton Dickinson, USA). On the same day, corresponding plasma and haemolysates were prepared as described previously.³⁰ Plasma and ghost free haemolysates were stored at -35°C .

Two hundred microlitres of haemolysate were diluted by addition of 1800 μl NaCl (0.9%). $\text{Cu}^{++}/\text{Zn}^{++}$ 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 $\text{Cu}^{++}/\text{Zn}^{++}$ 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

Variables	Miners without CWP		
	Exposed to low dust concentrations for ≥ 4 y at the time of the study (n=105)	Exposed to high dust concentrations for ≥ 14 y at the time of the study (n=58)	Retired miners with CWP (n=19)
Age (y, mean (range))	46.5 (39–53)	45.6 (39–50)	54.7 (50–61)*
Estimated cumulative exposure to dust (mg/m ³ .y, mean (range))	35.3 (4–180)	74.0 (25–180)	123.0 (48–272)†
Cigarette smoking (cigarettes/day, mean (range))	17.7 (2–50)	13.3 (2–30)	11.3 (6–20)‡
Smoking habits (%):			
Non-smokers	20.0	8.6	47.4
Ex-smokers	32.4	31.0	36.8
Current smokers	47.6	60.4	15.8§

*p=0.0001 Mann-Whitney U test.

†p=0.0001 Kruskal-Wallis test; p=0.0001 trend test.

‡p=0.007 Kruskal-Wallis test; p=0.003 trend test.

§p=0.002 χ^2 test.

the Cobas-Mira S analyser with a kit based on the method of Miller *et al*³¹ (Randox Laboratories Crumlin, UK). This method arose from the finding that when 2,2'-azinobis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS)) is incubated with hydrogen peroxide and a peroxidase such as metmyoglobin, the relatively long lived radical cation ABTS⁺ is formed. In the presence of antioxidant reductants and hydrogen donors (5 μ l 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,³⁰ 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 μ l of the upper aqueous phase. Human erythrocyte SOD (Sigma) was used as a standard.

Glutathione peroxidase activity was measured as previously described³⁰ in the Cobas-Mira S analyser.

Catalase activity was measured at 25°C in a UV 25 Spectrophotometer (Beckman Instruments, France) as previously described.³⁰

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.³⁰

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

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

	Miners exposed to low dust concentrations for ≥ 4 y at the time of the study (n=105)				Miners exposed to high dust concentrations for ≥ 14 y at the time of the study (n=58)			
	Estimated cumulative exposure to dust				Estimated cumulative exposure to dust			
	≤ 26 mg/m ³ .y (n=67)	26–71 mg/m ³ .y (n=23)	>71 mg/m ³ .y (n=15)	p Value	≤ 71 mg/m ³ .y (n=37)	>71 mg/m ³ .y (n=21)	p Value	
In erythrocytes:								
Catalase (k/g Hb)	91.8 (35.6–152.3)	96.2 (53.9–302.8)	90.0 (44.1–254.1)	0.76	89.4 (41.6–144.9)	103.8 (71.7–161.2)	0.04	
Cu ⁺⁺ /Zn ⁺⁺ SOD (U/g Hb)	140.0 (100.1–233.2)	148.1 (105.0–234.1)	143.8 (102.7–195.1)	0.53	147.1 (104.0–234.5)	130.4 (100.7–173.6)	0.02	
Glutathione peroxidase (U/g Hb)	38.0 (13.3–67.7)	39.3 (16.8–61.2)	41.7 (28.3–61.1)	0.68	34.1 (14.2–65.8)	41.4 (16.1–63.4)	0.08	
In plasma:								
Glutathione peroxidase (U/l)	741 (220–1294)	719 (378–1401)	656 (237–865)	0.47	692 (277–1103)	689 (329–1004)	0.96	
Total antioxidant concentration (mmol/l)	1.35 (0.90–1.68)	1.42 (0.92–2.29)	1.35 (1.07–1.65)	0.42*	1.35 (0.88–1.68)	1.34 (0.93–1.49)	0.77	

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

*p=0.86 without the outlier.

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

	Miners exposed to low dust concentrations for ≥ 4 y at the time of the study (n=105)					Miners exposed to high dust concentrations for ≥ 14 y at the time of the study (n=58)				
	Coefficient	(95% CI)	p Value	R ²	p Value	Coefficient	(95% CI)	p Value	R ²	p Value
Catalase (k/g Hb):				0.038	0.14				0.126	0.006
Constant	5.32	(4.52 to 6.12)	0.0001			4.37	(4.23 to 4.51)	0.0001		
Estimated cumulative exposure to dust (mg/m ³ .y)	0.87.10 ⁻³	(-0.94 to 2.69).10 ⁻³	0.35			2.39.10 ⁻³	(0.74 to 4.04).10 ⁻³	0.0063		
Age (y)	-17.54.10 ⁻³	(-34.99 to -0.08).10 ⁻³	0.05							
Cu ⁺⁺ /Zn ⁺⁺ SOD (U/g Hb):				0.06	0.042				0.141	0.004
Constant	4.99	(4.93 to 5.05)	0.0001			5.08	(4.98 to 5.18)	0.0001		
Estimated cumulative exposure to dust (mg/m ³ .y)	-0.015.10 ⁻³	(-1.21 to 1.18).10 ⁻³	0.98			-1.86.10 ⁻³	(-3.06 to -0.66).10 ⁻³	0.0036		
Cigarette smoking (cigarettes/day)	-4.89.10 ⁻³	(-8.71 to -1.07).10 ⁻³	0.014							

tertile. Previously,³² 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 X² 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.³³ 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).³⁴

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 between the subgroups of estimated cumulative exposure 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 4 Comparison of antioxidant enzyme activities and total plasma antioxidant concentration in subgroups of ILO profusion grades in all miners (n=182)

	ILO profusion grades			CWP		Kruskal-Wallis test, p value	Trend test, p value
	0/0 (n=133)	0/1 (n=18)	1/0 (n=12)	1/1 or 1/2 (n=12)	2/1, 2/2 or 3/3 (n=7)		
In erythrocytes:							
Catalase (k/g Hb)	91.5 (35.6–152.3)	103.1 (65.4–302.8)	97.0 (72.3–161.2)	107.9 (55.9–207.0)	126.6 (90.9–196.5)	0.13	0.002
Cu ⁺⁺ /Zn ⁺⁺ SOD (U/g Hb)	141.0 (100.2–234.5)	149.4 (105.0–224.0)	139.2 (100.7–187.7)	133.6 (91.0–200.3)	117.2 (90.4–145.2)	0.13	0.06
Glutathione peroxidase (U/g Hb)	38.2 (13.3–67.7)	38.4 (14.1–62.0)	34.9 (16.9–53.1)	37.2 (19.3–69.3)	38.5 (18.1–48.9)	0.91	0.72
In plasma:							
Glutathione peroxidase (U/l)	727 (220–1401)	660 (237–898)	631 (378–864)	780 (377–1339)	788 (540–996)	0.31	0.79
Total antioxidant concentration (mmol/l)	1.35 (0.88–2.29)	1.39 (1.22–1.63)	1.36 (0.93–1.82)	1.47 (1.28–1.75)	1.42 (0.87–1.71)	0.18*	0.06†

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.

Table 5 Multiple linear regression of catalase and Cu⁺⁺/Zn⁺⁺ SOD activities on the five subgroups of ILO profusion grades according to dust concentrations at the time of the study in all miners (n=182)

	Miners exposed to low dust concentrations for ≥ 4 y at the time of the study (n=105) and retired miners (n=19)					Miners exposed to high dust concentrations for ≥ 14 y at the time of the study (n=58)				
	Coefficient	(95% CI)	p Value	R ²	p Value	Coefficient	(95% CI)	p Value	R ²	p Value
Catalase (k/g Hb):				0.11	0.003				0.13	0.019
Constant	5.06	(4.35 to 5.76)	0.0001			4.40	(4.23 to 4.57)	0.0001		
ILO profusion grades (5 subgroups)	126.2.10 ⁻³	(58.8 to 193.6).10 ⁻³	0.0004			-43.79.10 ⁻³	(-167.9 to 80.32).10 ⁻³	0.49		
Estimated cumulative exposure to dust (mg/m ³ .y)	-0.94.10 ⁻³	(-2.48 to 0.61).10 ⁻³	0.23			2.67.10 ⁻³	(0.83 to 4.51).10 ⁻³	0.0062		
Age (y)	-14.02.10 ⁻³	(-30.18 to 2.14).10 ⁻³	0.09							
Cu ⁺⁺ /Zn ⁺⁺ SOD (U/g Hb):				0.076	0.023				0.15	0.011
Constant	5.03	(4.96 to 5.11)	0.0001			5.05	(4.92 to 5.17)	0.0001		
ILO profusion grades (5 subgroups)	-25.07.10 ⁻³	(-65.7 to 15.56).10 ⁻³	0.228			36.72.10 ⁻³	(-53.3 to 126.7).10 ⁻³	0.43		
Estimated cumulative exposure to dust (mg/m ³ .y)	-0.37.10 ⁻³	(-1.41 to 0.67).10 ⁻³	0.487			-2.09.10 ⁻³	(-3.43 to -0.76).10 ⁻³	0.0032		
Cigarette smoking (cig/day)	-5.02.10 ⁻³	(-8.82 to -1.22).10 ⁻³	0.011							

miners with estimated cumulative exposure to dust >71 mg/m³.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/m³.y were older than miners with estimated cumulative exposure to dust ≤ 71 mg/m³.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 ≥ 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 ≥ 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 ≥ 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 ≥ 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 ≥ 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 (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

Table 6 Comparison of antioxidant enzyme activities and total plasma antioxidant concentration in CT classes of micronodular score in miners without CWP (n=105+58)

	Micronodular score <4 (n=143)	Micronodular score ≥ 4 (n=20)	Mann-Whitney U test, p value
In erythrocytes:			
Catalase (k/g Hb)	91.3 (35.7–161.2)	107.0 (60.3–302.8)	0.14
Cu ⁺⁺ /Zn ⁺⁺ SOD (U/g Hb)	142.0 (100.2–234.5)	140.2 (100.7–211.4)	0.81
Glutathione peroxidase (U/g Hb)	37.8 (13.3–67.7)	39.2 (13.4–62.0)	0.45
In plasma:			
Glutathione peroxidase (U/l)	717 (220–1401)	675 (237–998)	0.67
Total antioxidant concentration (mmol/l)	1.35 (0.88–2.29)	1.40 (0.93–1.63)	0.09*

* $p=0.08$ without the outlier.
Values are mean (range).

between catalase and $\text{Cu}^{++}/\text{Zn}^{++}$ 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 $\leq 1/0$. However, catalase activity was related to the estimated cumulative exposure to dust.

$\text{Cu}^{++}/\text{Zn}^{++}$ 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.

RELATIONS BETWEEN ANTIOXIDANT ENZYME ACTIVITIES AND TOTAL PLASMA ANTIOXIDANT CONCENTRATION WITH CT CLASSES IN MINERS WITHOUT CWP

A comparison of the antioxidant enzyme activities and total plasma antioxidant concentration in CT classes of micronodular score showed that catalase activity and total plasma antioxidant concentration were higher in the group of miners with a micronodular score ≥ 4 (table 6). Moreover, catalase activity was negatively but not significantly associated whereas total plasma antioxidant concentration was positively but not significantly associated with micronodular scores ($r=-0.04$, $p=0.61$ and $r=0.13$, $p=0.10$, respectively).

Discussion

In this study, we found that erythrocyte catalase and $\text{Cu}^{++}/\text{Zn}^{++}$ 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 $\text{Cu}^{++}/\text{Zn}^{++}$ 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,¹⁴ 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 $\text{Cu}^{++}/\text{Zn}^{++}$ 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 $\text{Cu}^{++}/\text{Zn}^{++}$ SOD activities between underground miners and surface workers in three different mines.³⁵ As our studies are the only ones that have investigated the relations between occupational exposure and blood antioxidant enzyme activi-

ties, 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 $\text{Cu}^{++}/\text{Zn}^{++}$ 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 $\text{Cu}^{++}/\text{Zn}^{++}$ 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 $\text{Cu}^{++}/\text{Zn}^{++}$ SOD will return to normal.

The positive association found for catalase activity is concomitant with the negative association found for $\text{Cu}^{++}/\text{Zn}^{++}$ 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 H_2O_2 . As expected, an increase in catalase activity as a compensatory mechanism in response to the generation of H_2O_2 was found. The decrease in $\text{Cu}^{++}/\text{Zn}^{++}$ SOD activity can be interpreted with the findings of Tsan.³⁶ As $\text{Cu}^{++}/\text{Zn}^{++}$ SOD can be inactivated by its enzymatic reaction product H_2O_2 , we suppose that the high concentration of H_2O_2 in lungs partially inactivates $\text{Cu}^{++}/\text{Zn}^{++}$ SOD. Normal concentrations of $\text{Cu}^{++}/\text{Zn}^{++}$ 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.³⁶

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*¹¹ and Engelen *et al*¹² reported no significant differences in catalase activity

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,¹⁴ 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 between the results already described and those of our present study. Age and number of cigarettes smoked a day have not been taken into account in all these studies. Occupational exposure was expressed as years of underground work in miners all heavily exposed for ≥ 12 years,^{11,12} or as concentrations of respirable coal mine dust available only in active workers.¹⁴ Miners were considered as having pneumoconiosis if their ILO profusion grades were $>0/1$,^{11,12} $>1/0$,¹⁴ or $>1/1$ (this study). All or a part of these 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.*³⁷ found that alveolar macrophages from people with CWP who did not smoke were spontaneously releasing increased amounts of H_2O_2 . Dalal *et al.*³⁸ 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.*⁹ that the main function of catalase is to protect against high concentrations of exogenous H_2O_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 study¹⁴ 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.*^{11,12} 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,³² 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 1/0). Our results accord with

those of Schins *et al.*³⁹ who have studied the relation between several blood antioxidant parameters measured in coal workers and the development of CWP at 5 year follow up. 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.

We acknowledge the medical staff and all the French coal mine workers who made this study possible. We are grateful to Catherine Frette and Francine Kauffmann for helpful discussion or comments on this manuscript and to Anne Bara for revising it. This research was supported, in part, by grant number 7280.03.001 from the European Community of Coal and Steel.

- Doelman CJA, Leurs R, Oosterom WC, *et al.* Mineral dust exposure and free radical-mediated lung damage. *Exp Lung Res* 1990;16:41–55.
- Malech HL, Gallin JI. Neutrophils in human diseases. *N Engl J Med* 1987;317:687–94.
- Wallaert B, Lasalle P, Fortin F, *et al.* Superoxide anion generation by alveolar inflammatory cells in simple pneumoconiosis and in progressive massive fibrosis of no smoking coal workers. *Am Rev Respir Dis* 1990;141:129–33.
- Berg I, Schlüter T, Gercken G. Increase of bovine alveolar macrophage superoxide anion and hydrogen peroxide release by dusts of different origin. *J Toxicol Environ Health* 1993;39:341–54.
- Petit JC, Boettner JC. Evidence of H_2O_2 formation in the early stage of coal oxidation. In: Charcosset H, ed. *Advanced methodologies in coal characterisation*. Amsterdam: Elsevier, 1990:253–66.
- Sies H. *Oxidative stress*. London: Academic Press, 1985.
- Toth KM, Clifford DP, Berger EM, *et al.* Intact human erythrocytes prevent hydrogen peroxide-mediated damage to isolated perfused rat lungs and cultured bovine pulmonary artery endothelial cells. *J Clin Invest* 1984;74:292–5.
- Van Asbeck BS, Hoidal J, Vercelotti GM, *et al.* Protection against lethal hyperoxia by tracheal insufflation of erythrocytes: role of red cell glutathione. *Science* 1984;277:756–8.
- Agar NS, Sadrzadeh SMH, Hallaway PE, *et al.* Erythrocyte catalase: a somatic oxidant defence. *J Clin Invest* 1986;77:319–21.
- Halliwell B, Gutteridge JMC. Protection against oxidants in biological systems: the superoxide theory of oxygen toxicity. In: Halliwell B, Gutteridge JMC, eds. *Free radicals in biology and medicine*, 2nd rev ed. New York: Oxford University Press, 1989:86–187.
- Borm PJA, Meijers JMM, Swaen GMH. Molecular epidemiology of coal worker's pneumoconiosis: application to risk assessment of oxidant and monokine generation by mineral dusts. *Exp Lung Res* 1990;16:57–71.
- Engelen JJM, Borm PJA, Van Sprundel M, *et al.* Blood antioxidant parameters at different stages of pneumoconiosis in coal workers. *Environ Health Perspect* 1990;84:165–72.
- Evelo CTA, Bos RP, Borm PJA. Decreased glutathione content and glutathione S-transferase activity in red blood cells of coal miners with early stages of pneumoconiosis. *Br J Ind Med* 1993;50:633–6.

- 14 Perrin-Nadif R, Auburtin G, Dusch M, et al. Blood antioxidant enzymes as markers of exposure or effect in coal miners. *Occup Environ Med* 1996;53:41-5.
- 15 International Labour Office. *Guidelines for the use of ILO International classification of radiographs of pneumoconiosis*. Geneva: Occupational Safety and Health Sciences, 1980: 22.
- 16 Wagner GR, Attfield MD, Parker JE. Chest radiography in dust-exposed miners: promise and problems, potential and imperfections. *Occup Med* 1993;8:127-41.
- 17 Remy-Jardin M, Degreaf JM, Beuscart R, et al. Coal worker's pneumoconiosis: CT assessment in exposed workers and correlation with radiographic findings. *Radiology* 1990;177:363-71.
- 18 Bégin R, Ostiguy G, Fillion R, et al. Computed tomography scan in the early detection of silicosis. *Am Rev Respir Dis* 1991;144:697-705.
- 19 Remy-Jardin M, Remy J, Farre I, et al. Computed tomographic evaluation of silicosis and coal worker's pneumoconiosis. *Radiol Clin North Am* 1992;30:1155-76.
- 20 Lamers RJS, Schins RPF, Wouters EFM, et al. High-resolution computed tomography of the lungs in coal miners with a normal chest radiograph. *Exp Lung Res* 1994;20: 411-19.
- 21 Caillier I, Bruant A, Mahieu B, et al. Constitution d'un échantillon de sujets à l'aide d'une étude de concordance inter-observateurs. Application à des mineurs suspects de pneumoconiose. *Arch Mal Prof* 1991;52:541-7.
- 22 Rebstock-Bourgard E, Chau N, Caillier I, et al. Symptômes et fonction respiratoires des mineurs de charbon présentant des anomalies radiologiques pulmonaires. *Rev Epidemiol Santé Publ* 1994;42:533-41.
- 23 Pham QT, Bourgard E, Chau N, et al. Forced oscillation technique (FOT): a new tool for epidemiology of occupational lung diseases? *Eur Respir J* 1995;8:1307-13.
- 24 Kauffmann F, Frette C, Pham QT, et al. Associations of blood group-related antigens to FEV₁, wheezing, and asthma. *Am J Respir Crit Care Med* 1996;153:76-82.
- 25 Minette A. Questionnaire of the European Community for Coal and Steel (ECSC) on respiratory symptoms. *Eur Respir J* 1989;2:165-77.
- 26 Attfield MD, Morring K. The derivation of estimated dust exposures for US coal miners working before 1970. *Am Ind Hyg Assoc J* 1992;53:248-55.
- 27 Bernadac P, Hennequin L, Bertal A, et al. Radiologie et pneumoconioses. *Encyclopédie Médico-Chirurgicale Radiodiagnostic - Cœur - Poumon*. Paris: Elsevier, 1997:20. (32-450-A-10.)
- 28 Kleinerman J, Green F, Harley RA, et al. Pathology standards for coal workers' pneumoconiosis. Report of the pneumoconiosis committee of the College of American Pathologists to the National Institute for Occupational Safety and Health. *Arch Pathol Lab Med* 1979;103:375-432.
- 29 Frija J. Sémantique des pneumopathies infiltrantes diffuses étudiées par la tomodensitométrie. *Revue d'Imagerie Médicale* 1991;3:355-61.
- 30 Perrin R, Briançon S, Jeandel C, et al. Blood activity of Cu/Zn superoxide dismutase, glutathione peroxidase and catalase in Alzheimer's disease: a case-control study. *Gerontology* 1990;36:306-13.
- 31 Miller NJ, Rice-Evans C, Davies MJ, et al. A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. *Clin Sci* 1993;84:407-12.
- 32 Rebstock-Bourgard E. *Diagnostic précoce d'une progression des anomalies radiologiques pulmonaires chez des mineurs de charbon* [Thèse de Doctorat de l'Université de Paris XI, mention santé publique]. Paris: Université Paris XI, 1990: 170.
- 33 Guemouri L. Enzymes du métabolisme du glutathion, des radicaux libres et des peroxydes: développements analytiques et étude de la variabilité en physiopathologie humaine. Effets des médicaments et de l'alcool. [Thèse de Doctorat de l'Université de Nancy I, mention pharmacologie]. Nancy: Université de Nancy I, 1991:141.
- 34 SAS Institute. *SAS/stat user's guide, version 6, 4th ed*. Cary, North Carolina: SAS, 1990.
- 35 Perrin-Nadif R, Porcher JM, Dusch M, et al. Erythrocyte antioxidant enzyme activities in coal miners from three French regions. *Int Arch Occup Environ Health* 1988 (in press).
- 36 Tsan MF. Superoxide dismutase and pulmonary oxygen toxicity. *Proc Soc Exp Biol Med* 1997;214:107-13.
- 37 Rom WN, Bitterman PB, Rennard SI, et al. Characterization of the lower respiratory tract inflammation of non-smoking individuals with interstitial lung disease associated with chronic inhalation of inorganic dusts. *Am Rev Respir Dis* 1987;136:1429-34.
- 38 Dalal NS, Jafari B, Petersen M, et al. Presence of stable coal radicals in autopsied coal miners' lungs and its possible correlation to coal workers' pneumoconiosis. *Arch Environ Health* 1991;46:366-72.
- 39 Schins RPF, Keman S, Borm PJA. Blood antioxidant status in coal dust-induced respiratory disorders: a longitudinal evaluation of multiple biomarkers. *Biomarkers* 1997;2:45-50.

Correspondence and editorials

Occupational and Environmental Medicine welcomes correspondence relating to any of the material appearing in the journal. Results from preliminary or small scale studies may also be published in the correspondence column if this seems appropriate. Letters should be not more than 500 words in length and contain a minimum of references. Tables and figures should be kept to an absolute

minimum. Letters are accepted on the understanding that they be subject to editorial revision and shortening.

The journal also publishes editorials which are normally specially commissioned. The Editor welcomes suggestions regarding suitable topics; those wishing to submit an editorial, however, should do so only after discussion with the Editor.