Characterization by Scanning Transmission Electron Microscopy of Silica Particles from Alveolar Macrophages of Coal Miners

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The structure and composition of silica-rich particles recovered by lavage from the lungs of three active miners with different medical histories were studied using high-resolution electron microscopy and chemical microanalysis. The results are compared to the similarly determined structure and composition of respirable-size mineral particles obtained from roof-bolter dust-box samples from two coal mines of widely different bulk quartz concentrations. The results show that the lungs of the miners contain silica-based particles with structures not found in the mine samples. Also, the particle structures and compositions found in the macrophages were different in each of the miners. The results suggest the possibility that intracellular processes may affect the susceptibility of individuals to silica-induced pneumoconioses. *Key words:* alveolar macrophages, energy-dispersive X-ray analysis, quartz, scanning transmission electron microscopy, silica. *Environ Health Perspect* 102:862–868 (1994)

The cytotoxic and fibrogenic potentials of mine dusts result from the activity of their individual components. Whether this effect is additive or even mass (i.e., dose) related is questionable and has been the subject of many investigations. Particle size and size distribution of various constituent minerals, the surface structure and properties of individual particles, and the self-cleaning capability of the lung (i.e., the capacity of the macrophage system) may, among other variables, affect the pathogenic changes induced by dusts lodged inside lungs. Extensive epidemiological investigations conducted on miners from different coal-mining districts, where equivalent dust exposures have been shown to result in vastly different frequencies of lung disease incidence, also confirm this hypothesis (*1*).

In a recent study, Ferrer et al. (*2*) used energy-dispersive X-ray analysis (EDXA) to investigate the inorganic element content in pleura and lung in reference, silica-exposed nonpneumoconiotic, and silicotic populations. Constant depositions of silicon and calcium were detected in visceral pleura, parietal pleura, and lung of the refence group and in visceral pleura and lung of the exposed nonpneumoconiotic and silicotic groups. Comparison of the silicon content in pleura between silicotic and exposed nonpneumoconiotic subjects showed a nonsignificant probability of difference, whereas there were no differences with respect to the silicon content in lungs.

These results point to the likelihood that the principal parameters relating to the incidence of silicosis and mineral dust-induced pneumoconioses may be the characteristics of the inhaled silica particles (crystallinity, surface composition, particle size) and the ability of each individual's immunological system to respond to the presence of such particles in pleura and lungs.

The concentration in mine dusts of quartz and of other crystalline forms of silicon has been identified as a primary reference variable for anticipated pneumoconioses risk, resulting in regulations of the allowable quartz concentration in mine dusts. Although such regulation may reduce risk factors in a first approximation, there is reason to doubt that it can address particular situations where factors of particle size and morphology, surface characteristics, associated minerals, and particular characteristics of the immunological response system may outweigh the effect of bulk concentration of silica or quartz. Furthermore, *in vitro* studies of the membranolytic activity and cytotoxicity of mineral dust particles have shown no unique relationship with the bulk concentration of quartz in such dusts (*3–5*). On the other hand, many *in vitro* and *in vivo* studies of the effect on cytotoxicity of the surface characteristics of mineral particles have shown positive correlations with different related variables (*6–9*). Also, Rehn et al. (*10*) have shown that the presence of admixed minerals affects the rate of elimination of inhaled dusts from the lung.

Most laboratory studies conducted *in vitro* or *in vivo* use either synthetic mixtures of various minerals or natural dusts characterized only on a macroscopic scale. Only recently has attention been drawn to the importance of particle-by-particle analysis for a full characterization of respirable dusts (*11–15*).

Lung macrophages may be unable to transport particular types of dust particles out of the lungs. One proposed mechanism is that the dust particles cause the macrophage to generate enzymes that eventually lead to its destruction and the formation of abnormal tissue in the lung (*16,17*). Although one of the normal mechanisms for the elimination of foreign particles relies on the capability of macrophages to dissolve solid particles of low aqueous solubility (*18–20*), recent studies have shown that the opposite can also occur [i.e., that the lysosome of lung macrophages may concentrate and precipitate elements inhaled as a part of water soluble compounds (*21*)].

We conducted a particle-by-particle examination of dusts recovered from macrophages extracted by lavage from the lungs of coal miners with long-term exposure to coal mine dusts and compared the results with those obtained by similar analysis of mineral particles obtained directly from typical mining environments. By examining particles extracted from the lungs of miners with different lung-disease-related medical histories, we sought to establish any noticeable compositional and/or structural differences between them.

Materials and Methods

Electron microscopy and microanalysis permit precise determination of morphological, microstructural, and compositional characteristics of individual particles examined. We collected data with a VG HB5 field emission scanning transmission electron microscope (STEM), operated at 100 keV, with a probe size of 20 Å. We conducted X-ray analysis using an energy dis-

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persive X-ray analyzer through the characteristic excitation of X-rays caused by energy losses from the inelastic collisions of electrons with atoms. Elements of atomic number 5 (boron) and greater can be detected by this approach. X-ray spectra of elemental composition, element locational "maps," and images of particles were obtained using STEM. We obtained electron diffraction patterns of particles using a transmission electron microscope (TEM). The instrument used was a JEOL 200CX electron microscope, operating at 200keV, with a LaB_6 filament. Electron diffraction patterns of individual particles served to ascertain the degree of crystallinity of silica particles and to confirm the quartz phase.

We examined particles extracted from pulmonary macrophages, lavaged from the lungs of three coal miners with long-term exposure to quartz-containing dusts. The samples, labeled "A" and "B," were from miners diagnosed with pneumoconiosis, and sample "C" came from a miner with no apparent pulmonary disorders (Kuhn D, 1993, Hershey Medical Center, personal communication).

Patient A, 51 years old, was an active miner at the time of sampling, who had worked in northeastern Pennsylvania mines for 33 years and had been diagnosed with stage 3 black lung disease. Patient B, 40 years old, was also an active miner at the time of sampling and had worked at the mine face, with exposure to anthracite and hard rocks, for the previous 19 years. The chest X-rays of patient B showed round opacities consistent with pneumoconiosis. Patient C, 71 years old, had, at the time of sampling, worked for 35 years at the mine face (blasting, drilling, hauling) and subsequent 20 years in maintenance activities. During his lifetime, this patient had extensive exposure to cement, graphite, and coal. The medical report on the condition of the lungs of this patient classifies it as "normal."

The macrophage samples, supplied by Douglas Kuhn of the Hershey Medical Center, had been incubated in growth media, then resuspended in 0.1% sodium dodecylsulfate to digest the macrophage wall. Upon receiving the samples, they were washed, centrifuged, dried, and weighed. A small portion of each sample was ultrasonically resuspended in ethanol and deposited by dropper onto electron microscope grids for analysis. Each original sample was derived from an equal number of macrophages (approximately 20×10^6) to allow quantitative comparison of dust loadings. Sample A contained 1.0 mg and sample C 0.7 mg of particles, respectively. Sample B could not be similarly assessed because of accidental loss of part of the sample. The particles examined ranged in size from about 0.1–5 μm in diameter, well within the respirable range.

Within each sample, approximately 100 randomly selected mineral particles were surveyed and categorized by composition. We constructed composition maps for different elemental constituents for each particle examined based on EDXA. These maps show semiquantitatively the relative abundance of a specific element across a given particle. Mineral phases associated with silica-based particles were noted, and the occurrence of elements other than silicon above the instrumental detection limit (~2%) in these adjunct phases was determined for each sample.

Subsequently, we selected high silica content particles (over 80% SiO_2 by weight) within each sample (37, 55, and 56 from samples A, B, and C, respectively) and examined them in more detail through electron micrographs and by EDXA. A fraction of these (18, 32, and 26 from samples A, B, and C, respectively) were examined by electron diffraction. Among the silica-based particles examined, we found amorphous (or microcrystalline) materials, polycrystalline aggregates, and particles of single crystal alpha-quartz structure.

For comparison with typical mineral dust particles before inhalation, two dust samples prepared from material collected in roof-bolter dust boxes of two coal mines located in different geographic and geological regions were examined: sample 1 from the Lower Sunnyside seam, Apex Mine, Andalex Resources, Inc.; sample 2 from Illinois no. 6 seam, Brushy Creek Mine, Kennelis Energy. These samples were collected by the Mine Safety and Health Administration (MSHA) and supplied by Paul Parobek of the MSHA Pittsburgh laboratories.

The roof-bolter dust-box samples were milled and subsequently classified by Microtrac laser size-particle analysis at the Pennsylvania State University Mineral Processing Center (by courtesy of Richard Hogg). The dusts so generated were deemed to be representative of typical hard-rock structures proximate to coal seams. The mineral composition of the mine dusts was determined by the Rietveld method of X-ray diffraction. The respirable size fraction of each roof-bolter dust-box sample, prepared by milling and size classification, was ultrasonically suspended in ethanol. A sample of the suspended particles was then deposited by dropper onto an electron microscope grid. The structure and composition of individual silica-rich (>80% SiO_2) particles was determined by the same STEM/EDXA procedure used for the particles recovered from macrophages.

Results

Particles Recovered from Pulmonary Macrophages

Figures 1–3 illustrate the results of classification by composition of the approximately 100 randomly selected particles surveyed

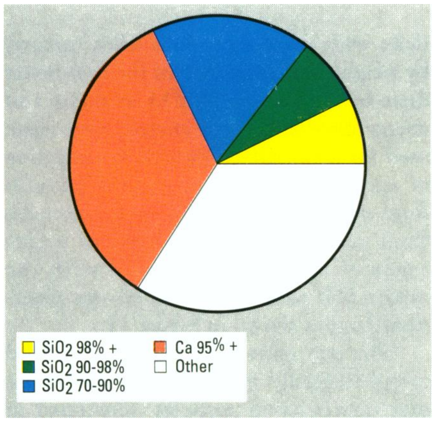

Figure 1. Sample A: percentage of particles by silica content and by principal component; 94 particles examined.

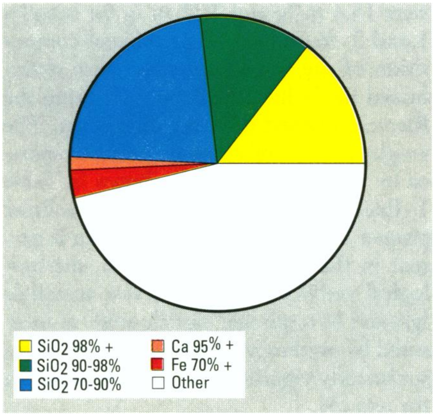

Figure 2. Sample B: percentage of particles by silica content and by principal component; 97 particles examined.

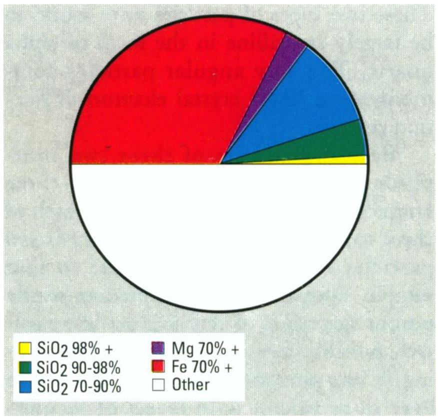

Figure 3. Sample C: percentage of particles by silica content and by principal component; 86 particles examined.

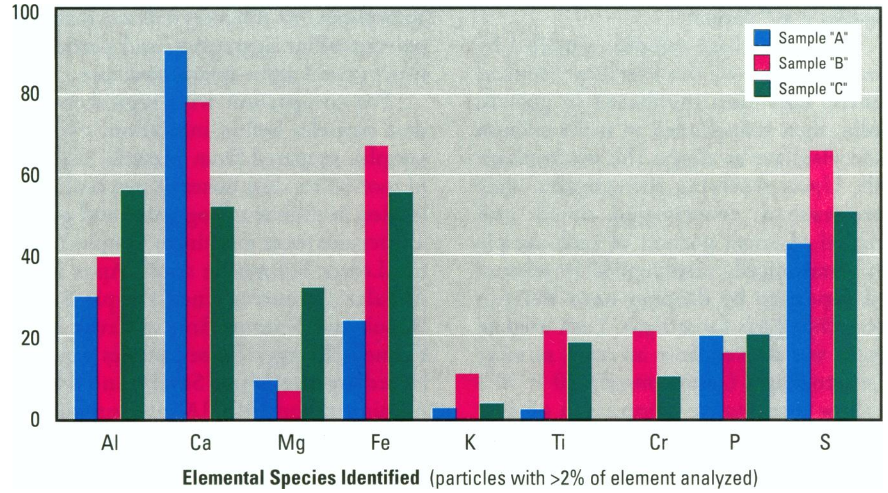

Figure 4. Human alveolar macrophage samples: frequency of elemental species associated with SiO_2 particles.

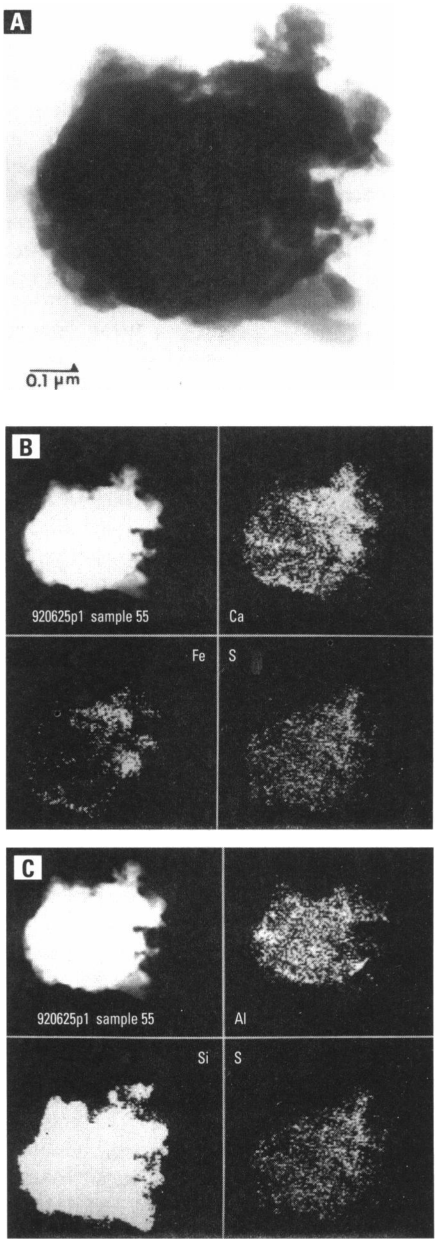

Figure 5. (A) Electron micrograph of a multiphased silica-based particle from a macrophage. (B,C) Elemental X-ray maps of the same particle.

from samples A, B, and C, respectively. It is noted that the particle samples from miners with diagnosed lung disease, samples A and B, have a significantly higher percentage of silica (quartz) particles than sample C, taken from a miner with normal lungs.

Figure 4 shows the percentage of silica-based particles within each sample with a detectable concentration (>2%) of secondary elements. The frequency of occurrence of aluminum and magnesium is higher for particles recovered from the lungs of the healthy miner, sample C, than for particles in samples A and B, taken from miners with diagnosed lung disease. The reverse case applies for the frequency of occurrence of calcium.

Two types of morphologies of silica-based particles were principally observed in all three samples derived from macrophages. One morphology, structurally complex and multiphased, is illustrated by the electron micrograph and associated elemental X-ray maps of Figure 5. The other common silica particle morphology is single-phased and sharply faceted, illustrated by the micrograph and compositional map in Figure 6. These two types of particles were found to be largely crystalline in the form of alpha quartz. The very angular particles commonly gave single crystal electron diffraction patterns.

Besides particles of these two morphologies commonly found in all three samples derived from macrophages, each of these samples also contained silica-based particles of morphology unique to that sample. Sample A contained predominantly porous aggregates of very fine gel-like particles, possibly formed by precipitation during *in vivo* processes. These aggregates were very pure silicon with levels of second-phase cations undetectable above the Bremsstrahlung in the X-ray spectrum. One such particle which, except for adhering calcium- and aluminum-rich fragments, is 98+% SiO_2, is illustrated in Figure 7.

Sample B contained agglomerates of nodular primary silica particles, frequently with high silica content (Fig. 8). Sample C contained aggregates of platelets, with low levels (~5%) of magnesium dispersed throughout. One such particle is shown in Figure 9.

It was noted also that the majority of particles with a high silica concentration (>98%) were also of amorphous or microcrystalline structure. The distributions of silica-rich particles between crystalline and amorphous structures, and by composition (>98% SiO_2 and ≤98% SiO_2) for the three samples examined are shown in Figure 10. The coincidence of these distributions indicates that the formation of amorphous or microcrystalline aggregates inside the macrophages is associated with the precipitation of nearly pure silica.

Dust Samples Collected from Mines

The specific surface areas of the roof-bolter-box mine dusts, as determined by Brunauer-Emmet-Teller (BET) analysis, were 15.4 m^2/g and 7.77 m^2/g for samples 1 and 2, respectively. The mineral composition of the two samples has been determined by Seehra and Babu (*22*) using the Rietveld method of X-ray diffraction. The weight percent of minerals present reported by these investigators is shown in Table 1. Except for a small amount of kaolinite present in the two samples, all silica is present in the form of quartz. Also, the biological activity of the same two materials (ground to respirable particle size), as measured by erythrocyte hemolysis, showed no statistically significant difference between the two (*5*).

To compare with the compositions and morphologies of the particles examined in the macrophage-derived samples, only particles with more than 80% silica (quartz) by weight were examined in the roof-bolter dust-box samples. STEM analysis and energy dispersive X-ray analysis were again used for a particle-by-particle investigation of approximately 50 quartz-rich particles from sample 1 and 30 quartz-rich particles from sample 2. The frequency of occurrence of elements associated with the quartz-rich particles examined was determined and is shown in Figure 11.

The two mine-dust samples contained morphologically and compositionally complex quartz-based particles, illustrated in Figures 12 and 13. Although the quartz-based particles from the two samples were morphologically indistinguishable, the

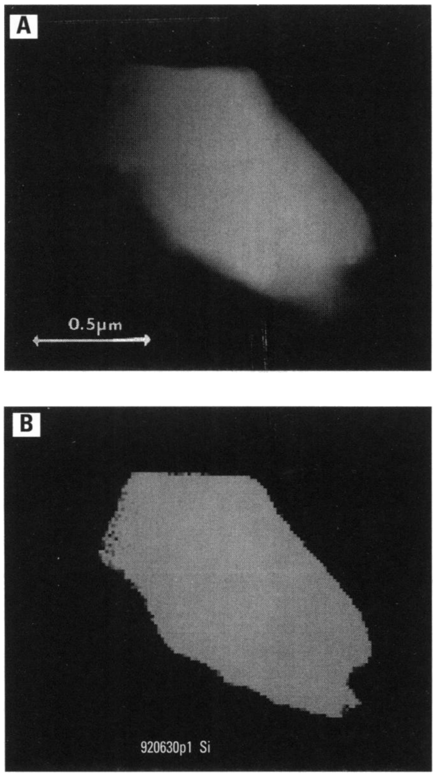

Figure 6. (A) Electron micrograph of a single-phased silica-based particle from a macrophage. (B) Compositional map of same particle.

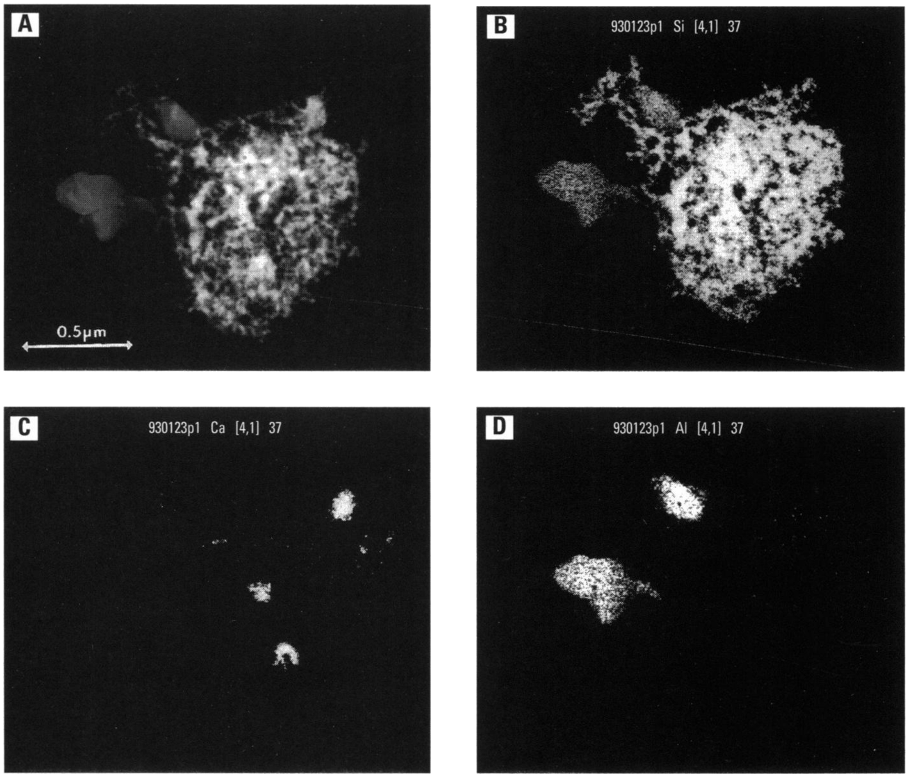

Figure 7. (A) Electron micrograph of a 98+% SiO_2 aggregate from a macrophage from sample A. (B–D) Elemental X-ray maps of same aggregate.

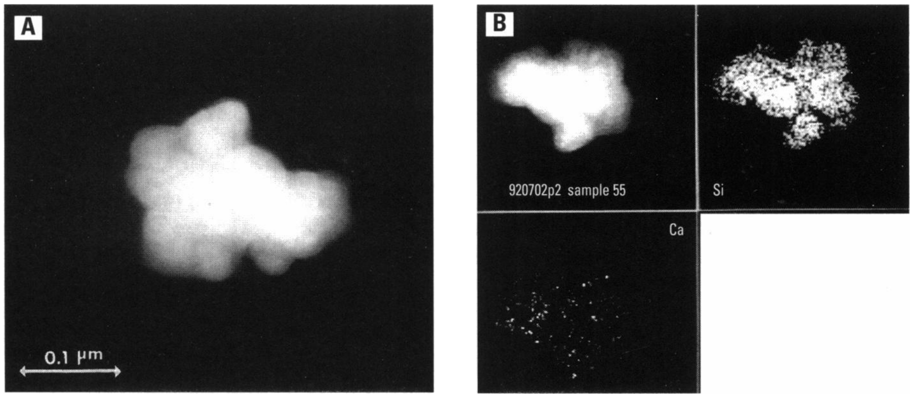

Figure 8. (A) Electron micrograph of agglomerates of nodular primary silica particles from sample B. (B) Elemental X-ray map of same agglomerate.

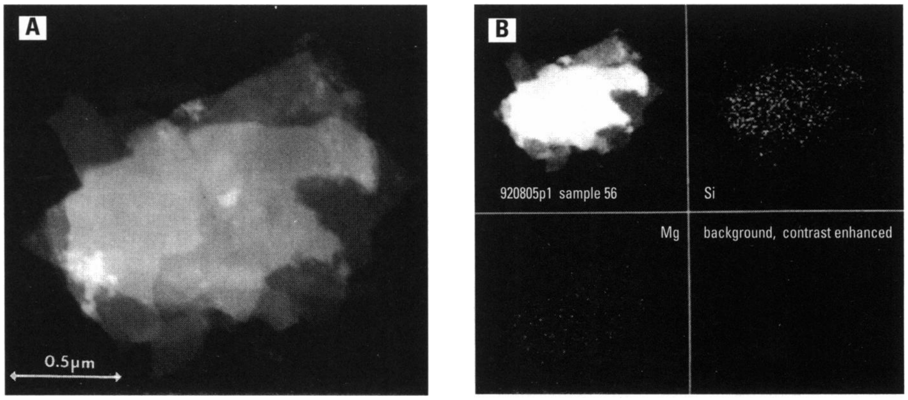

Figure 9. (A) Electron micrograph of aggregate of platelets containing low levels of magnesium from sample C. (B) Elemental X-ray map of same aggregate.

average size of quartz-based particles in sample 1 was estimated as approximately 0.6 μm, while compositionally similar particles in sample 2 were approximately an order of magnitude larger (~6 μm). This difference in the size of the quartz-based particles is probably a reflection of different mineralization of the source material. Despite the difference in the overall composition of the two samples shown in Table 1, the composition of the quartz-rich particles found in both samples was remarkably similar. This is illustrated in Figure 11, which shows the frequency of occurrence of various impurity cations or associated phases in the quartz-based particles examined in the two samples.

Contrary to the case of particles recovered from macrophages, the bolter-box dust samples contained very few quartz particles of high purity. Also, aluminum was found to be the most abundant secondary element in quartz-rich particles in the roof-bolter-box samples, while it was less abundant in the silica-rich particles recovered from macrophages.

The characteristics of mineral dust particles found in the two mine dust samples examined in this study are in general agreement with results of similar particle-by-particle studies conducted by other investigators. Smith et al. (*14*) examined the shape of respirable, high silica content (>75% SiO_2) particles collected near con-

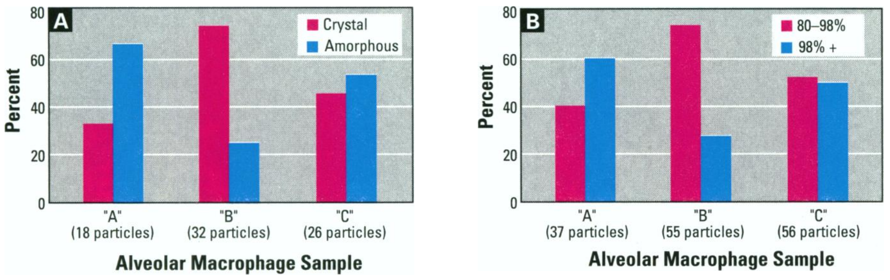

Figure 10. Human alveolar macrophage samples. (A) Distribution of crystalline and amorphous 80+% SiO_2 particles. (B) Distribution of 80–98% SiO_2 and 98+% SiO_2 particles.

Table 1. Mineralogical analysis of roof-bolter dust-box samples (*22*)[a]

	Weight %	
Mineral	Sample 1	Sample 2
Quartz	21.8	71.3
Calcite	15.8	5.7
Dolomite	50.5	9.9
Kaolinite	1.5	4.6
Illite	1.9	1.2
Carbonaceous material	8.5	7.3

[a]Sample 1 from Lower Sunnyside seam, Apex Mine, Andalex Resources, Inc. Sample 2 from Illinois no. 6 seam, Brushy Creek Mine, Kennelis Energy.

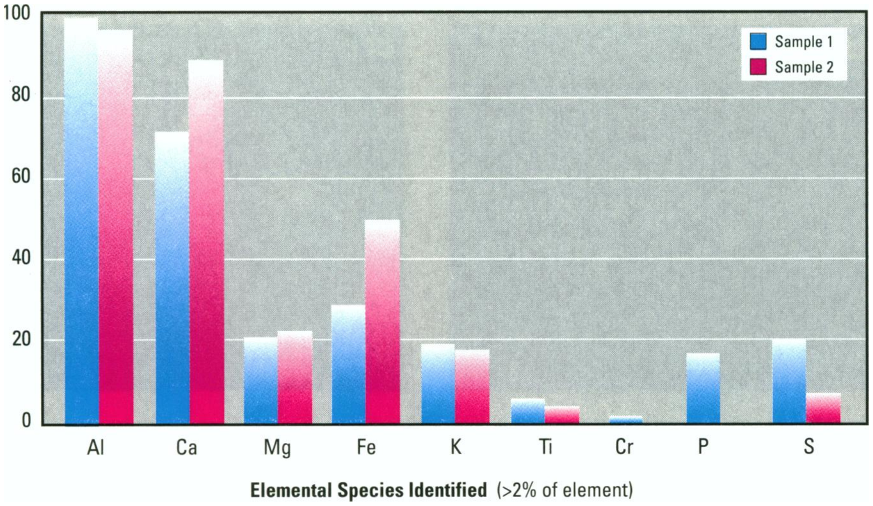

Figure 11. Roof-bolter-box samples. Frequency of elemental species associated with SiO_2 particles.

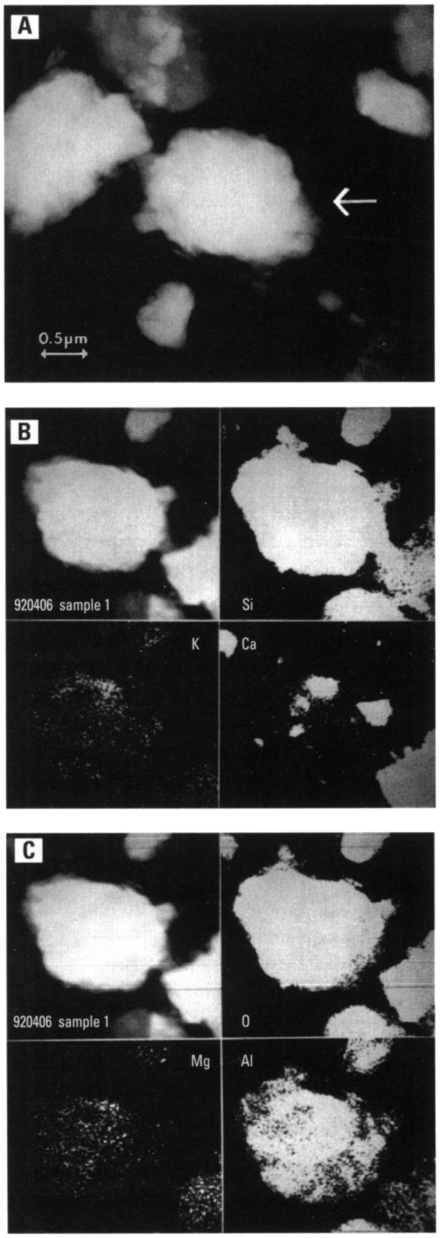

Figure 12. (A) Electron micrograph of mine-dust quartz-based particle. (B,C) Elemental X-ray maps of same particle.

tinuous miner operations in mines with high-quartz-content airborne dust. These investigators found no quartz particles with sharp facets, such as would be formed if the particle was formed by fracture during the mining operation. The observed rounded shape of the quartz-rich particles is indicative of long-term erosion and surface contamination of particles, typical of metamorphosed rocks. A similar absence of angular, freshly formed particles in dusts generated at longwalls of coal mines was reported by Grayson and Peng (*23*).

Probert et al. (*15*) used EDXA depth profiling to examine the surface and core compositions of 1000 particles from each of 5 different mines of a broad range of coal rank. They found that, on average, the frequency of occurrence of pure silica (i.e., quartz) particles (>98% SiO_2) was less than 1%, ranging from 0.1% to 1.5% in the five samples tested.

Discussion

The composition and morphology of silica-based particles recovered from macrophages is clearly different from that of equivalent particles found in roof-bolter-box dusts. The macrophages contained highly pure and crystalline particles of quartz not found in the mineral dust samples, in addition to complex multiphase quartz-based particles similar in morphology to the predominant quartz-based particle type found in the mineral dust. Furthermore, the macrophages contained unique forms of apparently dissolved and reprecipitated silica particles of amorphous or finely dispersed crystalline structures.

One simple hypothesis to explain this observation, consistent with the concept of macrophage-ingestible quartz particles, is that highly pure quartz-based particles are concentrated over time in the lung, as those with contaminated and otherwise modified surfaces are preferentially removed by the macrophages. Alternatively, the macrophage action on phagocytized particles may lead to the removal of contaminants and adjunct mineral phases from the particle surface, thus forming pure quartz particles *in situ*. These two mechanisms, acting separately or in conjunction, could also explain the elevated concentration of nearly pure quartz particles inside macrophages as compared to their scarcity in typical coal-mine dust samples.

On the other hand, the presence of pure or nearly pure silica-based particles with morphologies typical of reprecipitated phases (see Figs. 7–9) and not found in mine dust samples is evidence of more complex intracellular processes. Mechanisms have been proposed and studied *in vivo* for selective concentration of elements present in minerals as insoluble phosphate salts (*18,19,24*), and more recently, Galle and co-workers have characterized by elec-

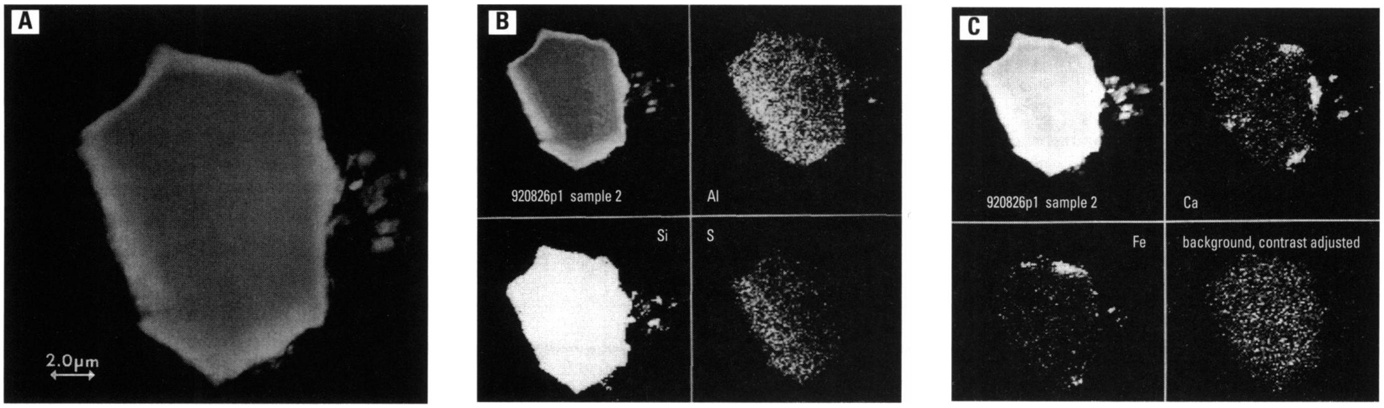

Figure 13. (A) Electron micrograph of mine-dust quartz-based particle. (B,C) Elemental X-ray maps of same particle.

tron microscopy and microanalysis the composition and morphology of dense phosphate deposits inside rat macrophages after inhalation of mineral salt-based aerosols (*20*). These precipitates exhibit granular morphologies not unlike those of the silica precipitates observed in the present experiments.

The phenomenon observed by Galle and co-workers derives from enzymatic processes inside macrophages that involve acid phosphates. A similar, but still undefined, enzymatic process may be involved in the dissolution and reprecipitation of silicon oxide particles.

Also noteworthy is the difference between the morphologies and compositions of the quartz-based particles recovered from macrophages of the three individuals tested. Although the number of samples is admittedly too small to draw general conclusions, these observations may suggest hypotheses about the mechanism of formation of the observed structures that deserve further experimental testing. The differing morphologies of the crystallites and agglomerates formed, as well as the prevalence of different cationic contaminants within each sample, suggest that the process of quartz particle elimination by the macrophage may depend on individual-specific particle–macrophage interactions related to susceptibility to quartz-induced pneumoconioses.

The determination of the bulk composition of a sample is not sufficient to infer the number of pure quartz or silica particles in the material. Even though the two roof-bolter dust samples had widely different silica (i.e., quartz) concentrations (21.8% versus 71.3%), the number of pure quartz particles was comparatively small in both. The samples recovered from macrophages, while relatively low in total silica content also, exhibited much larger numbers of pure silica particles (>98% SiO_2) than the roof-bolter dust samples.

The relative number of quartz-based particles contaminated with aluminum is much lower in the particles recovered from macrophages than in those found in the roof-bolter dust-box samples. This observation suggests either differential dissolution of elements from the surface of quartz-based particles or more efficient removal from the lung of aluminum-containing particles.

Although recovery of particles from lung macrophages and subsequent particle-by-particle characterization is a slow and laborious process, the results obtained in this preliminary study indicate that it may be useful both for a better understanding of macrophage–particle interactions and, possibly, as a tool for screening and diagnosis of lung disease potential in exposed individuals.

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