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THE CHEMICAL PROPERTIES OF SILICA PARTICLE SURFACE IN RELATION TO SILICA–CELL INTERACTIONS

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Although silicosis has been studied extensively, the mechanism is still not fully understood. Experiments do provide evidence that the actions of unique properties of silica surface on the cell membrane are the starting point of silicotic processes. This paper summarizes literature on chemical properties of silica surface, and the effect of particle size on silica toxicity. This paper also discusses the ways in which silica dusts are thought to interact with the cell membrane, with emphasis on freshness, hydrogen bonding, and free-radical interactions.

INTRODUCTION

Silicosis is a debilitating pulmonary disease that afflicts persons (miners, foundry workers, ceramic workers, heavy construction workers, etc.) who chronically inhale silica-containing dusts (Schepers, 1960; Silicosis and Silicate Disease Committee, 1988). Since silica is second only to oxygen in weight percentage of the earth's crust (about 28%) and is found in an enormous diversity of minerals, it is natural that silicosis is one of the major industrial health hazards all over the world. Although silicosis has been studied extensively, the mechanism by which the dusts exert their toxic actions on cells and the processes by which these actions progress

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to fibrogenesis are still poorly understood. The biological characteristics of the dusts are largely unknown, although it is suggested that the dust size, shape, surface charge, roughness, surface chemical composition, trace metal content, and crystallinity; dose of silica in the ambient air of the workplace; duration of exposure; genetic variation in exposed subjects; personal respiratory patterns; and presence of any coexisting disease (such as tuberculosis or rheumatoid arthritis) all influence the expression of silicosis (Koskinen et al., 1983; Noweir et al., 1980; Raask and Schilling, 1980; Reiser and Last, 1979; Thorne et al., 1985; Wolff et al., 1981). The actual biochemical mechanisms through which these factors operate are still unclear. However, the experiments do provide evidence that the actions of certain unique properties of silica surface on the cell membrane are the starting point of the silicotic process (Allison et al., 1966; Allison and Harrow, 1971; Bateman et al., 1982; Marks, 1957; Parazzi et al., 1968; Reiser and Last, 1979; Summerton et al., 1977). This article summarizes literature on chemical properties of silica dusts and how silica dusts are thought to interact with the cell membrane, with emphasis on hydrogen bonding and free-radical interactions.

THE GENERAL CHEMISTRY OF FIBROGENIC QUARTZ

In nature, silica occurs in three forms: quartz, tridymite, and cristobalite, of which the transition temperatures (°C) and specific gravities are given below:

quartz	← 870°C ←	tridymite	← 1470°C →	cristobalite (mp 1710°C)
2.65		2.26		2.32

Thus, silica melts at 1710°C and then forms a transparent glass on cooling. Chemically, it is fairly inert, but is attacked by alkali or by hydrofluoric acid according to the equations

$$SiO_2 + 2NaOH \rightarrow Na_2SiO_3 + H_2O \quad (1)$$

$$SiO_2 + 4HF \rightarrow SiF_4 + 2H_2O \quad (2)$$

The crystals of all three forms consist of three-dimensional SiO_2 tetrahedral networks joined such that each oxygen atom is common to two tetrahedral fragments and situated midway between the silicon atoms (Durrant, 1970). Thus, the empirical formula for such a substance would simply be $(SiO_2)_n$. The tetrahedral fragments form a spiral and are optically active (i.e., change plane of polarization of incident light).

Next to the chemical structure, two of the most important factors in

the cytotoxicity of silica are thought to be the chemical properties of the surface and the particle sizes.

Free-Radical Formation on the Surface of Silica Dust

For laboratory experiments, one of the methods of preparing a quartz dust sample is to grind the crystal. Grinding of large quartz particles causes breakages of Si—O bonds and generates silicon-based radicals (Bolis et al., 1983; Bystrikov and Streletskii, 1980; Dalal et al., 1986; Fubini et al., 1987; Hochstrasser and Antonini, 1972; Kolbanev et al., 1980; Radtsig and Bysterikov, 1978; Radtsig and Khalif, 1979; Vallyathan et al., 1988) and other defects at the surface. Using electron spin resonance (esr) spectroscopy Hochstrasser and Antonini (1972) have shown that grinding of quartz under an ultrahigh vacuum ($\sim 10^{-10}$ mm Hg) produces equal amounts of $Si\cdot$ and $SiO\cdot$ radicals. When these silicon-based radicals are produced, some competitive processes occur to reduce the stress due to the radical formation. One of these processes is surface reconstruction, which takes place during, or just after, the formation of the silica-based radicals. Hochstrasser and Antonini (1972) noted that the esr signal due to the broken bonds was stable for months in an ultrahigh vacuum or in an inert atmosphere, such as argon. However, introduction of air at atmospheric pressure reduces the signal amplitude by a factor of five instantaneously, and then the signal decays with a half-life of approximately 36 h. Hochstrasser and Antonini (1972) established that the quartz surface thus prepared may absorb various gases. By using esr, they found that the absorption of carbon dioxide does take place on a silica surface that has free $\equiv Si\cdot$ radicals with formation of $\equiv SiCOO\cdot$ complexes as follows:

$$\equiv Si\cdot + CO_2 \rightarrow \equiv SiCOO\cdot \quad (3)$$

The bonding between the $\equiv Si\cdot$ and CO_2 molecule is partially covalent and partially ionic as deduced from Si^{29} hyperfine coupling. Later Radtsig and Bystrikov (1978) obtained esr spectra demonstrating the interaction of the quartz surface with O_2 and CO. They suggested the following simple addition-type reactions:

$$\equiv SiO\cdot + O_2 \rightarrow \equiv SiO_3\cdot \quad (4)$$

$$\equiv SiO_3\cdot + CO \rightarrow \equiv SiCO_4\cdot \quad (5)$$

$$\equiv SiO\cdot + CO \rightarrow \equiv SiCO_2\cdot \quad (6)$$

$$\equiv SiCO\cdot + O_2 \rightarrow \equiv SiOO\cdot + CO_2 \quad (7)$$

$$\equiv Si\cdot + O_2 \rightarrow \equiv SiOO\cdot \quad (8)$$

Bystrikov and Streletskii (1980) have also used the high-vacuum esr technique for measuring the concentration of the radicals on the surface of quartz. They found that the ratio of the concentrations $[\equiv SiO\cdot] : [\equiv SiOO\cdot]$ on the oxidized quartz surface is close to 1 : 1.

More recently, Dalal et al. (1986) (Vallyathan et al., 1988) made esr measurements on quartz dust freshly generated in ambient air and found that grinding silica in an ambient environment leads to the formation of the $Si\cdot$ and $SiO\cdot$ types of radical. Moreover, their data further show that the concentration of the free radicals generated on the silica surface decreases on storing the silica dust in air or in biological buffers (Vallyathan et al., 1988).

Reaction of Silica Surface with Water and Hydrogen Peroxide

When the ground quartz surface comes into contact with water, hydrolysis of the surface silicon–oxygen bonds takes place, resulting in the formation of surface silanol ($\equiv SiOH$) groups (Aines and Rossman, 1984; Bolis et al., 1985; Cauwelaert et al., 1973; Hochstrasser and Antonini, 1972; Klier et al., 1973; Langer and Nolan, 1986; Marik-Korda et al., 1984; Morrow and Cody, 1973; Nash et al., 1966; Tsuchiya, 1982), as found via infrared spectroscopy (Morrow and Cody, 1973; Tsuchiya, 1982). The silanol group, depending on pH, should obey the following equilibrium:

$$\equiv SiOH \leftrightarrows \equiv SiO^- + H^+ \qquad (9)$$

Thus, the ratio of the partially ionized group ($\equiv SiO^-$) to $\equiv SiOH$ is pH dependent (Iler, 1978). Under physiological conditions, quartz particles are known to have negative charge (Nolan et al., 1981). It is worth mentioning here that a reduction in the surface charge causes an increase in cytotoxicity of quartz particles (Bergman and Langrish, 1972). The explanation may be that the reduction of negative charge reflects greater silanol group formation at the expense of the ionized form (Nolan et al., 1981). The surface charge can be reduced by cations other than H^+. Several investigators (Dalal et al., 1988; Nolan et al., 1981; Stalder and Stöber, 1965) have already shown that bonding of the quartz surface with a trace metal reduces the quartz cytotoxicity.

Other important reactions were also suggested between water and silicon-based radicals, such as $\equiv Si\cdot$, $\equiv SiO\cdot$, and $\equiv SiOO\cdot$ (Kolbanev et al., 1980; Shi et al., 1988; Vallyathan et al., 1988). For the $\equiv SiO\cdot$ radical, the bonding between the Si and O atoms is quite strong (108 kcal/mol) (Durrant, 1970). Hence, this bond will not easily be broken. Kolbanev et al. (1980) suggested the following reactions of silicon-based radicals with water:

$$\equiv SiO\cdot + H_2O \rightarrow \equiv SiOH + \cdot OH \quad (10)$$

$$\equiv SiO\cdot + \cdot OH \rightarrow \equiv SiOOH \quad (11)$$

$$\equiv SiOO\cdot + H_2O \rightarrow \equiv SiOH + HO_2 \quad (12)$$

It has also been suggested that hydrolysis of $\equiv$SiOOH produces H_2O_2 (Kolbanev et al., 1980) according to

$$\equiv SiOOH + H_2O \rightarrow \equiv SiOH + H_2O_2 \quad (13)$$

Reactions of the $\cdot$OH and HO_2 radicals with each other or with $\equiv$SiO$\cdot$ and $\equiv$SiOO$\cdot$ lead to the production of H_2O_2 and molecular oxygen (in the case of the HO_2 radical) (Kolbanev et al., 1980). The $\cdot$OH radical can also react with $\equiv$SiOOOSi$\equiv$ (Kolbanev et al., 1980), according to the following reaction:

$$\equiv SiOOOSi\equiv + \cdot OH \rightarrow \equiv SiOOH + \equiv SiOO\cdot \quad (14)$$

Thus, the net result is an increase in the yield of H_2O_2 (Kolbanev et al., 1980). The concentration of H_2O_2 is reported to be 3–5 times that of the Si$\cdot$ and SiO$\cdot$ types of radical. The yield of H_2O_2 is reported to be as high as 10^{18} molecules/g (Kolbanev et al., 1980). The methodology of measuring H_2O_2 production used by Kolbanev et al. (1980) was the standard method of wet analytical chemistry, as in the following equation:

$$\underset{\text{(pink)}}{2MnO_4^-} + 5H_2O_2 + 6H^+ \rightarrow 5O_2 + \underset{\text{(colorless)}}{2Mn^{2+}} + 8H_2O \quad (15)$$

Recently, Shi et al. (1988) (Dalal et al., 1989a) verified the reducing activity of quartz particle aqueous suspensions with respect to potassium permanganate. However, the H_2O_2 was measured to be an order of magnitude smaller (Dalal et al., 1989a; Shi et al., 1988). Moreover, using esr in conjunction with spin trapping methodology, Shi et al. (1988) detect hydroxyl ($\cdot$OH) and possibly superoxide (O_2^-) radical formation (Dalal et al., 1989a) from freshly ground quartz particle aqueous suspension. In addition, Shi et al. (1988) (Dalal et al., 1989a) demonstrate that the ability of the freshly ground quartz particles to generate oxygenated radicals decreases with a half-life of about 20 h. Metal chelators, such as desferal, inhibit the $\cdot$OH radical formation by more than 80%, suggesting that the Fenton reaction

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + \cdot OH + OH^- \quad (16)$$

may play a role in the $\cdot$OH radical generation (Dalal et al., 1989a; Morehouse and Mason, 1988). The Fe^{2+} may be present as a trace impurity or

impregnated in the chemical structure of quartz dust. In order to study the role of H_2O_2 in the ·OH radical formation, an H_2O_2 scavenger, catalase, was added into the fresh quartz particle aqueous suspension. A complete suppression of the ·OH radical formation was observed, showing the importance of H_2O_2 in the mechanism of ·OH radical formation. However, the dependence of the ·OH radical production on the dust's freshness suggests that the dust's surface characteristics (such as the radical content) play a significant role in the ·OH generation.

Gulumian and van Wyk (1987) have reported that reaction of stale quartz particles with H_2O_2 generates ·OH radicals, which can be inhibited by polyvinylpyridine-*N*-oxide (PVPNO). The PVPNO was reported to inhibit quartz-induced fibrosis in animals (Schlipköter and Brockhaus, 1960), and several laboratories have confirmed its therapeutic efficacy in silicosis (Kaw et al., 1975; Von Behren et al., 1983). Recently, Kennedy et al. (1989) have reported that silicates causing pneumoconiosis function as Fenton catalysts to generate ·OH radicals when incubated with H_2O_2 and a reducing substance. However, it is not clear as to why Kaolin, a silicate not known to cause fibrosis, also exhibited high Fenton activity in this study.

Influence of Sample Size and Preparation on Toxicity

It has been reported that quartz particles ranging in size from 0.5 to 2 μm in diameter are of great importance for the development of silicosis (Thorne et al., 1985). The majority of particles deposited to the terminal bronchioles measure less than 10 μm in diameter. Larger particles are effectively removed by the conducting airway (Melandri et al., 1975; Thorne et al., 1985; Wolff et al., 1981).

The particles deposited may be removed by one of the following routes: the lymphatic system or the bronchial tree. Most of the particles deposited proximally to the respiratory bronchioles are removed by the effective mucociliary stream. It has been shown that some agents either inhibit or stimulate this clearing system. For example, Lippman et al. (1975) have reported that cigarette smoking inhibits the system, whereas the SO_2 and NO_2 gases stimulate the system. The elimination of quartz particles from the lung continues for many years after the last exposure. Even apparently locked particles in the area of fibrosis are mobilized and cleared slowly (Heppleston, 1962). Robock and Klosterkötter (1973) reported that the toxicity of the quartz particles can be changed according to the method of preparation. They found that the toxicity of the quartz particles increased when the sample was ground in a mill instead of a press. The reason may be that after crushing in the press, the particle has a smooth surface, whereas the milled particle has sharp edges and a larger surface area.

HYDROGEN BONDING INTERACTION BETWEEN SILICA SURFACE AND THE CELL MEMBRANE

Once the quartz particle is within the human host and encounters cells, the particle is coated with certain host-derived proteins, which cause it to be identified by macrophages as a foreign particle. The host-derived proteins, called opsonins, are members of the immunoglobulin and its complement systems and are thought to be recognized by specific receptors present in the macrophage membrane (Nolan et al., 1981; Summerton et al., 1977). Once having been identified, the surface coated particle is engulfed by the macrophage. In the second step, when a silica-laden phagosome within the macrophage coalesces with a lysosome within the macrophage, the surface coating is removed from the particle by lysosomal enzymes. The unmasked silanol groups now react with the inner surface of the lysosome membrane, causing damage to the membrane (Langer and Nolan, 1986; Nolan et al., 1981; Summerton et al., 1977). The damaged lysosome releases destructive enzymes into the cytoplasm of the cell, resulting in breakdown of the macrophage, followed by the release of the dust particle. The released particle can be coated again and be engulfed by another macrophage to continue the cycle of engulfment, unmasking, and macrophage death, promoting the silicosis process (Nolan et al., 1981; Summerton et al., 1977).

In the above mechanism, understanding the interaction of a quartz particle with the cell membrane is a key because this is the first interaction of the quartz particle with the macrophage. It is suggested that the toxicity of the quartz particle is due to the fact that silanol groups are formed on the surface of the quartz particle by interaction with water (Aines and Rossman, 1984; Bolis et al., 1985; Cauwelaert et al., 1973; Hochstrasser and Antonini, 1972; Klier et al., 1973; Marik-Korda et al., 1984; Morrow and Cody, 1973; Nash et al., 1966; Tsuchiya, 1982). These silanol groups might act as sites for strong hydrogen bonding with a cell membrane (Summerton et al., 1977). The reaction between the silanol groups and the cell membrane can be visualized in terms of the Lewis acid-base theory wherein an acid is any substance that can accept an electron pair, and a base is any substance capable of donating an electron pair. In a biological system, a large number of sites exist that have electron pairs on their functional groups or atoms such as oxygen or nitrogen. These electron pairs can form hydrogen bonds with a weak acid:

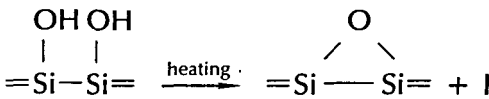
$$\equiv SiOH + O{=}N- \rightarrow \equiv Si{-}O{-}H \cdots O{=}N- \quad (17)$$

$$\equiv SiOH + O{=}P\equiv \rightarrow \equiv Si{-}O{-}H \cdots O{=}P\equiv \quad (18)$$

$$\equiv SiOH + HN{=} \rightarrow \equiv Si{-}O{-}H \cdots NH{=} \quad (19)$$

The above interpretation of the toxicity of quartz particles is supported by the observation that a quartz particle can react with proteins, phospholipids, and biological membrane systems (Bonner et al., 1974; Harley and Margolis, 1961; Heppleston and Styles, 1967; Marasas and Harington, 1960; Stalder and Stöber, 1965). It has been reported that stronger interactions are those between the silanol groups and the phosphate ester groups of phospholipids (Nash et al., 1966). However, Summerton and co-workers present another viewpoint. They studied the silica-membrane interaction by using sheep red blood cells as a model of the membrane system. Their experimental finding favors the silica-protein interaction rather than the silica lipid interaction. Chemically, the evidence favoring hydrogen bonding or some other weak bonding over ionic or covalent bonding between the quartz surface and cell membrane is fairly convincing. Since covalent and ionic bonds are much stronger than hydrogen bonds, the quartz particle would not easily be released upon the death of the macrophage if the bonding were covalent or ionic. Furthermore, cell lysis depends on pH. At a low pH (4.5–7.0), when the silica surface has a high density of neutral silanol groups [eq. (9)], red blood cell lysis is considerably increased compared with that at physiological pH (7.2–7.4) (Summerton et al., 1977) thus supporting the importance of hydrogen bonding in the cell lysis.

Sakabe et al. (1971) have reported that the toxicity of silica particles was greatly reduced if the particles had been previously heated at 800 K for an hour. This loss of toxicity can be explained by considering the decrease in the number of silanol groups on the surface of the particle:



(20)

(Dalal et al., 1988; Sakabe et al., 1971). Further evidence in support of the hydrogen bonding mechanism comes from the studies involving a direct coating of quartz particles (Schlipköter et al., 1963; Wallace et al., 1985). When macrophages are incubated with the quartz particles in serum-containing media, cytotoxic effects are delayed. The explanation offered (Schlipköter et al., 1963; Wallace et al., 1985) is that the quartz particles become coated with proteins or lipid and the interactions between the quartz particle and the cell membrane are delayed until the proteins are digested and the bare particles are exposed (Wallace et al., 1985). Further support is provided by the effects of polyvinylpyridine-*N*-oxide (PVPNO) on the reduction of cytotoxicity (Allison et al., 1966; Nash et al., 1966). Pyridine-*N*-oxide, the basic unit, has a strong electronegative oxygen atom capable of forming strong bonding with the silanol groups on the quartz surface, thus reducing the hydrogen bonding between the silanol groups and the cell membrane (Nash et al., 1966). Because of this reduc-

tion in the quartz–membrane bonding, the phagocytosis processes are delayed, and hence also the eventual cell breakdown by the quartz particles.

FREE-RADICAL–MEDIATED CELL INJURY

After the quartz particles are engulfed in phagosomes, both neutrophils and macrophages increase oxygen consumption in what is called the "respiratory burst" (Fantone and Ward, 1982). During this process the hexose monophosphate (HMP) shunt is activated, resulting in an increase in glucose oxidation [eq. (21)] (Iyer et al., 1961; Lehninger, 1982; Sbarra and Karnovsky, 1956). In this metabolic pathway,

$$\text{glucose-6-phosphate} + 2\text{NADP}^+ + \text{H}_2\text{O} \rightarrow \text{ribulose-5-phosphate} + \text{CO}_2 + 2\text{NADPH} + 2\text{H}^+ \quad (21)$$

glucose is oxidized to CO_2 and a five-carbon sugar, with $NADP^+$ as an oxidant, i.e., an electron acceptor (Fig. 1). Thus, the shunt activation increases the oxidation of NADPH/$NADP^+$ during the respiratory burst. The increase in the oxygen uptake is not solely to provide ATP as a source of energy for phagocytosis (Goldstein et al., 1977), but also to

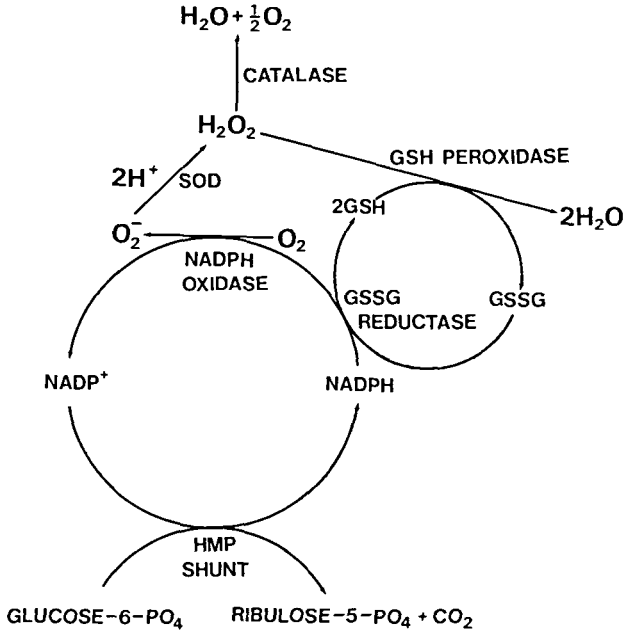


FIGURE 1. Cellular free-radical generation and defense mechanism. SOD accelerates the dismutation of O_2^- into H_2O_2. The catalase and glutathione (GSH) redox system degrades H_2O_2. The HMP shunt provides NADPH for the reduction of GSH and oxygen. GSSG = oxidized glutathione.

form O_2^-. It is known that almost all the oxygen consumed during the respiratory burst is changed into the O_2^-, and most of the O_2^- thus produced is converted to H_2O_2 according to the following equations (Fig. 1):

$$2O_2 + NADPH \rightarrow 2O_2^- + NADP^+ + H^+ \quad (22)$$

$$2O_2^- + 2H^+ \rightarrow O_2 + H_2O_2 \quad (23)$$

(Babior, 1978; Briggs et al., 1977; Flebanoft, 1980; Fantone and Ward, 1982; Fridovich, 1976; Goldstein et al., 1977). The increase in NADPH production results in the activation of the hexose monophosphate shunt.

The O_2^- formation is the result of one-electron reduction of molecular oxygen. According to the substance it reacts with, it can be either oxidant or reductant. O_2^- also exists in its protonated form (HO_2). The ionization pKa is 4.8; hence, at near biological pH the HO_2 radicals are almost entirely in the O_2^- form. Because of the toxicity of the O_2^- radical, superoxide dismutase (SOD) provides a protective role (McCord, 1983; McCord and Fridovich, 1969). The generally accepted protective mechanism is that the SOD catalyzes the following reaction (Fig. 1):

$$2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2 \quad (23)$$

The reaction occurs at maximum speed at a pH equal to pKa = 4.8. H_2O_2 is formed when oxygen accepts two electrons, via either dismutation of O_2^- or through two-electron reduction of O_2 (without the formation of O_2^-) (Fridovich, 1976; Weiss and LoBuglio, 1982). The hydrogen peroxide can be "detoxified" by the glutathione peroxidase-glutathione reductase system and catalase. Catalase can directly degrade H_2O_2 according to the following reaction (Fig. 1):

$$2H_2O_2 \rightarrow 2H_2O + O_2 \quad (24)$$

Glutathione (GSH) catalyzes the reduction of H_2O_2 (Fig. 1):

$$H_2O_2 + 2GSH \rightarrow 2H_2O + GSSG \quad (25)$$

The oxidized glutathione (GSSG) reacts with NADPH and glutathione reductase to produce GSH (Babior, 1978):

$$GSSG + NADPH + H^+ \rightarrow 2GSH + NADP^+ \quad (26)$$

These reactions [Eqs. (22–26)] result in the utilization of H_2O_2 to drive the hexosemonophosphate shunt through the reoxidation of NADPH (Fig. 1).

The amount of O_2^- and H_2O_2 produced by neutrophils and macrophages plays an important role because O_2^- facilitates killing of the en-

gulfed substance, such as bacteria. Experiments indicate that SOD and catalase can indeed inhibit bacterial killing by the macrophages (Babior et al., 1973; Johnston et al., 1975). Although the biochemical mechanisms underlying the bacterial killing by the macrophages are not yet fully understood, there is experimental evidence suggesting that the damaging effects are caused by H_2O_2 and not O_2^- (Gregory et al., 1973; Mandell, 1975; Nathan et al., 1979; Simon et al., 1981). Under normal conditions, the macrophages protect themselves by releasing SOD (McCord, 1983; Roose et al., 1980), glutathione peroxidase-glutathione reductase systems and catalase from potential oxidative damage by their own bacteriocidal products (Baehner et al., 1977; Nelson et al., 1979). The amount of O_2^- and H_2O_2 produced by neutrophils and macrophages depends on many factors and experimental conditions (Johnston et al., 1978).

As discussed in previous sections, when quartz is ground in air, silicon-based radicals are generated on the silica surface. These radicals subsequently react with atmospheric components and water to generate H_2O_2, $\cdot OH$, and possibly O_2^-. In addition, iron and other transition metals that are present as trace impurities or impregnated in the chemical structure of silica surface catalyze the generation of $\cdot OH$ radicals from H_2O_2 which the lung itself supplies. The obvious toxicity of oxygenated reactive species ($\cdot OH$, O_2^-, and H_2O_2) leads Dalal and co-workers (Dalal et al., 1989a; Shi et al., 1988; Vallyathan et al., 1988) to suggest the following model of the initial events in the reaction of the quartz particle with a cell membrane. The free radicals ($Si\cdot$ and $SiO\cdot$) on the surface of silica dusts and their associated oxygenated reactive species are involved in the interaction of the cell membrane with quartz dust (Fig. 2). The result of this reaction would be the release of reactive oxygenated species (H_2O_2, O_2^-, $\cdot OH$, $R\cdot$, and $RO\cdot$). These reactive oxygenated species would further react with the cell membrane, leading to additional release of these species and to lipid peroxidation (Fantone and Ward, 1982). As to the sites of the reactions between the cell membranes and the quartz dust, it is noted that the reaction of quartz particles with H_2O produces silanol (SiOH) groups on the particle surface, as detected by infrared spectroscopy (Tsuchiya, 1982). These silanol groups could form hydrogen bonds with the nitrogen or oxygen atomic sites on the cell membrane. This is supported by the report of a weak (hydrogen) bond formation between a secondary amide (peptide) of proteins and silanol moieties (Summerton et al., 1977). Such hydrogen bonding could bring the silica surface and cell membrane close enough to provide a favorable environment for the initiation of lipid peroxidation by the silicon-based radicals and their associated oxygenated radicals. The concentration of the reactive oxygenated species produced might be high enough to be beyond the cell defense. The unsaturated fatty acids, phospholipids, glycolipids, glycerides, sterols of the membrane, and oxidizable amino acid groups and sulfhydryl groups of the transmembrane proteins are easily dam-

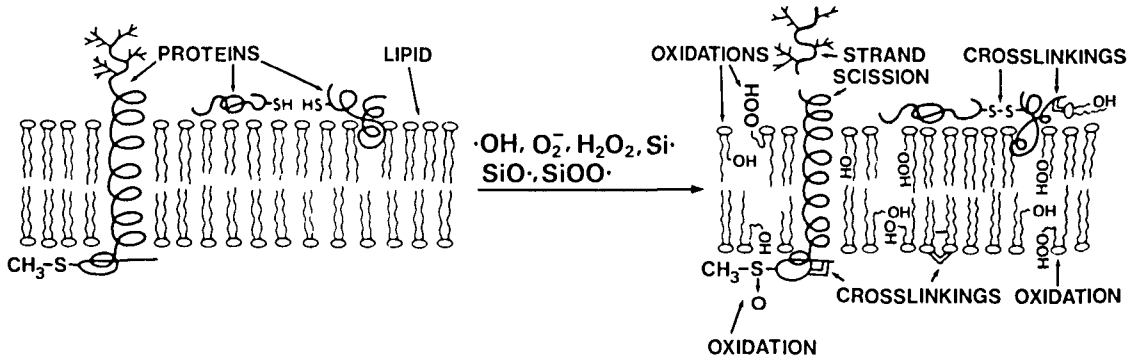


FIGURE 2. Free-radical-mediated cell membrane damage. Free radicals can damage lipid by initiating peroxidation. Free radicals can also damage membrane proteins by catalyzing amino acid oxidation, protein-protein cross-linking, and protein strand scission.

aged by the oxygenated reactive species (Fig. 2) (Freeman and Larpo, 1982). The oxidation of transmembrane proteins may cause the membrane permeability to increase, allowing more free-ion migration and breaking down transmembrane ion gradients in the cell, eventually resulting in cell death (Freeman and Larpo, 1982).

Some support for the role of free radicals in the silica fibrogenicity was presented by Velichkovskii et al. (1985). They used an esr spin probing method for measuring the rate of O_2 consumption by rat peritoneal macrophages after activation with fibrogenic dust particles. The effects of dust particles with different fibrogenic activities were compared with respect to O_2 consumption. The O_2 consumption rate of the peritoneal macrophages activated with the highly fibrogenous quartz dust particles was considerably higher in comparison with weakly fibrogenous TiO_2 dust. Also, the O_2 consumption was proportional to the concentration of the dust particles used. Gulumian and van Wyk (1987) have reported that in the presence of H_2O_2, silica dusts generate $\cdot OH$ radicals, which were suggested to be an initiator of macrophage membrane lipid peroxidation. Gabor et al. (1975) have studied the in vitro action of quartz particles on alveolar macrophage lipid peroxidation by measuring malonaldehyde formation. They reported that incubation of quartz particles with macrophages produced a higher amount of lipid peroxides, whereas no significant production of lipid peroxides was observed when corundum (Al_2O_3) dust was used. In another experiment, Gabor et al. (1985) reported that treatment of macrophages with antioxidant (selenite) significantly reduced the toxicity and lowered the rate of lipid peroxidation. Their explanation was that selenium is an essential constituent of the peroxide-reducing enzyme, glutathione peroxidase. Since this selenium-containing enzyme acts on hydroperoxides of unsaturated fatty acid and hydrogen peroxide, the enzyme plays an important role in protecting the cell membrane from damage (Gabor et al., 1985; Morosava et al., 1984). Reduction of lipid peroxidation may protect cell membranes and reduce fibrosis because it slows macrophage breakdown. However, once macrophages are broken down, an additional process comes into play that promotes fibrosis (Reisey and Last, 1979).

Evans and Yano (1985) have used a specially constructed computerized luminometer to monitor the associated luminal dependent chemiluminescence (CL) to assess the in vitro production of oxygenated reactive species by phagocytic cells, macrophages, and polymorphonuclearcytes. They reported a general positive correlation between the phagocytic cell, macrophage, and polymorphonuclearcyte CL response. The inert TiO_2 produces the least response, and the highly pathogenic erionite dusts the most, with silica and asbestos causing an intermediate response. Very recently, Dalal et al. (1988a) have reported that freshly crushed silica exhibits luminescence which decreases with the dust's aging and correlates with toxicity. It should be noted that the lumines-

cence originates directly from the silica surface and is not a CL response. These results also support the role of radicals in the silica toxicity.

As mentioned in the earlier sections, the concentrations of both silicon-based radicals and their associated ·OH radicals decrease with aging in air (Dalal et al., 1986; Dalal et al., 1989a, 1989b; Shi et al., 1988). Dalal and co-workers (Dalal et al., 1989b; Vallyathan et al., 1988) show that freshly ground silica is more biologically reactive than aged silica because freshly crushed silica activates a greater respiratory burst in alveolar macrophages than aged silica. For example, storage of freshly ground dust in air decreases silica-induced superoxide anion secretion, hydrogen peroxide release, and nitroblue tetrazolium (NBT) reduction by 25, 68, and 43%, respectively. Furthermore, compared to aged silica, freshly ground silica exhibits a greater cytotoxic effect on cellular membrane integrity, i.e., a 1.5-fold increase in lactate dehydrogenase (LDH) release from macrophage (Dalal et al., 1989b; Vallyathan et al., 1988). These results indicated that fractured-generated silicon-based radicals and their associated ·OH and possibly O_2^- radicals may play a significant role in the pathogenesis of silicosis (Dalal et al., 1989b; Vallyathan et al., 1988).

It should be noted that occupational exposure to silica can be associated with either chronic or acute silicosis. Chronic silicosis becomes manifest 20–40 years after the first exposure, whereas acute silicosis becomes manifest within a few years after the first exposure (Ziskind et al., 1976). It does not seem likely that acute silicosis can be explained simply as the response of the lung to high levels of silica. Acute silicosis is commonly associated with sandblasting, rock drilling, tunneling, and silica mill operations where freshly crushed or sheared silica is produced. Freshly generated silica, which has a high concentration of silicon-based radicals and an enhanced ability to generate oxygenated reactive species, is more reactive toward the lung tissue than aged silica. The silicon-based radicals and oxygenated reactive species may play a major role in the manifestation of acute silicosis (Dalal et al., 1989b; Vallyathan et al., 1988).

It also should be noted that one major difference between human exposure and laboratory studies is that the human, in most cases, breathes fresh silica, whereas the animal and cells are exposed to stale silica. Here, freshly generated silica is named "fresh silica," and the same silica kept in air for several hours or longer "stale silica." Dalal and co-workers (Dalal et al., 1986, 1989a, 1989b; Shi et al., 1988; Vallyathan et al., 1988) use these terminologies because of the observed biological difference between the fresh and stale silica. This difference is probably the reason that laboratory studies sometimes do not correlate with the human experiences and for the often conflicting results of different workers (Dalal et al., 1986). Although much further work remains to be done in explaining the role of silicon-based radicals and their related oxygenated reactive species, Dalal and co-workers have reported that the "storage" time of the silica before inhalation must be seriously considered in

planning any studies of silicosis in laboratory animals or other species (Dalal et al., 1989b; Vallyathan et al., 1988). It may also be noted that freshly ground coal dust is also more cytotoxic, and the underlying cause seems to be free radical mediated (Dalal et al., 1989c).

Kennedy and co-workers (1989) reported that dusts causing pneumoconiosis generate ·OH radicals by acting as Fenton catalysts. They hypothesized that ·OH radical formation might be a common mechanism of pneumoconiosis induced by all silicates. They used hemolysis as a model of their studies and observed that silicate-induced hemolysis was decreased by either the H_2O_2 scavenger, catalase, or the O_2^- scavenger, SOD. In a similar experiment Dalal and co-workers also observed the protective ability of catalase and SOD on silica-induced hemolysis (N. S. Dalal, X. Shi, and V. Vallyathan, unpublished results). However, boiled SOD (inactive) or albumin have a great protective ability toward silica-induced hemolysis. The protective ability of these proteins might be due to the surface coating of silica particles. In addition, the parallel cytotoxicity and free-radical measurements on silica suggested that silanol groups on the silica surfaces and not the free radicals play a major role in silica-induced hemolysis (Dalal et al., 1988a). However, this result does not necessarily mean that silanol groups play a major role in silica-induced lung injury since it is reported that silica-induced hemolysis and lipid peroxidation, which can be initiated by free radicals, proceed via independent mechanisms (Kilroe-Smith, 1974; Singh and Rahman, 1987).

In conclusion, although the initial interaction between the silica dust and cell membrane was suggested to be through the hydrogen bonding or free-radical-mediated reaction or both, the reaction mechanism is largely unknown. Clearly, further work is needed in this area. For example, the esr nitroxide spin probe method might be used to monitor the dynamics of lipid peroxidation (Gendek et al., 1984; Leyko and Gendek, 1985; Subczynski and Kusumi, 1985; Yamaguchi et al., 1982). The spin trapping technique (Janzen, 1971; Rosen and Freeman, 1984; Rosen and Rauckman, 1981; Yamada et al., 1984) may be used to identify and characterize the free radicals involved in the dust-cell interaction and the effects of the radical scavenger. Much work is also needed to identify the sites responsible for the light emission from freshly ground silica (Dalal et al., 1988b), and the effects of metal ions on silica toxicity. These experiments might yield significant new information on the mechanism of dust-cell interaction for quartz, and suggest new strategies for combating silicosis and related diseases.

REFERENCES

Aines, R. D., and Rossman, G. R. 1984. Water in mineral? A peak in the infrared. *J. Geophys. Res.* 89:4059-4071.

Allison, A. C., Harington, J. S., and Birbeck, M. J. 1966. An examination of the cytotoxic effects of silica on macrophages. *J. Exp. Med.* 124:141–153.

Allison, A. C., and Harrow, M. D. 1971. Lysosomes and the toxicity of particulate pollutants. *Arch. Intern. Med.* 128:131–139.

Babior, B. N. 1978. Oxygen-dependent microbial killing by phagocytes. *N. Engl. J. Med.* 298:659–668.

Babior, B. R., Kipnes, R., and Curnutte, J. 1973. Biological defense mechanism: The production by leukocytes of superoxide, a potential bactericidal agent. *J. Clin. Invest.* 52:741–744.

Baehner, R. L., Boxer, L. A., Allen, J. M., and Davis, J. 1977. Autooxidation as a basis for altered function by polymorphonuclear leukocytes. *Blood* 50:327–335.

Bateman, E. D., Emerson, R. J., and Cole, P. J. 1982. A study of macrophage-mediated initiation of fibrosis by asbestos and silica using a diffusion chamber technique. *Br. J. Exp. Pathol.* 63:414–425.

Bergman, I., and Langrish, B. 1972. Silica powders of respirable size: The effect of methods of comminution and pretreatment on electrophoretic mobility in solutions of varying ionic strength, acidity and metal-ion content. *J. Electronanal. Chem. Interfacial Electronchem.* 34:203–210.

Bolis, V., Fubini, B., and Venturello, G. 1983. Surface characterization of various silica. *J. Thermal Anal.* 28:249–257.

Bonner, W. A., Kavasmaneck, P. R., and Martin, F. S. 1974. Asymmetric absorption of alanine by quartz. *Science* 186:143–144.

Briggs, R. T., Karnovsky, M. L., and Karnovsky, M. J. 1977. Hydrogen peroxide production in chronic granulomatous disease. *J. Clin. Invest.* 59:1088–1098.

Bystrikov, A. V., and Streletskii, A. N. 1980. Mechanochemistry of quartz surface. *Kinet. Katal.* 21:1148–1153.

Cauwelaert, F. H., Jacobs, P. A., and Uytterhoeven, J. B. 1973. A-type hydroxyls on silica surfaces. *J. Phys. Chem.* 77:1470–1471.

Dalal, N. S., Suryan, M. M., Jafari, B., Shi, X., Vallyathan, V., and Green, F. H. Y. 1986. Electron spin resonance detection of reactive free radicals in fresh coal dust and quartz dust and its implications to pneumoconiosis and silicosis. In *Proc. Int. Symp. Respir. Dusts Mineral Ind.*, Pennsylvania State University, University Park, Pa., pp. 25–29.

Dalal, N. S., Shi, X., and Vallyathan, V. 1988a. Do silicon-oxygen radicals play a role in the quartz-induced hemolysis and fibrogenicity. In *Proc. VIIth Int. Pneumoniosis Conf.*, Pittsburgh, Pa. (in press).

Dalal, N. S., Vallyathan, V., and Castranova, V. 1988b. Chemiluminescence and biological reactivity of fractured silica. In *Proc. VIIth Int. Pneumoniosis Conf.*, Pittsburgh, Pa. (in press).

Dalal, N. S., Shi, X., and Vallyathan, V. 1989a. Oxygenated radical formation by fresh quartz dust in a cell-free aqueous medium and its inhibition by scavengers. In *Effects of Mineral Dusts on Cells*, eds. B. T. Mossman and R. Bégin, NATO ASI Series. New York: Springer-Verlag (in press).

Dalal, N. S., Shi, X., and Vallyathan, V. 1989b. Potential role of silicon-oxygen radicals in acute lung injury. In *Effects of Mineral Dusts on Cells*, eds. B. T. Mossman and R. Bégin, NATO ASI Series. New York: Springer-Verlag (in press).

Dalal, N. S., Suryan, M. M., Vallyathan, V., Green, F. H. F., Jafari, B., and Wheeler, R. 1989c. Detection of reactive free radicals in fresh coal mine dust and their implication for pulmonary injury. *Ann. Occup. Hyg.* 33:79–84.

Durrant, P. J. 1970. *Introduction to Advanced Inorganic Chemistry*, p. 633. New York: Wiley.

Evans, P. H., and Yano, E. 1985. Phagocyte-derived reactive oxygen metabolites: Stimulation by pathogenic mineral dusts. *Biochem. Soc. Trans.* 13:1205–1206.

Fantone, J. C., and Ward, P. A. 1982. Role of oxygen-derived free radicals and metabolites in leukocyte-dependent inflammatory reactions. *Am. J. Pathol.* 107:397–418.

Flebanoft, S. J. 1980. Oxygen metabolism and the toxic properties of phagocytes. *Ann. Intern. Med.* 93:480–489.

Freeman, B. A., and Larpo, J. D. 1982. Biology of disease: Free radicals and tissue injury. *Lab. Invest.* 47:412–416.

Fridovich, I. 1976. Oxygen radicals, hydrogen peroxide, and oxygen toxicity. In *Free Radicals in Biology,* ed. W. A. Pryor, vol. 1, pp. 239–277. New York: Academic Press.

Fubini, B., Bolis, V., and Giamello, E. 1987. The surface chemistry of crushed quartz dust in relation to its pathogenicity. *Inorg. Chim. Acta* 138:193–197.

Gabor, S., Anca, Z., and Zugravu, E. 1979. In vitro action of quartz on alveolar macrophage lipid peroxides. *Arch. Environ. Health* 30:499–501.

Gabor, S., Ciugudeanu, M., and Survel, D. 1985. Effects of selenium on quartz-induced cytotoxicity in macrophage. *Environ. Res.* 37:293–297.

Gendek, E., Bartosz, G., and Layko, W. 1984. A spin label study of the effect of chrysotile asbestos on erythrocyte membrane. *Br. J. Ind. Med.* 41:46–50.

Goldstein, I. M., Cerquiera, M., Lind, S., and Kaplan, H. B. 1977. Evidence that the superoxide-generating system of human leukocytes is associated with the cell surface. *J. Clin. Invest.* 59:249–254.

Gregory, E. M., Yost, F. J., and Fridovich, I. 1973. Superoxide dismutase of Escherichia coli: Intracellular localization and functions. *J. Bacteriol.* 115:987–991.

Gulumian, M., and van Wyk, A. 1987. Free radical scavenging properties of polyvinylpyridine-*N*-oxide: A possible mechanism for its action in pneumoconiosis. *Med. Lav.* 78:124–128.

Harley, J. D., and Margolis, J. 1961. Hemolytic activity of colloidal silica. *Nature* 189:1010–1011.

Heppleston, A. G. 1962. The disposal of dust in the lungs of silicotic rats. *Am. J. Pathol.* 40:493–506.

Heppleston, A. G., and Styles, J. A. 1967. Activity of macrophage factor in collagen formation by silica. *Nature* 214:521–522.

Hochstrasser, G., and Antonini, J. F. 1972. Surface states of pristine silica surface. *Surface Sci.* 32:644–664.

Iler, R. K. 1978. Cellular reaction with silica. In *Biochemistry of Silicon and Related Problems,* eds. G. Bendz and I. Lindquist, pp. 357–359. New York: Plenum.

Iyer, G. N. Y., Islam, M. F., and Quastel, J. H. 1961. Biochemical aspects of phagocytosis. *Nature* 192:535–541.

Janzen, E. G. 1971. Spin trapping. *Accounts Chem. Res.* 4:31–40.

Johnston, R. B., Godzk, C. A., and Cohn, Z. A. 1978. Increased superoxide anion production by immunologically activated and chemically elicited macrophages. *J. Exp. Med.* 148:115–127.

Johnston, R. B., Keele, B. B., Misea, H. P., Lehmeyer, J., Webb, L. S., Bachner, R. L., and Rajagopolam, K. V. 1975. Role of superoxide anion generation in phagocytic bactericidal activity. Studies with normal and chronic granulomatous disease leukocytes. *J. Clin. Invest.* 55:1375–1372.

Kaw, J. L., Beck, E. C., and Bruck, J. 1975. Studies of quartz cytotoxicity on peritoneal macrophages of guinea pigs pretreated with polyvinylpyridine-*N*-oxide. *Environ. Res.* 9:313–320.

Kennedy, T. P., Dodson, R., Rao, N. C., Ky, H., Hopkins, C., Baser, M., Tolly, E., and Hoidal, J. R. 1989. Dusts causing pneumoconiosis generate ·OH and produce hemolysis by acting as Fenton catalysts. *Arch. Biochem. Biophys.* 269:359–364.

Klier, K., Shen, J. H., and Zettlemoyer, A. C. 1973. Water on silica and silicate surface. I. Partially hydrophobic silicas. *J. Phys. Chem.* 77:1458–1465.

Kilroe-Smith, T. A. 1974. Peroxidative action of quartz in relation to membrane lysis. *Environ. Res.* 7:110–116.

Kolbanev, I. V., Berestetskaya, I. V., and Butyagin, P. Yu. 1980. Mechanochemistry of quartz surface. *Kinet. Katal.* 21:1154–1158.

Koskinen, H. A., Tiilikainen, A., and Nordman, H. 1983. Increased prevalence of HLA-Aw19 and B18 in advanced silicosis. *Chest* 83:848–852.

Langer, A. M., and Nolan, R. P. 1986. Physicochemical properties of quartz controlling biological activity. In *Silica, Silicosis and Cancer,* eds. D. F. Goldsmith, D. M. Winn, and C. M. Shy, pp. 125–135. New York: Praeger.

Lehninger, A. L. 1982. *Principles of Biochemistry,* eds. S. Anderson and J. Fox, p. 457. New York: Worth.

Leibovich, S. J., and Ross, R. 1975. The role of the macrophage in wound repair. *Am. J. Pathol.* 78:71–100.

Leibovich, S. J., and Ross, R. 1976. A macrophage-dependent factor that stimulates the proliferation of fibroblasts in vivo. *Am. J. Pathol.* 84:501–513.

Leyko, W., and Gendek, E. 1985. A spin label study of the effects of asbestos, quartz and titanium dioxide dusts on the bovine erythrocyte membrane. *Br. J. Ind. Med.* 42:281–284.

Lippmann, M., Albert, R. E., Yeates, D. B., Berger, J. M., Foster, W. M., and Bohning, D. E. 1975. Factoring effecting tracheobronchial mucociliary transport. In *Inhaled Particles IV,* ed. W. H. Walton, vol. 1, p. 305. New York: Pergamon.

Mandell, G. L. 1975. Catalase, superoxide dismutase and virulence of staphylococcus aureus. *J. Clin. Invest.* 55:561–566.

Marasas, L. W., and Harington, J. S. 1960. Some oxidative and hydroxylative actions of quartz: Their possible relationship to the development of silicosis. *Nature* 188:1173–1174.

Marik-Korda, P., Eross-Kiss, K., and Strauss, H. 1984. Study and determination of the water content of finely dispersed silicic acids. *J. Thermal Anal.* 29:157–165.

Marks, J. 1957. The neutralization of silica toxicity in vitro. *Br. J. Ind. Med.* 14:81–84.

McCord, J. M. 1983. Pulmonary oxygen toxicity: Bronchoalveolar lavage demonstration of early parameters of alveolitis. *Chest* 83:35s–37s.

McCord, M., and Fridovich, I. 1969. Superoxide dismutase: An enzyme function for erythrocuperin. *J. Biol. Chem.* 244:6046–6050.

Melandri, C., Prodi, V., Tarroni, G., Formigna, M., Dezaiaco, T., Bompane, G. F. Maestri, G., and Giacomel, G. 1975. On the deposition of unipolarly charged particles in the human respiratory tract. In *Inhaled Particles IV,* ed. W. H. Walton, vol. 1, p. 193. New York: Pergamon.

Morehouse, K. M., and Mason, R. P. 1988. The transition metal-mediated formation of the hydroxyl free radical during the reduction of molecular oxygen by ferredoxin-ferredoxin: $NADP^+$ oxidoreductase. *J. Biol. Chem.* 263:1024–1211.

Morosava, K. I., Katsnelson, B. A., Rotenberg, Y. S., and Belobragina, G. V. 1984. Further experimental study of the antisilicotic effect of glutamate. *Br. J. Ind. Med.* 41:518–525.

Morrow, B. A., and Cody, I. A. 1973. Infrared spectra of the isolated hydroxyl groups on silica. J. Phys. Chem. 77:1465–1469.

Nash, T., Allison, A. C., and Harington, J. S. 1966. Physico-chemical properties of silica in relation to its toxicity. *Nature* 210:260–261.

Nathan, C. F., Bruckner, L. H., Silverstein, S. C., and Cohn, Z. A. 1979. Extracellular cytolysis by activated macrophages and granulocytes I. Pharmacologic triggering of effector cells and the release of hydrogen peroxide. *J. Exp. Med.* 149:84–89.

Nelson, R. D., Simmons, R. L., McCormack, R. T., Fiegel, V. D., Herron, M., Simmons, R. L., and Quie, P. G. 1979. Chemotactive deactivation of human neutrophils: Possible relationship to stimulation of oxidative metabolism. *Infect. Immunol.* 23:282–286.

Nolan, R. P., Langer, A. M., Harington, J. S., Oster, G., and Selikoff, I. J. 1981. Quartz hemolysis as related to its surface functionalities. *Environ. Res.* 26:503–520.

Noweir, M. H., Moselhi, M., and Amine, E. K. 1980. Role of family susceptibility, occupational and family histories and individuals' blood groups in the development of silicosis. *Br. J. Ind. Med.* 37:399–404.

Parazzi, E., Secchi, G. C., Pernis, B., and Vigliani, E. 1968. Cytotoxic action of silica dusts on macrophage in vitro. *Arch. Environ. Health* 17:850–859.

Raask, E., and Schilling, C. J. 1980. Research findings on the toxicity of quartz particles relevant to pulverized fuel ash. *Ann. Occup. Hyg.* 23:147–157.

Radtsig, V. A., and Bysterikov, A. V. 1978. ESR study of chemically active centers on the surface of quartz. *Kinet. Katal.* 19:713–719.

Radtsig, V. A., and Khalif, V. A. 1979. Investigation of gas chemisorption processes on the surface of finely divided quartz by ESR spectroscopy and microcalorimetry. *Kinet. Katal.* 20:705–713.

Reiser, K. M., and Last, J. A. 1979. Silicosis and fibrogenesis: Fact and artifact. *Toxicology* 13:51–72.

Robock, K., and Klosterkötter, W. 1973. Specific toxicity of different silicon dioxide and silicate dusts. *Stub-Reinhalt. Luft* 33:60–63.

Roose, D., Weening, R. S., Wyss, S. R., and Aebi, H. E. 1980. Protection of human neutrophils by

endogenous catalase: Studies with cells from catalase-dependent individuals. *J. Clin. Invest.* 65:1512–1522.

Rosen, G. M., and Freeman, B. A. 1984. Detection of superoxide generated by endothelial cells. *Proc. Natl. Acad. Sci. USA* 81:7269–7273.

Rosen, G. M., and Rauckman, E. J. 1981. Spin trapping of free radicals during hepatic microsomal lipid peroxidation. *Proc. Natl. Acad. Sci. USA* 78:7346–7349.

Sakabe, H., Koshi, K., and Hayashi, H. 1971. Cell toxicity of mineral dusts. In *Inhaled Particles III*, ed. W. H. Walton, vol. 1, pp. 423–435. England: Unwin Brothers.

Sbarra, A. J., and Karnovsky, M. L. 1956. The biochemical basis of phagocytosis. *J. Biol. Chem.* 234:1355–1362.

Schepers, G. W. 1960. Theories for the causes of silicosis. *Ind. Med. Surg.* 29:326–333.

Schlipköter, H. W. and Brockhaus, A. 1960. The action of polyvinylpyridine-*N*-oxide on experimental silicosis. *Dtsch. Med. Wochenschr.* 85:920–923.

Schlipköter, H. W., Dolgner, R., and Brockhaus, A. 1963. The treatment of experimental silicosis. *German Med. Monthly* 8:509–514.

Shi, X., Dalal, N. S., and Vallyathan, V. 1988. ESR evidence for the hydroxyl radical formation in aqueous suspension of quartz particles and its possible significance to lipid peroxidation in silicosis. *J. Toxicol. Environ. Health* 25:237–245.

Silicosis and Silicate Disease Committee. 1988. Disease associated with exposure to silica and nonfibrous silicate minerals. *Arch. Pathol. Lab. Med.* 112:673–720.

Simon, R. H., Scoggin, C. H., and Patterson, D. 1981. Hydrogen peroxide causes the fetal injury to human fibroblasts exposed to oxygen and its bearing on silicosis. *Exp. Mol. Pathol.* 26:113–128.

Singh, S. V., and Rahman, Q. 1987. Interrelationship between hemolysis and lipid peroxidation of human erythrocytes induced by silicic acid and silicate dusts. *J. Appl. Toxicol.* 12:91–96.

Stalder, K., Stöber, W. 1965. Hemolytic activity of suspensions of different silica modification and inert dusts. *Nature* 206:874–875.

Subczynski, W. K., and Kusumi, A. 1985. Detection of oxygen consumption during very early stages of lipid peroxidation by ESR nitroxide spin probe method. *Biochem. Biophys. Acta* 821:259–263.

Summerton, P. C., Hoenig, S. A., Butler, C., and Chvapil, M. 1977. The mechanism of hemolysis by silica and its bearing on silicosis. *Exp. Mol. Pathol.* 26:113–128.

Thorne, P. S., Lightfoot, E. N., and Albrecht, R. M. 1985. Physicochemical characterization of cryogenically ground, size separated, fibrogenic particles. *Environ. Res.* 36:89–110.

Tsuchiya, I. 1982. Infrared spectroscopic study of hydroxyl groups on silica surfaces. *J. Phys. Chem.* 86:4107–4112.

Vallyathan, V., Shi, X., Dalal, N. S., Castranova, V., Irr, W. 1988. Generation of free radicals from freshly fractured silica dust: Potential role in acute silica-induced lung injury. *Am. Rev. Respir. Dis.* 138:1213–1219.

Velichkovskii, B. T., Yakhyaev, A. V., Azizova, O. A., Osipov, A. N., and Korkina, Yu. A. 1985. Effect of fibrogenous dust on the rate of oxygen consumption by peritoneal macrophages: ESR study. *Gig. Tr. Prof. Zabol.* (10):31–34.

Von Behren, L. A., Chaudhary, S., Rabinovich, E., Shu, M. O., and Tewari, R. P. 1983. Protective effect of poly-2-vinylpyridine-*N*-oxide on susceptibility of silica-treated mice to experimental histoplasmosis. *Infect. Immunol.* 42:818–823.

Wallace, W. E., Vallyathan, V., Keane, M. J., and Robinson, V. 1985. In vitro biological toxicity of native and surface-modified silica and kaolin. *J. Toxicol. Environ. Health* 16:415–424.

Weiss, S. J., and LoBuglio, A. F. 1982. Biology of disease: Phagocyte-generated oxygen metabolites and cellular injury. *Lab. Invest.* 47:5–8.

Wolff, R. K., Kanapilly, G. M., DeNee, P. B., and McClellan, R. O. 1981. Deposition of 0.1 fm chain aggregate aerosols in beagle dogs. *J. Aerosol. Sci.* 12:119–129.

Yamada, T., Niki, E., Yokoi, S., Tsuchiya, J., Yamamoto, Y., and Kamiya, Y. 1984. Oxidation of lipids. XI. Spin trapping and identification of peroxy and alkoxy radicals of methyl linoleate. *Chem. Phys. Lipid.* 36:189–196.

Yamaguchi, T., Koga, M., Takehara, H., and Kimoto, E. 1982. Conformational changes of spin-labelled membrane proteins in human erythrocytes. *FEBS Lett.* 141:53–55.
Ziskind, M., Jones, R. N., and Weill, H. 1976. Silicosis. *Am. Rev. Respir. Dis.* 113:643–645.

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