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Presence of Stable Coal Radicals in Autopsied Coal Miners' Lungs and Its Possible Correlation to Coal Workers' Pneumoconiosis

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ABSTRACT. Stable coal radicals (SCRs) were detected by electron spin resonance (ESR) spectroscopy in the lung tissue of autopsied coal miners. The SCR concentrations were measured in the lung tissues from 98 coal miners with and without (a) coal workers' pneumoconiosis (CWP), (b) cancer, and (c) a history of cigarette smoking. Concentrations of SCRs were also determined in the lungs of nonminer controls. The SCR concentration was related to longer mining tenure, CWP disease severity, lung cancer, and cigarette smoking. The mean concentration of SCRs in the lung tissues of miners with 30 $\pm$ 1.4 y of coal mining exposure was 5.3 $\pm$ 1.3 $\times$ 10^{17} spins/g versus controls who had a nondetectable level (< 10^{15} spins/g). An increase in disease severity was accompanied by a progressive increase in SCR concentration. A SCR concentration of 4.8 $\pm$ 0.7 $\times$ 10^{17} spins/g was found for simple CWP (with moderate coal macules) versus 7.8 $\pm$ 4.6 spins/g lung tissue for complicated CWP (with progressive massive fibrosis). Significantly higher (i.e., 10 $\times$ 10^{17}) concentrations of SCR in the coal miners' lung tissues were associated with an exposure history in the anthracite regions of northeastern Pennsylvania. These results indicate a possible role for SCRs in the disease process. Furthermore, ESR appears to be an adequate methodology for the quantitation of coal dust retained in the lung and for distinguishing exposures to anthracite and/or bituminous coal.

THE PREVALENCE AND SEVERITY of coal workers' pneumoconiosis (CWP) differ markedly between geographic locations, mines, and coal fields irrespective of comparable levels of exposure to coal mine dust.[1,2] These differences cannot be fully explained by the presence of silica and other minerals in the coal mine

dust.[3] Rank of coal is also an important factor in the pathogenesis of CWP. Equally important is that the prevalence of CWP decreases from east to west in the United States,[4] and South Wales miners develop more severe CWP than miners in Scotland.[5] However, there are conflicting reports that cast uncertainty on the influence of rank of coal in the development of CWP.[6-9] These problems are complicated but will be solved as new technology emerges and as more is learned about the disease process.

As new ideas regarding the pathogenesis of CWP have emerged, we and other investigators have reported the presence and generation of free radicals during crushing and grinding of coal.[10-14] Grinding and crushing operations are involved in coal mining; therefore, it has been postulated that the reactive free radicals generated during coal mining may play a role in the early biochemical injury leading to CWP.[10,12] In particular, we have shown that the concentrations of reactive free radical sites generated during the grinding of coal decrease with time and that there is a concurrent decrease in toxicity as evidenced by hemolysis.[10,11] However, a high concentration of stable coal radicals (SCRs) that had chemical characteristics similar to those generated by the grinding remained detectable in the coal for long periods thereafter.[10] The present study was undertaken to determine possible relationships between severity of CWP and concentration of SCRs and the relationship between cigarette smoking and SCR concentration in the lungs of miners who did and who did not have lung cancer. The presence of SCRs in the lung tissue of autopsied coal miners[10,11,15] and stable radicals in the lungs of urban dwellers[16] has been reported previously. However, this is the first study in which an attempt was made to correlate the concentration of SCRs present in the lung tissue with CWP disease pattern, severity of CWP, cancer of the lung, smoking, and type of coal exposure.

Materials and methods

Coal miners. Lung tissues from 98 coal miners were obtained from the National Coal Workers' Autopsy Study (NCWAS). These tissues were submitted to the National Institute for Occupational Safety and Health (NIOSH) from 1971 through 1980. Only deceased coal miners who had at least 1 y of underground exposure to coal mining were included in the program, and each case was submitted with detailed demographic, occupational, and smoking histories. An autopsy report and lung tissues were submitted for each case. The pulmonary tissues included a minimum of three paraffin blocks and slides from representative sites of the lungs. The relationship between CWP severity, age, mining tenure, and SCRs in the lung tissue was determined from 98 coal miners' autopsy cases that were selected from a subpopulation of 106 primary lung cancer cases. These cases were matched with an equal number of coal miners who (a) did not have lung cancer for age at death (± 2), (b) years of underground coal mining (± 2), and (c) pack-years of smoking (± 2). Among these 98 autopsies, 49 cases had primary lung cancer, 5 had non-lung cancer, and 30 deaths resulted from cardiac diseases. The remaining 14 deaths were caused by pneumoconiosis (n = 4), accidents (n = 3), pneumonia (n = 2), and other diseases (n = 5). Ages ranged from 48 to 84 y in the combined study population, and underground coal mining exposure ranged from 1 to 55 y. Smokers in the group had a smoking history of 1–134 pack-years.

Non-coal miner controls. Lung tissues from 11 autopsied non-coal miners were obtained by matching age at death and sex with coal miners from a local hospital. The medical files of these 11 referent non-coal miner controls were screened to ensure that there was no known history of employment in coal mining or occupational exposure to coal. The information on the smoking habits of these referent controls was unsatisfactory.

Animal controls. Lung tissue samples from pathogen-free Fisher 344 rats that were exposed only to coal dust (2 mg/m^3) or filtered air for 7 h/d for 5 d/wk for 24 mo were obtained from an ongoing independent study at NIOSH.[17] These samples were compared with human tissue and human data on SCR concentration. Subsequent to the termination of the 24-mo exposure, the histopathologic evaluation of the lungs demonstrated that coal dust particles were deposited in the lungs and that macules had developed.

Electron spin resonance (ESR). All tissue samples were prepared from paraffin blocks, and the same protocols were employed for electron spin resonance studies. Paraffin sections (20–30 μ) were cut and deparaffinized in three changes of xylene for 1 h and air dried for 24 h at room temperature (22 °C ± 2 °C). Air-dried samples that had an average weight of 20–25 mg were packed in duplicate ESR quartz tubes and were labeled. Duplicate samples from the lungs that were obtained from subjects who experienced an increased severity of disease showed 40–64% variability in the SCR concentration. Samples of lungs from subjects who had minimal disease showed less than 20% variability between samples. Tissue sections were sometimes pooled from two or more paraffin blocks from the same case if the subject had minimal disease and cancer of the lung. The samples were randomized, and SCR concentration was measured with a Bruker ER 200D, X-band (9.5 GHz) ESR spectrometer by two of the authors (ND and BJ) who did not have prior knowledge of case history, smoking history, or disease status of the cases. The SCR concentration for each sample was obtained from the ESR signal intensity, i.e., area under the ESR peaks, via a double integration technique for which an ASPECT 2000 microcomputer was used. The SCR concentration was expressed in spins per gram dry lung tissue.[10,11] The SCR concentration in the air-exposed rat lungs, which were fixed and processed by employing the same protocol, was undetectable. Therefore, no significant effects of processing were apparent.

Spectral differences and similarities between SCR and fracture-induced free radicals were compared. Anthracite (95% carbon) and bituminous (72% carbon) coals

were obtained from the Pennsylvania State University, Generic Respirable Dust Technology Center, State College, Pennsylvania. Coals were hand ground in an agate mortar with pestle for 30 min and sieved to 20-μ size. Comparisons of lung tissue SCRs for which freshly ground and aged coal mine dust were used were made according to the methods reported earlier.[10,11]

Histopathologic analysis. At least three hematoxylin- and eosin-stained sections were available for all cases and controls. In addition, sections stained with special stains were used in the typing and classification of lung tumors. The presence, type, and severity of CWP was evaluated independently and without any prior knowledge of historical information by two pathologists (FHYG and VV), who adhered to the criteria and standards recommended by the College of American Pathologists and NIOSH.[18] Lung cancer was typed and classified according to the World Health Organization criteria.[19]

Statistical analysis. The relationship between SCR concentration in the lung tissue and its possible association with disease processes and smoking was evaluated by controlling the influence of confounding variables. Concentrations of SCRs in tissues showed extreme non-normal variations from case-to-case. To determine the effects of age, years of mining, pack-years of cigarettes smoked, severity of CWP, and lung cancer on SCR concentrations, we first calculated the cube root of the log (SCR $\pm$ 1). This variable had a fairly normal-shaped distribution and was used only for the testing of the hypothesis. The values presented in Tables 1-5 represent SCR data. Each of the independent variables (i.e., age, years of mining, pack-years of cigarettes smoked, severity of CWP, cancer) was then tested separately, either by analysis of variance or by simple linear regression, for its influence on the transformed variable. Variables were excluded from further analysis if a p value greater than .10 was found. The remaining variables and cancer by severity of CWP interaction were then tested simultaneously. If the p value was less than or equal to .05, an effect was considered to be significant.

Results

The 98 coal miners in the study group had an average age of 64 $\pm$ 0.8 y (mean $\pm$ standard error of the mean) and an average underground mining exposure of 30 $\pm$ 1.4 y. Among these 98 coal miners, 18 were nonsmokers. The average pack-years of those individuals who had smoked was 35 $\pm$ 2.9.

Lung tissues from autopsied coal miners were grouped into those without disease (n = 35), macular disease (n = 31), CWP and silicosis (n = 10), and progressive massive fibrosis (PMF) (n = 22). This grouping was based on the highest category of disease type and its severity; hence, subjects with PMF may also have had silicosis, nodules, and macules, and subjects with silicosis may also have had nodules and macules, whereas the subjects grouped under macular disease only had macules.

The mean age, mining tenure, and the SCRs, ranked by disease group, for the 98 coal miners and 11 controls who had no coal mining experience are shown in Table 1. It is evident that in the 11 controls with no known exposure to coal mine dust, there were no SCRs detected in the lung tissue. However, in 35 miners who did not have CWP and who were exposed to varying amounts of coal dust, the mean SCR concentration was (1.6 $\pm$ 0.7) $\times$ 10^{17} spins/g dry lung, whereas in 63 miners who had CWP and who were exposed to a greater amount of coal dust, the SCR concentration was increased approximately fivefold, i.e., (7.3 $\pm$ 2.0) $\times$ 10^{17} spins/g dry lung. In comparison, accumulations of focal black pigmented alveolar macrophages adjacent to the respiratory bronchioles were found in the lungs from rats exposed for 24 mo to 2 mg/m^3 coal dust for 5 d/wk, 7 h/d, and a mean SCR concentration of 0.6 $\times$ 10^{17} spins/g dry lung was found. This was approximately one-half the concentration of SCRs observed in the lungs from miners that showed no evidence of CWP (Table 1). It is interesting that the SCR concentration in autopsied lungs, obtained from urban Tokyo dwellers, with black dust deposits was of the same order of magnitude as that found in rats that were exposed to coal dust for 24 mo.[16] The combined group with CWP showed a fivefold increase in SCR concentration com-

Table 1.—SCR Concentrations in the Lungs of Coal Miners, by Type of CWP

Group	Age	Mining years	SRC conc. 10^{17} spins/g dry lung
Controls (11)	65 $\pm$ 1.5†	0 $\pm$ 0.0	0.0 $\pm$ 0
Miners without CWP (35)	63 $\pm$ 1.2	29 $\pm$ 2.3	1.6 $\pm$ 0.7
All miners (98)	64 $\pm$ 0.8	30‡ $\pm$ 1.4	5.3 $\pm$ 1.3
Miners with CWP (63)	64 $\pm$ 1.1	30 $\pm$ 1.8	7.3 $\pm$ 2.0
Macular CWP (31)	62 $\pm$ 1.5	27 $\pm$ 2.6	4.8§ $\pm$ 1.7
PMF (22)	68 $\pm$ 1.8	33 $\pm$ 3.3	7.8§ $\pm$ 4.6
Silicosis with CWP (10)	64 $\pm$ 1.6	34 $\pm$ 3.3	14.2// $\pm$ 5.2

*Numbers within parentheses indicate number of subjects studied.
†Standard error of the mean.
‡Sample size = 97.
§Different from mean for miners without CWP group, $p < .05$.
//Different from mean for miners without CWP group, $p < .01$.

pared with the group without disease ($p < .01$) (Table 1). Groups with macular CWP and PMF showed higher concentrations of SCRs ($p < .05$) compared with the group without CWP. The group with silicosis and CWP showed a ninefold increase in SCR concentration compared with the group without CWP ($p < .01$).

Typical ESR spectra obtained from dry lung tissues from an autopsied nonminer control, coal miner with macular CWP, coal miner with PMF, and coal miner with macular CWP and silicosis are illustrated in Figure 1 a–d, respectively. The SCR concentration (as can be deduced from the areas under the ESR spectra) was the least for macular disease and the highest for the coal miner with macular CWP and silicosis (Fig. 1). The SCR concentrations for all the samples were calculated by area measurements, which were obtained by a double integration technique completed with the aid of an ASPECT 2000 microcomputer.[10]

After other factors were adjusted form, pack-years of cigarettes smoked and the presence of lung cancer were significantly related to SCR concentration. Age was not related to SCR concentration. Years of mining were related to SCR concentration only when other factors were not adjusted for. The significance of the relationship between CWP and SCR concentration was dependent on the smoking status and the presence or absence of cancer in the lung. The mean SCR concentration in miners who did and did not have lung cancer, by category of pneumoconiosis when pack-years of smoking was controlled for, is shown in Table 2. A strong relationship between SCR and severity of CWP was apparent only for subjects without cancer. Generally, lung cancer cases had lower SCR concentrations than did noncancer cases who had similar CWP. It is not apparent from the preliminary analysis of data on lung cancer cases whether the different cell types had any significant effect on the SCR concentration.

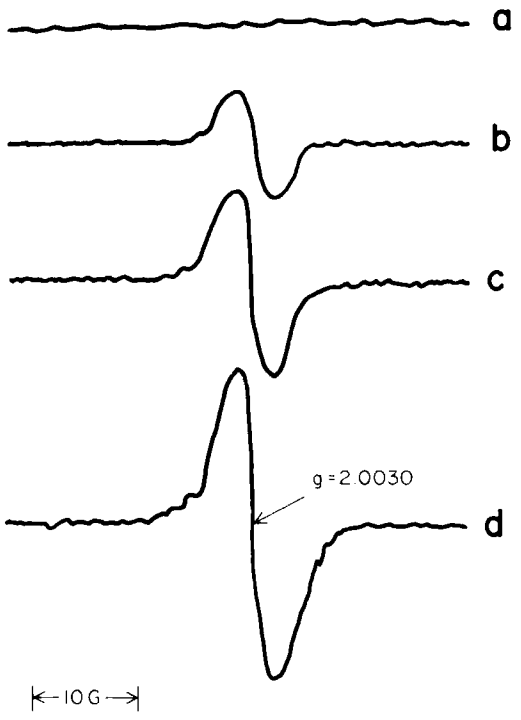

Fig. 1. Typical X-band (9.5 GHz) ESR spectra of air-dried lung tissue samples (25 mg) from (a) control, 72-y-old man, nonminer; (b) 84-y-old man, with 7 y underground coal mining exposure, who had macular lesions; (c) 64-y-old man with 34 y underground coal mining exposure who had macules and PMF; and (d) 58-y-old man with 38 y underground coal mining exposure who had macules and silicosis. The *g* value was 2.0029 + 0.0005. The magnetic field scale was similar in all measurements.

Table 2.—SCR Concentrations in the Lungs of Coal Miners Who Did and Did Not Have Lung Cancer, Adjusted for Smoking Pack-Years

CWP type	SCR concentration 10^{17} spins/g dry lung* Without cancer	With lung cancer
Without CWP	9.0 ± 0.3 (17)	0.3 ± 0.1 (18)
Macular	0.8 ± 0.3 (13)	1.0† ± 0.3 (16)
CWP with silicosis	3.1‡ ± 1.5 (9)	— (0)
PMF	7.1§ ± 5.8 (5)	0.6 ± 0.2 (14)

*Numbers within parentheses indicate number of subjects studied. Means represent inverse transformation of adjusted means, and standard errors represent one-half the width of the confidence interval of the mean, which was formed by taking the inverse transformation of the 67% confidence intervals endprints of the transformed data.
†Higher than the mean for no CWP group, $p < .05$.
‡Higher than the mean for no CWP and macular groups, $p < .05$.
§Higher than the mean for no CWP and macular groups, $p < .01$.

Table 3.—SCR Concentration in the Lungs of Smokers and Nonsmokers

Group	Age	Mining	SRC conc. 10^{17} spins/g dry lung
Nonsmokers (18)*	66 ± 2†	37‡ ± 2	11.7 ± 3.8
Smokers (79)	63 ± 1	28 ± 2	3.9§ ± 1.4

*Numbers within parentheses indicate number of subjects studied.
†Standard error of the mean.
‡Sample size = 17.
§Lower than mean for nonsmokers, $p < .01$.

The relationship of smoking status with SCR concentration in the lung of coal miners is shown in Table 3. The smokers in the study group showed a threefold decrease in SCR concentration ($p < .01$). This decrease was also clearly evident on a dose-dependent basis, as illustrated in Figure 2, which represents the least squares curvilinear relationship between SCR and pack-years unadjusted for other factors (SCR = Exp $[.977 - .0040PY]^3 - 1$). Smoking appeared to be associated with a decrease in the concentration of SCR.

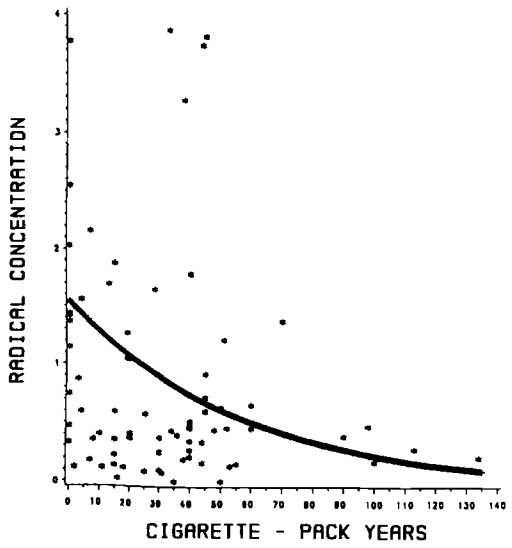

Fig. 2. Effect of cigarette smoking on the concentration of coal stable radicals in lung tissue. The concentration of stable radical decreased on a dose-dependent basis at a statistically significant level ($p < .01$). The estimated dose-response equation was ESR = exp [.97732 - .00396681(*PY*)]^3 - 1.

The decrease in SCR concentration, when adjusted for the influence of other factors (i.e., cancer, CWP severity, and cancer by disease severity interaction), was approximately 0.0024 units in the transformed variable for each pack-year of cigarettes smoked, compared with .004 before adjustment.

The data analysis provided evidence that the smokers had lower levels of SCR and that they tended to experience a lower prevalence of CWP. A higher prevalence of CWP in nonsmokers and a higher SCR concentration are reported in Table 4 ($p < .001$). We sought to confirm this by matching 17 nonsmokers with 17 smokers with respect to age and years of mining tenure, and we compared the prevalence of CWP by type and severity. The mean age of nonsmokers was 66.4 y, and the mean tenure in mining was 36.9 y versus 66.7 y and 36.9 y, respectively, for smokers. A higher prevalence of each CWP disease category in nonsmokers and an overall higher SCR concentration were found in these age-matched analyses ($p < .003$, Table 5).

Discussion

The major hypothesis in this study was that the concentration of SCRs in lung tissue would be related to the severity of CWP. It is evident from the data presented in Table 1 that this was the case. As previously discussed, there appeared to be a direct relationship between the formation of free radicals at the freshly fractured surfaces and the concentration of long-lived SCRs[10,11]; and between rank of coal, free radical formation, and cytotoxicity.[10] Hence, our data suggest indirectly that the fracture-induced free radicals might be involved in the initiation of CWP but that the SCRs contained within the particle may be involved in the propagation of cytotoxic reactions and the progression of CWP. It is known that severity of CWP correlates with total lung dust burden.[3] Therefore, it is probable that SCR in the lung tissue will increase as dust levels increase. The analysis of data showed an exposure-tenure-dependent and disease-severity-associated increase in SCR concentration (when other factors were not adjusted for).

In addition to this possible relationship of SCR concentration in the lungs with CWP severity, the lung tissues from coal miners with silicosis had a significantly higher SCR concentration than that found in the PMF cases. This higher SCR concentration was very evident in all the silicosis cases despite their lower coal mine

Table 4.—Comparison of All Nonsmokers and Smokers for the Severity of CWP and SCR Concentrations

	Without disease	Macule	PMF	Silicosis	SCR conc. 10^{17} spins/g dry lung
Nonsmokers	0 (0%)	6 (33%)	6 (33%)	6 (33%)	11.7 ± 3.8
Smokers	35 (44%)	25 (32%)	15 (19%)	4 (5%)	3.9 ± 1.4

Table 5.—Age and Years of Mining Comparison of 17 Nonsmokers and 17 Smokers for the Severity of CWP and SCR Concentration*

	Without disease	Macule	PMF	Silicosis	SCR conc. 10^{17} spins/g dry lung
Nonsmokers (17)	0 (0%)	6 (35%)	5 (29%)	6 (35%)	12.4 ± 16.2
Smokers (17)	8 (47%)	5 (29%)	2 (12%)	2 (12%)	4.2 ± 7.6

*The mean age of nonsmokers was 66.4, and the mean number of years spent mining was 36.9. The mean age for smokers was 66.7, and the mean number of years spent mining was 36.9.

dust burden present in the lung sections, compared with PMF cases. Aged silica does not contain SCRs as does aged coal[11]; therefore, we conjectured that this increased concentration of SCRs in silicosis may have resulted from a high concentration of anthracite coal in the lung. Earlier observations[10,11] from this laboratory and by others[12] indicated that higher concentrations of SCRs are present in the anthracite coal and that fracturing generated significantly greater concentrations of free radicals from anthracite coal than from bituminous coal.[11] Electron spin resonance spectral shapes and signal intensities noted for the lung tissues obtained from subjects with silicosis were comparable to anthracite coal samples that had 95% carbon content, which indicated that there was a relationship between anthracite coal mine exposure and a higher concentration of SCR in lungs. Support for this hypothesis was obtained from the observation that all the silicosis cases in this study had worked in the northeastern and north central coal mines of Pennsylvania. A high prevalence of pneumoconiosis and silicosis in the anthracite miners of northeastern Pennsylvania[20,21] have been documented in epidemiologic and pathologic studies. Because there were a limited number of silicosis cases in our study, we did not attempt to further breakdown these analyses to detect differences between specific mines or coal seams. Also interesting is that all the cases (16/96) with a relative SCR concentration greater than 10×10^{17} spins/g dry lung had work histories in northern and north central Pennsylvania coal mines, which indicated a relationship between higher SCR concentration and anthracite coal mine dust exposure.

The SCR concentration in the lungs of miners showed a progressive increase proportional to the length of coal mining tenure and disease severity, but lung tissues of miners who had a history of smoking cigarettes had significantly lower SCR concentrations. This decrease in SCR concentration was dose-dependent, and significantly lower SCR concentrations were observed in the lungs from miners who smoked heavily ($p < .01$). However, this lower SCR concentration was also very evident in lung cancer cases adjusted for pack-years of smoking. The miners who had lung cancer and who did not have CWP and miners who had lung cancer and who did not have PMF had lower concentrations of SCRs than did those who did not have lung cancer. Therefore, it appears that this lower SCR concentration in lung cancer cases is not entirely the result of smoking and may be influenced by lung cancer and other factors.

Although the data in Table 5 would appear to indicate that smoking and cancer protect against CWP, we maintain this is not the case. Given the data on SCRs for smokers and nonsmokers in this study, several factors should be considered before the interpretations can be generalized to the entire population. A reasonable explanation of the relationship between SCR concentrations and both smoking and lung cancer involves the biased nature of this autopsy population. The lung tissues submitted to the NCWAS from nonsmoking coal miners were from coal miners who probably died of CWP and, therefore, whose lung tissue had high SCR values. However, smokers submitted to the NCWAS may have died prematurely from cancer of the lung or from heart disease rather than from CWP; they would have had a reduced mining tenure and low SCR values. Another possibility is that the NCWAS population may have been biased because it included only a small proportion of all miners who died with an exposure history in coal mines. Available estimates based on expected death rates of retired and active coal miners for the years 1970 to 1980 indicated that less than 10% of the coal miners' deaths were reported and submitted to the NCWAS program. It is possible that, because this was not a random sample, the majority of miners submitted to the NCWAS program had less CWP and were more severely affected by smoking-related diseases (e.g., cancer, emphysema, bronchitis). Therefore, the lower SCR concentrations observed in the smokers may have resulted from this biased sample of smokers who had minimal CWP. In support of this hypothesis, we found a significant decrease in the SCR concentration and prevalence of overall CWP in 17 smokers, compared with nonsmokers matched for age and years of mining. Comparison of all the smokers and nonsmokers in the study group also produced a chi-square significance of $p < .001$ for less CWP in smokers who had lower SCR concentrations.

Consideration of other independent contributions to the possible increase or decrease in SCR concentration may also be important. Among these, smoking, cancer, anthracite exposure, and other interactions of biomolecules should be considered. It is possible that increased mobility of macrophages, clearance, destruction of lung tissue, obstructive airways or hypersecretion of sputum that causes the deposition of inhaled dust in the bronchi and prevents its deposition deep in the alveoli might impact SCR concentration. Furthermore, it is possible that the lower SCR concentration in smokers may have resulted from the radical interactions or quenching effect of some chemical species present in the cigarette smoke. In support of this view, it was shown recently that cigarette smoke contained many short-lived highly reactive free radicals.[22] Such radicals could quench the SCRs via radical-radical termination reactions.

Other alternate mechanisms might be postulated to have caused a decrease or increase in SCRs in smokers and nonsmokers. A possible relationship of anthracite coal mine exposure and increased SCR concentration could be conjectured from the fact that all the coal miners in this study with a SCR concentration greater than 10×10^{17} spins/g dry lung had a work exposure in the coal mines of northeastern or north central Pennsylvania. We have shown previously that the free radical concentration of freshly ground anthracite coal is approximately 300% more than that of bituminous coal.[11] We also determined that the ESR spectral width and line intensities of the 18 cases with SCR concentrations above 10×10^{17} spins/g dry lung weight were comparable to either anthracite coal mine dust or a mixture of anthracite and bituminous coal, which indicated mixed exposures.

In conclusion, the ESR results presented in this study

provide evidence for the presence of SCRs embedded in the lung tissue even after years of residence in the lung. Generally, organic radicals such as those generated by fracture of the coal would have short lifetimes. However, their lifetime may be long because they are stabilized within coal because of being entrapped in the particle. Normally such entrapped stable radicals are not expected to react with tissue. Because coal is porous, a possibility of reactivity with these entrapped SCRs cannot be ruled out. In this context, Pryor et al.[23] have recently reported on ESR detection of stable radicals in pyrolyzed perfluoro polymer particles. In agreement with our present results on the SCRs in coal, these authors showed that the radicals in the burned polymer particles are stabilized by entrapment within the particle lattice. Without entrapment, the radicals would be too short-lived to reach the lower respiratory units. The particles act as carriers for radicals. From these studies, Pryor et al.[23] have suggested a combination of particle-mediated and particle-associated radical-mediated damage as the cause of the unusual toxicity of perfluoro polymer smoke.[23] Our data and the conclusions of the present study concur with this suggestion, which indicates that the entrapped SCRs might indeed be involved in the biochemical reactions of coal that lead to lung injury.

Finally, the ESR methodology can be used to provide quantitative measurements of coal dust load and to distinguish between anthracite and bituminous coal mine exposures.

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