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IMPACT OF DIFFERENT ASBESTOS SPECIES AND OTHER MINERAL PARTICLES ON PULMONARY PATHOGENESIS

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Abstract—Factors that are potentially important in the pulmonary pathogenesis of asbestos and other mineral particles are: 1) morphology, 2) Fe-content, 3) solubility under intra-phagosomal conditions, 4) value and sign of the surface potential of the particle, 5) hydrophobicity or hydrophilicity, 6) capacity to activate phagocytic leukocytes, and 7) duration of exposure to the particles. The order of importance of these factors in causing severe or fatal pulmonary pathogenicity is estimated to be: $1 > 3 > 7 > 6 \gg 5 > 4 > 2$. The order of pathogenicity of the minerals is estimated as: amphibole asbestos: crocidolite, tremolite, amosite > erionite > serpentine asbestos: chrysotile > talc > silica > simple metal oxides. Particle length, duration of exposure to the particles, and pre-treatment of the particles may however enhance the pathogenic potential of any of the lower-ranked particles.

Key Words—Asbestos, Clays, Lung, Neutrophils, Pathology, Phagocytosis, Physicochemical, Silica, Talc.

INTRODUCTION

Various chemical and physical characteristics of mineral particles, which may be responsible for many types of pulmonary pathologies, include: 1) morphology of thin asymmetrical fibers with diameters <0.25 μm and lengths >8.0 μm (Stanton *et al.*, 1981; *cf.*, Nolan and Langer, 1993); 2) high iron content (Hardy and Aust, 1995); 3) low solubility at low pH (Veblen and Wylie, 1993); 4) value and sign of the electrical surface potential (Light and Wei, 1977a, 1977b; Pundsack, 1955; Martinez and Zucker, 1960; Hochella, 1993); 5) hydrophobicity *vs.* hydrophilicity (Giese and van Oss, 1993; van Oss and Giese, 1995; Giese *et al.*, 1996); and 6) *in vitro* activation of phagocytic leukocytes (Lehnert, 1993). Based on previously published data augmented by experimental results reported here, a synthesis is given which identifies properties 1, 3, and 6 as crucial for classifying species of asbestos and other mineral particles as Category I particles (exceedingly dangerous), or Category II particles (dangerous after continuous and protracted exposure). Some asbestos species, such as the amphiboles, crocidolite, amosite, and tremolite, are pathogenic after only a short (*e.g.*, less than one year) exposure (Category I); serpentine asbestos (*e.g.*, chrysotile) and other mineral particles (Category II) are only pathogenic after long-term (many years) exposure. For both categories, the onset of overt disease in man usually occurs after one to several decades. There is one non-asbestos clay particle, a zeolite, erionite, which is also a Category I particle, based on its pathogenicity. Category I particles are those especially liable to cause mesothelioma, a rare form of pleural cancer (Konig, 1960; Wagner *et al.*, 1960; Kane, 1993).

PROPERTIES OF PARTICLES ELICITING PULMONARY PATHOGENESIS

Size and asymmetry

Stanton *et al.* (1981) implicated the size and asymmetry of mineral particles as extremely important factors in their pathogenicity (see also Mossman *et al.*, 1990). However, Nolan and Langer (1993) criticized some of the animal experiments supporting this hypothesis mainly because of experimental flaws. These flaws are not easily avoidable when reproducing pathogenic processes in humans, which may take decades to complete, since animal experiments have durations ten times shorter. Nonetheless, we know that needle-shaped particles with an axial dimension greater than their diameter (10–12 μm) cannot be eliminated by phagocytic cells (Churg, 1993). However, even the most indigestible particles with all dimensions smaller than those of phagocytes are eliminated by phagocytic transport (Singer *et al.*, 1969, 1972), at an apparent rate that is inversely proportional to the size of the particles. For particles near micron size, total elimination may require more than a year (Singer *et al.*, 1969).

High iron content

The importance of iron in the pathogenicity of some types of asbestos and other mineral particles (Hardy and Aust, 1995) is less than convincing. Most pathogenic varieties of asbestos, tremolite, amosite, and crocidolite have a high iron content. Iron content of the

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Table 1. Estimated iron content of erionite and various species of asbestos minerals.

Iron (%, w/w)	Mineral particles
1.57	Erionite[1]
28.5	Amosite[2]
27.3	Crocidolite[2]
0.7	Chrysotile[2]

[1] R.A. Sheppard and A.J. Gude (1967).
[2] Hardy and Aust (1995).

Table 2. Dissolution rate of erionite and various asbestos species, per 28 d, at pH 3.4 and 37°C, judged by the dissolution of Mg and of Fe (in %, w/w).

Mineral	% Dissolved	
	Mg	Fe
Erionite	6.90	29.93
Amosite	7.23	4.18
Crocidolite (Hope Town, South Africa)	1.15	2.08
Chrysotile (Globe, Arizona)	40.0	0.30
Chrysotile (Salt River, Arizona)	18.36	10.85
Chrysotile (Quebec)	29.95	11.09

less pathogenic asbestos, chrysotile, is low, but the iron content of the exceedingly pathogenic erionite is also low (Table 1). The *in vivo* build-up of layers of iron-containing protein surrounding amphibole asbestos fibers in particular result from peroxidase-producing phagocytes, and is discussed below.

Low solubility rate at low pH

For the *in vivo* removal of long, needle-shaped mineral particles, the solubility rate must be considered under the conditions that prevail inside of phagocytic cells, *i.e.,* inside the phagosomes, at pH ~3–4 and at a temperature of 37°C. Thus, boiling in 4 M HCl (Veblen and Wylie, 1993), which dissolves chrysotile fairly quickly but amosite and especially crocidolite more slowly, provides insight but does not necessarily reflect phenomena occurring *in vivo.* Hume and Rimstidt (1992) found that at 37°C and "in the acidity range (pH 4 to 7) of fluids found in the lung" (Veblen and Wylie, 1993), chrysotile dissolves much more quickly than glass fibers (see also Mossman *et al.,* 1990).

The dissolution rate of Mg and Fe for some of the mineral fibers studied under phagosomal conditions of pH 3.4 and 37°C is given in Table 2. For three different chrysotile varieties, the constituent Mg decreases quickly (average: 29.4%) (see also Light and Wei, 1977a, 1997b) and Fe also disappears relatively quickly (~11% for two of the three samples). Note that the starting Fe content is already low in chrysotile (Table 1). Dissolution of Mg shows crocidolite as the least soluble and relatively low solubilities are found also for amosite and erionite. Note also the rapid dissolution of Fe from erionite. However, the Fe in erionite (Table 1) occurs as a loosely-bound form adsorbed in the large cavities in the zeolite structure. Thus the rate of dissolution of Mg is on the whole a more reliable criterion for overall solubility.

Thus, chrysotile is capable of being eliminated relatively quickly by successive phagocytic engulfments of particles by different phagocytic cells (probably mainly macrophages, and some neutrophils). Erionite, tremolite, amosite, and crocidolite are impossible to eliminate by the phagosomal dissolution efforts of multiple phagocytes, even after a period of years. Chrysotile, therefore, will only cause pulmonary damage if continuously inhaled over long periods (years to decades), whereas erionite, amosite, tremolite, and crocidolite may cause pulmonary damage after only brief exposure (a year or less). Note that these solubility phenomena are important mainly for long needle-shaped particles that cannot be eliminated by phagocytic cells through step-wise dissolution. As noted above, particles that have a more spherical shape can be phagocytically ingested in their entirety and can be eliminated without being digested.

Value and sign of the electrical surface potential

Chrysotile is positively charged at neutral pH, as determined by streaming-potential measurements on chrysotile from Quebec (Martinez and Zucker, 1960), and by microelectrophoresis on chrysotiles of unknown origin (Light and Wei, 1977a, 1977b). However, of the six chrysotile samples of different origins (Table 3), three were positively and three were negatively charged. The positively charged chrysotiles were from Canada, (see also Martinez and Zucker, 1960) and the three negatively charged chrysotiles were from Southern Africa (Zimbabwe and the Republic of South Africa) and from Arizona. Two different varieties of crocidolite were both negatively charged at neutral pH (Table 3). All other particles were also negatively charged at neutral pH (Giese *et al.,* 1996). The capability of mineral particles to activate leukocytes seems unconnected to the sign or strength of their electrical surface (ζ) potential (Tables 3 and 4).

The ζ-potentials of all asbestos particles measured were <50 mV. The positive sign of chrysotile (Light and Wei, 1977a, 1977b; Pundsack, 1955; Martinez and Zucker, 1960) compared to the negative sign of crocidolite at neutral pH, may be a possible origin of the difference in pathogenicity of the two minerals (Hochella, 1993). The origin of the chrysotile samples used by Light and Wei (1977a, 1977b) is not easily traceable (they were obtained from the MRC Pneumoconiosis Unit, Penarth, South Wales, and the Johns-Manville Research and Development Center, Denver, Colorado). The chrysotile used by Martinez and Zucker (1960) was from Quebec. Table 3 shows three of the chrysotile samples, two from Quebec, one from On-

Table 3. Surface properties of clay and other mineral particles (Giese *et al.*, 1996).

Mineral particles	γ^{LW} (mJ/m²)	γ^{+} (mJ/m²)	γ^{-} (mJ/m²)	ΔG_{iwi} (mJ/m²)	ζ[1] mV	Hydrophobicity
Clay particles						
Serpentine asbestos						
Chrysotiles						
Globe, Arizona	35.1	0	31.2	+7.7	−31.0	−
Salt River, Arizona	42.7	0	5.0	−63.8	−32.8	++
Deloro, Ontario	40.4	0.5	25.8	−5.2	+36.1	(+)
Carey Mine, Quebec	35.2	2.9	15.4	−18.3	+35.2	+
Jacob's Mine, Quebec	38.3	0	23.9	−6.3	+46.3	(+)
Shabani, Zimbabwe	37.7	0	6.2	−56.0	−41.2	++
Amphibole asbestos						
Crocidolites						
Cape Town, South Africa	37.4	0.9	23.9	−6.5	−38.0	(+)
Prieska, South Africa	40.0	0.6	20.1	−15.2	−52.0	+
Amosite	38.5	0.8	18.3	−17.6	−33.6	+
Zeolites						
Erionite	36.1	0.2	12.7	−31.0	−24.2	+
Talc						
Gouverneur, New York	31.5	2.4	2.7	−49.5		++
Fisher (26.3 m²/g)	30.7	1.8	5.9	−40.4	−29.5	++
Other metal oxides						
Glass powder	31.1	0.4	37.1	+16.8	−52.7[2]	−
Silica (165 m²/g)	39.2	0.8	41.4	+17.9	−53.2	−
ZrO_2 (3.7 m²/g)	34.8	1.3	3.6	−52.3	−27.0	++
SnO_2 (5.2 m²/g)	31.1	2.9	8.5	−30.2	−38.0[2]	++
Al_2O_3	31.6	0.6	27.2	+1.0	−45.6[2]	−
Fe_2O_3[3] (monosized hematite)	46.1	0.1	50.1	+29.4	−42.6	−

[1] Measured in 0.015 M NaCl, pH 7.0.
[2] Measured in phosphate-buffered saline 0.015 M NaCl, pH 7.0
[3] From Costanzo *et al.* (1995).

tario, Canada, were positively charged. However, three other chrysotile samples, two from Arizona and one from Zimbabwe, were negatively charged. Still, >50% of the chrysotile produced worldwide (data 1953–1967) came from Canada; ~3–5% of the world-asbestos production was crocidolite, and ~2–4% was amosite (van Thoor, 1971). Canadian chrysotile (but not chrysotile from the United States or southern Africa) is indeed positively charged, and this is unusual, since it is the only positively charged asbestos mineral and also one of the only positively charged particles (at neutral pH) among other clays tested by Giese *et al.* (1996). In addition, most of the metal oxides and other minerals tested were also found to be negatively charged (Table 3; see also Giese *et al.*, 1996).

Nonetheless, although half the chrysotile used worldwide during the middle part of this century was positively charged, it does not explain why chrysotile is less acutely pathogenic than, *e.g.*, crocidolite, tremolite, amosite, and erionite. The latter four are negatively charged, therefore the sign of electric charge does not appear to correlate with the degree of pulmonary pathogenicity. (See also *Interaction with phagocytes,* below).

Hydrophobicity vs. hydrophilicity

Absolute hydrophobicity and hydrophilicity were defined (Giese and van Oss, 1993; van Oss, 1994; van Oss and Giese, 1995) in terms of the interfacial free energy (ΔG) of interaction between particles (i), immersed in water (w); for $\Delta G_{iwi} > 0$, the particles are hydrophilic (*i.e.*, they repel each other in water) and for $\Delta G_{iwi} < 0$, the particles are hydrophobic (*i.e.*, they attract each other in water). The hydrophobic/hydrophilic properties influence the interfacial interactions between particles and biopolymers when both are present in free solution. These comprise parts of the glycocalices, which form the outer surfaces of mammalian cells, such as leukocytes. Biopolymers (*e.g.*, proteins) adsorb more strongly to hydrophobic than to hydrophilic surfaces (MacRitchie, 1972; van Oss, 1995; van Oss *et al.*, 1995). Also, the adsorption of proteins, for example, onto hydrophobic surfaces is more denaturing and irreversible than adsorption onto hydrophilic surfaces (MacRitchie, 1972; Morrissey and Stromberg, 1974; van Oss, 1995). Finally, more hydrophobic microorganisms and particles are more avidly phagocytized by human phagocytic cells then

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Table 4. Neutrophil activation.

Particle or fiber	% of positive control[1]			
Crocidolite (Cape Town, South Africa)		128.0		
Crocidolite (Prieska, South Africa)		105.0	87.4	60.4
Erionite			45.0	30.6
Chrysotile (Globe, Arizona)		113.6	90.5	73.0
Chrysotile (Salt River, Arizona)		72.0		
Chrysotile (Deloro, Ontario)		97		
Chrysotile (Carey Mine, Quebec)		71.2		
Chrysotile (Jacob's Mine, Quebec)		125.0		
Chrysotile (Shabani, Zimbabwe)		81.8		
Amosite			86.2	51.5
Filter paper (Whatman 3MM)		56.1	39.9	36.7
Filter paper (Whatman 17)		72.7		
Talc (Gouverneur, New York)	93.2			
Talc (Fisher)	65.2			
Glass powder	65.2			
ZrO_2 (0.5 m^2/g surface area)	67.4			
ZrO_2 (3.7 m^2/g surface area)	63.6			
SnO_2	57.6			
Al_2O_3	51.5			
Silica (165 m^2/g surface area)	65.2			
Silica (286 m^2/g surface area)	54.4			
Negative Control, PBS/glucose	24.2 Stationary	31.8 Stationary	29.3 Stationary	10.9 Agitated

[1] Results given in the first two columns were obtained in different experiments done at different times. The results given in the last two columns were obtained at the same time.

Particles and fibers were used as received.

more hydrophilic microorganisms or particles (van Oss and Gillman, 1972; van Oss *et al.,* 1975). Surface tension properties (apolar, or Lifshitz-van der Waals, γ^{LW}, and Lewis acid-base, γ^{AB}), and the ζ-potential (van Oss, 1994) are given in Table 3. Surface-tension properties were measured by contact angle determination by means of thin-layer wicking (van Oss *et al.,* 1992). No striking differences in, for example, hydrophobicity or hydrophilicity are apparent between most chrysotile samples compared with the two crocidolite samples, and erionite, or amosite. Talc and zirconia appear to be more hydrophobic mineral particles, (*cf.,* strongly negative ΔG_{iwi} value), whereas glass powder, silica (165 m^2/g), and alumina, and chrysotile from Globe, Arizona, are clearly hydrophilic (positive ΔG_{iwi} values).

Interactions with phagocytes

Although red blood cells are not phagocytic cells, the measurement of their destruction (hemolysis) by mineral particles is probably the easiest (but not the most useful) of *in vitro* cytotoxicity tests. Driscoll (1993) reviewed results obtained by various authors with *in vitro* evaluation methods using mineral cytotoxicity. Silica particles have the strongest hemolytic activity, followed by chrysotile; crocidolite and amosite are less active. The hemolytic activity of chrysotile was correlated with positive surface charge (Light and Wei, 1977a, 1977b) although the majority of chrysotile samples are not positively charged (see also Table 3). The hemolytic activity of mineral particles, although easily determined, has little bearing on the degree of pathogenicity.

Measurements of the ability of mineral particles to activate phagocytic cells (macrophages, neutrophils) *in vitro* correlates more closely with the ability of the minerals to cause *in vivo* pathogenic changes in humans, than with *in vitro* measurements of hemolysis (Driscoll, 1993). Quartz particles and all varieties of asbestos were shown to activate macrophages of animal and human provenance, as measured by superoxide production (Driscoll, 1993; Table 4), but no convincing differences emerged between the degrees of macrophage activation caused by moderately pathogenic (*e.g.,* quartz), pathogenic (*e.g.,* chrysotile), or strongly pathogenic (*e.g.,* crocidolite, erionite) particles.

We decided to use human phagocytic cells, in a highly sensitive *in vitro* test, and to apply this test to a large variety of mineral particles, under the same or comparable conditions. Peripheral blood polymorphonuclear leukocytes (PMNs, or neutrophils) are human phagocytic cells which are readily available in large quantities. Human peripheral blood monocytes (precursors of macrophages) are too few to be useful for this purpose. Human alveolar or bronchial macrophages (sessile cells) are not easily obtainable without discomfort to the donor, are few in number, and are usually already partly pre-activated by the extraction procedure. The measurement of reactive-oxygen species

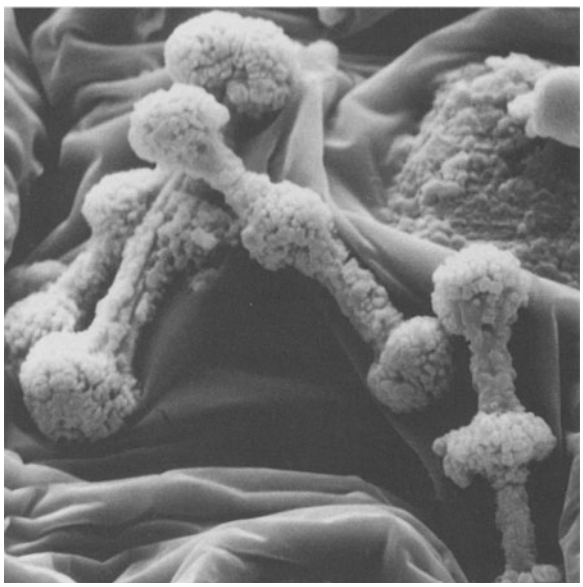

Figure 1. Electron micrograph of asbestos fibers from a human lung, covered by a ferruginous proteinaceous layer. Scanning electron micrograph of several amphibole fibers, coated with a ferruginous layer, and showing remains of macrophages encircling the fibers at intervals of 2–4 μm. The diameters of the macrophages are ~10 μm.

(ROS) from activated neutrophils (cells that are in the process of engulfing particles) may be a more reliable index of the potential biological hazards of various mineral fibers and particles. These ROS were shown to cause not only tissue and cellular damage, but also may play an important role in initiating malignancy and fibrosis in the lung and pleura (Vallyathan and Shi, 1997).

EXPERIMENTAL PROCEDURE

A fluorescence assay was used to measure the oxidative burst induced in human neutrophils by various mineral particles. The assay (Rodgers, 1995) measures hydrogen peroxide produced during oxidative metabolism induced by materials binding to neutrophil membranes by means of dichlorofluorescein diacetate (DCFH-DA), which is a stable non-fluorescent compound that freely enters cells. DCFH-DA becomes trapped inside cells after cleavage of the acetyl group, which forms the polar molecule dichlorofluorescein (DCFH). In the presence of hydrogen peroxide, DCFH forms oxidized dichlorofluorescein (DCF) which is fluorescent and can be measured with a spectrofluorometer. Heparinized human peripheral blood was obtained from volunteers. Neutrophils were isolated by dextran sedimentation or by sedimentation of the red cells with hexamethylstarch (van Oss *et al.*, 1981).

The dichlorofluorescein assay was performed as follows: The neutrophils were adjusted to a concentration of 10^6 cells/mL in phosphate-buffered saline (PBS) with 5 mM D-glucose (PBS/glucose), loaded with 5 μM of dichlorofluorescein diacetate (DCFH-DA), and incubated at 37°C for 15 min. The cells were washed of excess DCFH-DA and a sample of neutrophils was tested for viability by trypan blue staining. The various oxide and fiber materials were placed in a solution of PBS/glucose at a concentration of 0.2% (w/v). One half mL of each of the oxide/fiber solutions was separately added to 2.5 mL of the neutrophil suspension (2.5×10^6 cells). One half mL of phorbol myristate acetate PMA (100 ng/mL) was used as the positive control and 0.5 mL PBS/glucose was used as the negative control (blank). The cells and sample materials were incubated in a water bath at 37°C for 45 min, then centrifuged (250 × g). The supernatant was discarded and the cell pellet was dissolved with 3 mL of 0.1% Triton X for 15 min. The fluorescence readings were made on 1 mL samples using a Perkin-Elmer LS-5B Fluorescence Spectrometer (488 nm excitation, 525 nm emission). Each assay was performed in triplicate.

INTERACTIONS BETWEEN PHAGOCYTES AND FIBERS OR PARTICLES *IN VIVO*

The *in vivo* interaction between phagocytic cells and fibers or non-fibrous particles determines if serious pulmonary pathogenicity or a relatively mild transient inflammatory response will ensue. Therefore, it is useful to make a distinction between phagocytic engulfment and phagocytic digestion. Complete phagocytic engulfment of particles is only possible with nearly symmetrical particles with a greatest dimension <10 μm. Phagocytic cells, *i.e.,* monocytes/macrophages, as well as neutrophils, have diameters varying between ~8–12 μm. Nonetheless, phagocytes will continuously attempt to engulf foreign bodies such as needle-like (fibrous) particles that are thinner but much longer than themselves, but each single phagocytic cell is only partially successful (Figures 1 and 2). However, more symmetrical particles of smaller diameter can be completely engulfed by phagocytic cells.

Phagocytic digestion, which aims at killing ingested microorganisms (*e.g.,* bacteria) is usually only feasible after *ingestion.* Ingested particles are enclosed in phagosomes, which unite with intracellular granules that contribute a variety of biocidal enzymes, such as oxidases and peroxidases, to the phagosomal liquid medium. At the relatively low phagosomal pH of 3.5–4.0, these enzymes generally succeed in oxidizing and killing or inactivating most ingested infectious agents. Inorganic particles, such as metal oxides however, usually cannot be oxidized additionally and any minor increase in their oxidized state does not materially increase their solubility at pH ~3.5–4.0. This solubility at pH 3.5 and 37°C is a determining factor in the disposal of mineral particles or fibers *in vivo.* Soluble particles disappear soon after phagocytic engulfment; soluble fibers disappear somewhat more slowly, after

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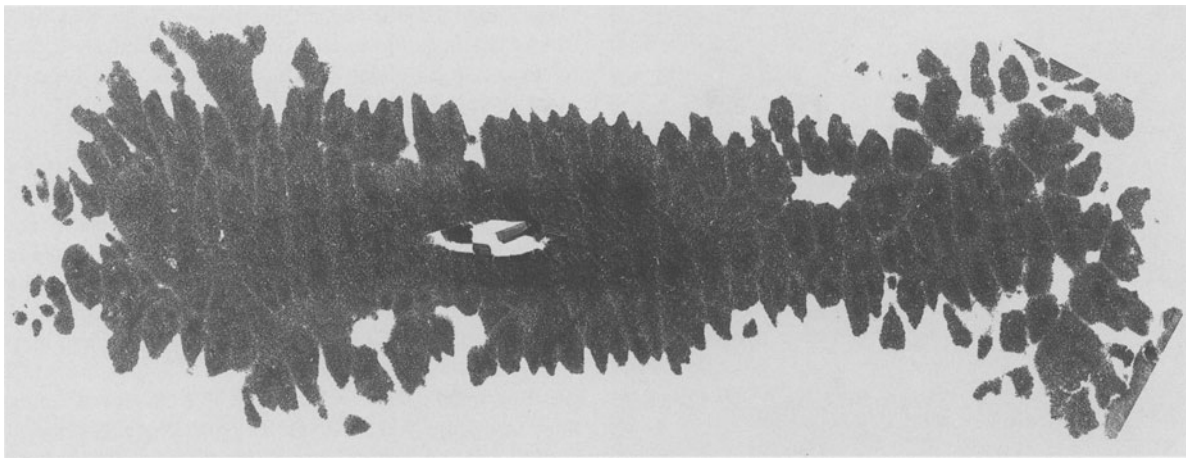

Figure 2. Transmission electron micrograph of an oblique section of an asbestos fiber showing a striated ferruginous layer and the remains of several macrophages. The diameters of the macrophages are ~10 μm.

being partly engulfed by a series of phagocytes (Table 5).

Insoluble particles that become completely engulfed are disposed of by phagocyte-mediated transport (*cf.,* Table 5). Insoluble fibers, however, cannot be transported by phagocytes, which can only engulf a small portion of them (Figures 1 and 2). Furthermore, rather rigid fibers tend to become imbedded in lung tissue, impeding their removal *in vivo.* In addition, amphibole asbestos fibers (crocidolite, tremolite, amosite), as well as the zeolite, erionite, are especially straight and rigid, and thus tend to pierce the lung tissue, to penetrate and lodge in the pleural cavity where, after many years, they ultimately cause pleural cancer, or mesothelioma. Serpentine asbestos fibers (chrysotile) are less rigid and more pliable. Additionally, chrysotile's much greater solubility under phagosomal conditions (*cf.,* Table 2), prevents these fibers from entering the pleural sac.

Finally, there are *sequelae* to the non-removability of amphibole asbestos fibers by phagocytic cells, and to their essential inertness to the continuous but vain biocidal attempts by phagocytic enzymes (oxidases and peroxidases; *cf.* Klebanoff, 1992). These *sequelae* are the *in vivo* deposition of multiple layers of iron-containing proteins (*e.g.,* ferric hemosiderin) which, over time, transforms these amphibole asbestos fibers (and probably also erionite fibers) into "ferruginous bodies" (see, *e.g.,* Churg, 1993; Kane, 1993).

Note that assays done on different days, with a different preparation of neutrophils, are not directly comparable. Therefore, for four assays (Table 4, right side) where the degrees of neutrophil activation by different particles are only comparable within a given column, each case shows a comparison with the negative and positive controls. Using relative positions from Table 4, particles can be ranked by neutrophil activation capacity (Table 6).

DISCUSSION

Degree of pulmonary pathogenicity of various mineral particles

Various epidemiological studies (*e.g.,* Feigin, 1989; Kane, 1993; Ross *et al.,* 1993; Churg, 1993) can be used to classify the various mineral particles and fibers discussed in approximately the following order of severity of pulmonary pathogenic potential: crocidolite and other amphiboles, including tremolite and amosite > erionite > chrysotile, which are all asymmetrical and which are more strongly pathogenic than the more symmetric particles: talc > silica > other metal oxides (see also Table 6).

Table 5. Summary of phagocytosis of fibrous and non-fibrous particles *in vivo* as a function of particle shape and solubility.

	Mineral particles	
Degree of solubility	Fibrous (asymmetrical, at least 10–20 μm long, not more than 1–2 μm wide)	Non-fibrous (small, symmetrical, maximum diameter 1 or a few μm)
Insoluble under phagosomal conditions	not engulfed by phagocytes, not dissolved by phagocytes, formation of persistent ferruginous bodies (Figure 1)	engulfed by phagocytes, but not dissolved by phagocytes, ultimately disposed of (Adlersberg *et al.,* 1969; Singer *et al.,* 1972)
Soluble under phagosomal conditions	not engulfed by phagocytes, dissolved slowly	engulfed and dissolved

Table 6. Summary of rank orders of a number of salient properties of pathogenic particles.

Estimated neutrophil activation order	Order of insolubility at pH 3.4 at 37°C (per Mg^{2+})	Order of asymmetry needle or fiber shape, with long axis >20 μm	Order of iron content
Crocidolite	Crocidolite (very insoluble)	Chrysotile	Hematite
Chrysotile	Amosite (fairly insoluble)	Crocidolite	Amosite
Amosite	Erionite (fairly insoluble)	Amosite	Crocidolite
Talc	Chrysotile (much more soluble than the three others)	Erionite	Chrysotile
Erionite			Erionite
Silica, glass powder			
Zirconia, Tin Oxide			
Alumina, filter paper			

Asymmetrical fibrous particles are potentially pathogenic upon inhalation (*cf.,* Stanton 1974), as they cannot be phagocytized in their entirety. If not completely dissolved under intraphagosomal conditions (see Figures 1 and 2), they remain forever lodged in the lungs. One of the most insoluble fibrous particles is crocidolite (see Table 2). Even after only brief exposure, this insolubility may cause delayed mesothelioma (the actual and invaribly fatal mesothelioma may only manifest itself many decades after the last exposure, *e.g.,* 20–60 y, *cf.,* Kane, 1993). The delayed pulmonary pathology of amphiboles is possibly related to the insolubility of crocidolite under intraphagocytic conditions. Only amphiboles (crocidolite, amosite) and erionite are sufficiently insoluble (Table 2) and sufficiently asymmetrical (Table 6) to avoid removal by phagocytes for decades. Thus considering brief exposure pathogenicity, crocidolite and other amphiboles, as well as erionite, are the main Category I particles (see the Introduction, above).

Phagocytic leukocytes are strongly activated by crocidolite and chrysotile (Tables 4 and 6). The ferruginous coating of the asbestos fibers (*cf.,* Figures 1 and 2), caused by continuous deposition of Fe involved in intraphagocytic oxidation may ultimately coat the fibers with a layer that continues to cause phagocytic activation for years. Neoplasm formation can take a few decades with crocidolite and erionite. With the much more soluble chrysotile, any ferruginous coating is unlikely to have a significant effect when enveloping a quickly dissolving fiber. Continued and prolonged exposure to erionite, with low initial phagocyte-activating capacity, and to chrysotile, with pronounced solubility, would be the strongest factor controlling their pathogenicity. Nonetheless, the slightly soluble but low cytotoxic erionite, and the more soluble but high cytotoxic chrysotile differ in another way. Erionite almost always gives rise to mesothelioma (Baris, 1987) which is not the case with pure chrysotile (Ross, 1981, 1984; Mossman *et al.,* 1990). For fibers to cause mesothelioma, they must come in contact with the mesothelium within the pleural space (Kane, 1993). Since the primary route of entry of the fiber is through inhalation, fibers that lodge deep in the lower lung must then traverse lung tissue to reach the pleural cavity. Most likely, only the most durable and rigid fibers (*i.e.,* the amphiboles, crocidolite, tremolite, amosite, as well as erionite) will be able to escape the confines of the lung and become disseminated within the pleural cavity and thus cause malignant mesothelioma. Also, chrysotile is more "curly" and pliable and thus much less prone to penetrate into the pleural cavity (Mossman *et al.,* 1990; Mossman and Gee, 1989).

Effect of prolonged exposure to fibrous particles

After prolonged exposure (*e.g.,* one or more decades) to asymmetrical particles (Stanton, 1974; Nolan and Langer, 1993), crocidolite appears the most pathogenic, followed by amosite (although few data exist, owing probably to low usage), and by erionite. Pulmonary pathogenicity is extremely high in the few villages in Turkey (Baris, 1987), where erionite abounds. Although needle shaped and insoluble, erionite does not particularly strongly activate phagocytic leukocytes. The continuous exposure since birth of the Turkish villagers may be a factor in the extreme pathogenicity of this zeolite (see also Feigin, 1989). Although erionite is somewhat more soluble than crocidolite (although not quite as soluble as chrysotile), and much less irritating to phagocytic leukocytes, it may become more irritating after coverage with a ferruginous layer (Table 5). For the inhabitants of Karain and Tuzkoy in Turkey (Baris, 1987), and to a lesser extent the inhabitants of Sarihidiz and a few other Cappadocian villages, prolonged and continued exposure to these fibrous particles makes erionite an extremely dangerous material.

Small symmetrical particles

Small, nearly symmetrical particles (*e.g.,* talc, silica, *etc.*) are not dangerous following only brief exposure; particles with high solubility at pH 3–4 (*e.g.,* silica) are not very pathogenic after only brief and temporary exposure. Freshly fractured silica, however, becomes more hydrophobic (Wu *et al.,* 1996) and thus less sol-

Table 7. Summary of estimated pulmonary pathogenicity as a function of exposure time.

Category I particles Short exposure (*e.g.*, months to a year)	Category II particles Long and repeated exposure (several years to decades)
Amphiboles	Chrysotile
Erionite	Talc
	Silica and other Metal Oxides
	Cellulose

uble, leading to more injury and inflammation than aged silica powder (Vallyathan *et al.,* 1995; see also Table 7). However, even extremely insoluble particles such as talc particles, can be disposed of after ingestion by phagocytic leukocytes. Studies on the intravenous injection of insoluble polystyrene latex particles of similar size (0.2–1.0 μm diameter), or of colloidal gold particles after phagocytic ingestion (Adlersberg *et al.,* 1969: Singer *et al.,* 1972) indicate that ultimately, after many cycles of ingestion and reingestion by other phagocytes, the particles are removed via the upper respiratory and lower digestive tracts where, curiously, the former pathway seems more important than the latter. Although talc is used in vast amounts in a large array of food, cosmetic, and pharmaceutical applications (Zazinski *et al.,* 1995), exceedingly untoward *sequelae* of its continuous use, *e.g.,* in cosmetics, seem rare. For instance, life-time perineal use *may* increase the risk of ovarian cancer but does not appear to be a major cause (Harlow *et al.,* 1992; see also Wehner, 1994). The pulmonary toxicity of talc for babies who somehow inhale excessive amounts can be severe and lasting (Hollinger, 1990). When talc is inappropriately used intravenously, severe respiratory disability can ensue (Hollinger, 1990); this fits well with the preferential transport by phagocytes of insoluble particles to the lungs, reported by Adlersberg *et al.* (1969).

Untoward effects caused by inhaling more soluble particles, such as silica, only occur after very prolonged exposure (see, *e.g.,* Feigin, 1989; Ross *et al.,* 1993). Of course, however soluble a particle may be, and notwithstanding the fact that it will be ultimately disposed, continuous exposure over, *e.g.,* decades, will defeat all these biological defense mechanisms, and will finally cause lasting pulmonary pathology.

Connection between the physical and physicochemical properties of mineral particles and their pulmonary pathogenicity

The connection between physical and physicochemical properties of mineral particles including the duration of their exposure is discussed below, in the order of importance.

Size and asymmetry. One of the most important contributors to pulmonary pathogenicity clearly is strong asymmetry, *i.e.,* fiber or needle-shape (Stanton, 1974; Nolan and Langer, 1993; Mossman *et al.,* 1990). Low solubility of particles is important (see below), but even insoluble, small symmetrical particles that can be completely engulfed by phagocytic cells ultimately get eliminated (Adlersberg *et al.,* 1969; Singer *et al.,* 1972). Insoluble fibrous particles such as "blue" asbestos, crocodolite, and erionite are prone to stay trapped in the lungs for the life of the patient.

Talc, silica, and the other metal oxide powders usually do not consist of fibers, but of particles with a shape and dimension that allow for complete phagocytic engulfment. Thus, whether soluble or not, they ultimately get removed by phagocytic transport (Singer *et al.,* 1969) and are only likely to cause pulmonary (or other) pathogenesis upon repeated and lengthy exposure. Talc, with pronounced leukocyte activation and very low solubility, would be more dangerous than silica. However, industrial exposure to silica typically is of much longer duration than exposure to talc (Table 7) and freshly ground silica is also not without danger (see below).

Low dissolution rate. With respect to fibrous particles, low dissolution rates (Table 2) prevent their phagocytic removal even in progressive steps (Figures 1 and 2), so that the combination of fibrous shape and low solubility rates places crocidolite and other amphiboles (tremolite, amosite), as well as erionite, into the category of the most dangerous mineral particles. Small, symmetrical particles ultimately do get removed (see above), but the rather slow removal of insoluble particles (Adlersberg *et al.,* 1969; Singer *et al.,* 1972) places, for instance, talc, in a potentially more dangerous category than, *e.g.,* silica, especially after only relatively brief but massive exposure, *e.g.,* via injection (Hollinger, 1990).

Chrysotile is more soluble than the fibrous particles mentioned above, which most probably accounts for its lower pathogenicity than crocidolite upon brief exposure, although chrysotile appears to be as potent as crocidolite in activating phagocytic leukocytes (Tables 4 and 6), it is therefore mainly dangerous upon prolonged repeated exposure, especially for long-term smokers (Mossman *et al.,* 1990). Note, however, that in certain instances chrysotile was reported to have been associated with small amounts of the amphibole, tremolite (Mossman and Gee, 1989; Mossman *et al.,* 1990). In the lungs of patients who were mainly exposed to chrysotile, mostly amphiboles were found (Mossman *et al.,* 1990), which probably was a consequence of the low *in vivo* solubility of amphiboles, in contrast with the marked solubility of chrysotiles (Table 2).

Cellulose is not very soluble and it has a fibrous shape, but it does not significantly activate leukocytes. Also cellulose fibers are more pliable than most min-

eral fibers so that the phagocytic ingestion may be somewhat easier. However, cellulose fibers are insoluble so that only extremely long, repeated, and massive exposure to cellulose fibers would be detrimental. Cellulose has been used as a negative control, *i.e.,* a material with little phagocytic activity (Table 7).

Length of exposure. This factor is most important in counteracting solubility (Table 7). Fairly soluble particles, whether fibrous or symmetrical, pose no serious risk, unless there is lengthy and repeated exposure, which can effectively obviate their continuous intraphagosomal elimination. This factor appears to be of great importance in lengthy exposure to chrysotile, which must be considered to be harmless upon brief and/or occasional exposure. Thus, the massive-scale removal of chrysotile, or "white" asbestos, may be counter-productive and may even be harmful for construction workers who are constantly engaged in removing this material from buildings where, if left alone, it would be essentially harmless.

Erionite, although less soluble than chrysotile, is fairly harmless upon brief exposure (Feigin, 1989) owing to its rather lower power to activate phagocytic leukocytes (Table 4). However, this is obviated by the very long exposure inhabitants undergo (from birth) in the few Turkish villages where this zeolite is abundant (Baris, 1987). Finally, fibers often acquire a ferruginous layer *in vivo* (Table 5). These factors produce an exceedingly high incidence of mesotheliomas among inhabitants between 20–40 years of age (Table 7).

Activation of leukocytes. Note from Table 4 that especially crocidolite and chrysotile strongly activate phagocytic leukocytes. This clearly plays a role in pulmonary pathogenicity of both asbestos varieties. As evidence for this, a recent study by Driscoll *et al.* (1997) demonstrated exposure of rats to doses of particles producing neutrophilic activation was associated with increased mutations in rat alveolar cells. However, given appropriate conditions of length of exposure and, possibly importantly, the age at which the exposure begins, erionite still can cause massive pathology, in as much as about half of the exposed population die before reaching middle age (Baris, 1987; Ross, 1981, 1984). Crocidolite can remain dormant while trapped in the pleural cavity for decades. At this location, it is probably coated with a ferruginous proteinaceous layer which may be the factor masking its leukocyte-activating tendencies. Among the small, symmetrical mineral particles, talc seems to be especially prone to activate phagocytic leukocytes, although freshly fracture silica can also be dangerous (Vallyathan *et al.,* 1995; Table 7). Talc has a tendency to activate and convene phagocytic leukocytes *in situ.* This has led to the instillation of talc suspensions and a successful alleviation of recurrent malignant pleural effusions by pleurodesis, a process that is largely based on the provocation of a strong localized intrapleural inflammation (Adler and Sayek, 1976).

Hydrophobicity and hydrophilicity. Among fibrous particles, hydrophobicity or hydrophilicity has little relation to pulmonary pathogenicity (Table 3). With small, symmetrical particles, however, it is striking that talc is among the most hydrophobic, which is of course also linked to its lower solubility. It is the latter property that is the most strongly connected to their putative pathogenicity.

With serpentine and amphibole asbestos varieties, the connection between hydrophobicity, hydrophilicity, and solubility is less clear. Note, however, a relative hydrophobic outer surface does not prohibit the ultimate penetration of water and the dissolution from the inside of the fiber. In general, however, more hydrophobic particles become more readily phagocytized (van Oss *et al.,* 1975) and thus, more readily activate phagocytic cells. In addition, hydrophobic particles are less soluble. While talc is more hydrophobic than silica (Table 3), and probably concomitantly activates leukocytes more strongly than silica (Table 4), it is noted that the measurements reported in Tables 3 and 4 were made with particles that were kept in storage for at least several months. Vallyanthan *et al.* (1995) showed that the inhalation of freshly fractured quartz leads to increased injury and inflammation. More recently, we showed (Wu *et al.,* 1996) that relatively coarse silica particles, which are hydrophilic, become hydrophobic when freshly ground. An increase in hydrophobicity (Table 3) tends to cause a stronger leukocyte activation (Table 4), and also gives rise to a decrease in solubility (see Table 7).

Value and sign of the surface potential. Light and Wei (1977a, 1977b) were doubtless correct that the chrysotile sample they studied was positively charged. However, differences in pathogenicity between crocidolite and chrysotile may not be related to crocidolite being negatively charged and chrysotile being positively charged. Table 3 shows that of the six chrysotile samples of different origins, three were positively and three negatively charged at neutral pH. On balance, the exceptional positive charge of *some* chrysotile samples, is most likely not relevant to the degree of their pathogenicity.

The iron content. Crocidolite has a high iron content, but chrysotile and erionite contain only minor amounts (Table 1). The iron content of particles clearly is not directly related to leukocyte activation (Table 4) and also has little to do with particle solubility. The least soluble particles under consideration are crocidolite (high iron) and talc (little or no iron). On the whole, the iron content appears unconnected to the pathogenicity of the particle in disagreement with Hardy and Aust (1995). It is not known, however, whether a high

iron content, caused by a ferruginous layer (Table 5; Figures 1 and 2), will affect the activation of phagocytes.

CONCLUSIONS

Pulmonary pathogenesis resulting from exposure to mineral particles is associated with, in the order of importance: 1) asymmetrical morphology and rigidity, 2) insolubility under intra-phagosomal conditions, 3) length of exposure, 4) capacity to activate phagocytic leukocytes, and 5) hydrophobicity *vs.* hydrophilicity of the particles. The iron content of the mineral fiber is in itself not important, but the development of a ferruginous coating *in vivo* caused by the enzymatic action of phagocytic oxidases and peroxidases may ultimately contribute to the pathogenic process leading to mesothelioma. No correlation was found between either the sign or the magnitude of the electrical surface potential of a particle or fiber and pulmonary pathogenicity. Most asbestos fibers (including crocidolite) and other mineral particles are negatively charged, whereas some chrysotile samples are negatively charged, and others are positively charged.

The amphibole asbestos fibers, crocidolite, tremolite, amosite, as well as the zeolite, erionite are the most dangerous. All of these are asymmetrical, rigid, and insoluble under phagosomal conditions and they are prone to become coated with a ferruginous layer. Chrysotile is normally much less pathogenic except upon long-term exposure, and when accompanied by heavy smoking. In addition, contamination of a chrysotile sample by amphibole fibers, such as tremolite, will increase its pathogenicity.

In time, symmetrical particles all become phagocytically engulfed and are ultimately disposed, even when insoluble, under intra-phagosomal conditions. However, long-term continuous exposure to such particles can of course counteract their ultimate disposal by phagocytic cells, and thus can still lead to pathological *sequelae.* Very hydrophobic talc particles appear to be the most dangerous. Silica is usually hydrophilic and somewhat less dangerous, but it becomes much more hydrophobic when freshly ground and is then more prone to be more pathogenic.

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