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Pulmonary Toxicology of Silica, Coal and Asbestos

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Mineral particles are customarily inhaled as mixtures, though one component may predominate and determine the response. Although the lesions often possess a characteristic structure, according to the main type of particle deposited, morphology affords little indication of pathogenesis. Being a major element in the evolution of dust lesions, macrophage behavior has been examined extensively *in vitro* after treatment with mineral particles, attention being directed to membrane and biochemical changes; however, no clear lead to the origin of the lesions has emerged.

Pulmonary fibrosis, as one of the ultimate consequences of dust accumulation, required a direct *in vitro* approach in which the products of the macrophage-particle interaction were utilized to provoke collagen formation by fibroblasts in a two-phase system. By this means, silica and asbestos stimulated connective tissue formation and application of the technique to coal dusts appears promising. Coal workers may develop a peculiar type of emphysema in relation to lesions whose fibrous content is comparatively small.

Type II alveolar epithelium is also stimulated by inhaled particles and lipid accumulation follows. Alveolar lipidosis interferes with the fibrotic response by preventing contact between macrophage and particles. This phenomenon may account in part for anomalies, apparent in coal workers, between epidemiological findings and dust composition.

Carcinogenesis is a well-recognized feature of asbestos exposure, but, as with fibrosis, risk prediction on the basis of *in vitro* tests of cytotoxicity is premature and may not be valid.

The Minerals

A mineral may be defined as a naturally occurring crystalline, inorganic compound or element and, in the present context, the important fraction is the respirable one, i.e., that fraction capable of penetrating to the alveolar region. In the case of compact particles, such as coal or silica, the respirable diameter is ≤ 5 μm. For fibrous particles, such as asbestos, diameter predominantly determines falling speed and an upper limit of about 3 μm regulates respirability.

Silicon and oxygen are the major elements of the earth's crust, so that any extraction procedure presents a potential risk of silicosis, as do the uses to which the mineral is put. Silicon dioxide exists in a tetrahedrally coordinated form, a silicon atom lying centrally and its four oxygen atoms being shared with neighboring atoms of silicon. The term free silica refers to the uncombined state, which exists in crystalline, cryptocrystalline and amorphous forms. Each form possesses its own arrangement of the tetrahedra. At atmospheric pressure, quartz is the stable crystal with tridymite and cristobalite being metastable types formed at higher temperatures than quartz. Coesite and stishovite are crystalline varieties produced by very high temperatures and pressures, the former having a tetrahedral structure but the latter being octahedral (like titanium dioxide) in which six shared oxygen atoms are attached to each atom of silicon. Flint and agate fall into the cryptocrystalline category, while diatomite, vitreous and sublimed silica are amorphous, in that the tetrahedra lack regular arrangement. Combined silica refers to silicon dioxide bound in complex ways to various cations, that is to silicates among which asbestos is biologically most important.

Airborne dust in coal mines is a complex mixture, mainly derived from coal seam but also from associated rock strata. Coal itself is not a uniform substance, lignite, bituminous coal and anthracite representing rising ranks that exhibit increasing content of carbon and decreasing content of oxygen. The minerals in respirable dust from coal mines are derived mainly from the non-coal strata, and in British samples, quartz comprised a mean of 4.5% with higher proportions of kaolin and mica (*1*). In Welsh collieries, however, the airborne dust contained about 2% free silica (*1,2*).

Asbestiform minerals exhibit high aspect (length: diameter) ratios, the fibers being flexible and possessing high tensile strength. Asbestos (meaning unquenchable) is the collective term for varieties of certain hydrated silicates, which also exist in nonfibrous form. Chrysotile (serpentine) asbestos is a layered silicate in which an outer layer of octahedrally coordinated magnesium cations, presenting as brucite [$Mg(OH)_2$], is linked by shared oxygen atoms with an inner tetrahedrally coordinated silicate layer, the fibril possessing a scroll or tubular structure on section and longitudinal curvatures.

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Amphibole types of asbestos are strong chains of silica tetrahedra connected by octahedrally coordinated cations, which in crocidolite comprise sodium and iron, in amosite magnesium and iron, in anthophyllite magnesium and in tremolite calcium and magnesium.

Fibers have customarily been defined as possessing an aspect ratio of 3:1, but many minerals cleave into such fragments and a ratio of 20:1 would ensure that most asbestos particles were included (*3*). Counting fibers by optical and electron microscopy gave excellent correlation with fibers > 5 μm long, and most fibers had an aspect ratio > 10:1 (*4*). Whiskers, which are metallic or ceramic filaments of single crystals, represent the synthetic analog of asbestos fibers.

The mining and use of asbestos, especially chrysotile, has increased enormously over the last 60 years. Apart from direct industrial exposure, which includes lagging and shipbreaking, workers near to processing plants may inhale asbestos, while nonindustrial and domestic exposures have occurred from proximity to mines or factories and from dust-impregnated clothing.

Silica

Silica as inhaled by man is usually in the stable, crystalline form of quartz and provides a valuable tool in the analysis of fibrogenesis.

Morphology

The mature silicotic nodule appears as a relatively inactive structure, typically located in relation to respiratory bronchioles. A small nodule usually exhibits three zones, the inner one consisting of hyaline connective tissue with a whorled arrangement, a midzone of concentrically disposed fibers, and a more cellular outer zone of dust-laden macrophages (often bearing carbonaceous material as well as silica and so appearing black) mingled with a loose and irregular network of collagen or reticulin. Particles of silica, as revealed by microincineration, are concentrated in the outer zone. Enlargement of nodules may continue after cessation of exposure. The most characteristic aspect of the classical silicotic nodule is its exuberant and peculiarly disposed fibrosis. Since respiratory bronchioles are thereby compressed, emphysema is not an integral feature.

The evolution of the silicotic nodule is difficult to judge from human material, but experimental evidence suggests that the sequence of events comprises (a) phagocytosis of quartz particles deposited widely over the alveolar surface, (b) aggregation of laden macrophages around respiratory bronchioles, especially the more proximal ones, (c) disintegration of macrophages with liberation of their load, (d) progressive accumulation of phagocytes in the usual location to reingest the particles, (e) the formation of collagen among the phagocytes and (f) hyalinization. These events are not sharply divided, and, because they overlap, the precise connections between them cannot be discerned *in vivo*; to do so requires the parallel deployment of *in vitro* techniques. The deposition and translocation within the lung and lymphatic system of particles, whether highly or poorly siliceous in content, has been considered elsewhere (*5–9*).

Cytotoxicity

Signs of cell damage may be detected by changes in membrane permeability and in metabolism. The former may be recognized by penetration of a dye while the latter is reflected largely by release of cellular enzymes. A variety of cell types has been employed, especially peritoneal or alveolar macrophages primarily derived from rodents or lagomorphs. The use of various agents to increase the yield of cells is to be deprecated since such cells, having already been provoked, may not react qualitatively or quantitatively to particle ingestion as do unstimulated cells. Natural sources of cells have lately been supplemented by permanent phagocytic lines derived from a mouse macrophagelike tumor ($P388D_1$), Chinese hamster lung (V79-4) and a human Type II alveolar epithelial tumor (A549), the intention being to standardize the target. However, variations in response to particles sometimes exhibited by cells from natural sources—possibly according to species, individuals or site of origin—may themselves be important *in vivo*, and, under natural conditions, alveolar Type II epithelium cannot be considered as phagocytic, its function being essentially secretory (*10*). To isolate the membrane effect, the reaction of particles with erythrocytes from different species has been utilized extensively.

Because both the red cell membrane and silica carry net negative surface charges—in erythrocytes probably contributed mainly by sialic acid residues—simple electrostatic attraction may be eliminated. The toxicity of silica has been attributed to hydrogen donation by polymeric silicic acid, forming hydrogen-bonded complexes notably with phospholipids of cell membranes (*11*). The effect of the polymer polyvinylpyridine-N-oxide (PNO) bore out this view, since the compound, first used in experimental pneumoconiosis (*12,13*) and later applied to macrophages in culture (*14,15*), prevented the destructive action of silica. PNO was considered to establish hydrogen bonds preferentially with silicic acid, which could not then react with biological membranes whose integrity was thereby preserved. PNO greatly diminished quartz hemolysis (*16*). Opposed to a silica-lipid interaction at the cell surface, was the view that the reaction occurred between silica and a protein component, which being abstracted weakened the red cell membrane (*17*).

The high affinity of silica, colloidal or particulate, for the positively charged trimethylammonium groups on the membrane surface may be responsible for hemolysis, since this effect was inhibited by tetramethylammonium ions, which were thought to be adsorbed onto silica (*18–20*). Aluminated silica was found to be as toxic as pure silica to erythrocytes with a low sialic acid content

(as in sheep) but much less damaging than silica when the cell membrane possessed a high level of sialic acid (as in the horse), an effect eliminated by neuraminidase. Rather than hydrogen-bonding of PNO and silica, adsorption was believed to depend on hydrophobic and electrostatic interactions. However, while silica and titanium dioxide carry negative charges, only silica is hemolytic, indicating that an additional determinant is concerned. Another view of the mechanism of hemolysis involves adsorption onto the particle of cell constituents such as red cell ghosts or synthetic liposomes of dipalmitoyl lecithin (DPL) (*21*), though the conditions of culture affected hemolysis by both compact and fibrous particles (*22*). The toxicity of particles, especially in medium containing serum, has tentatively been ascribed to changes of membrane potential and cell input resistance in guinea pig alveolar macrophages and $P388D_1$ cells (*23,24*), but the electrical changes could well be a secondary effect of dust contact and ingestion. Peroxidation of membrane lipids is not believed to explain the hemolytic or fibrogenic effects of quartz (*25,26*).

All the chemical or physicochemical reactions considered focus attention on surface phenomenon, and the old solubility theory, possessing too many inconsistencies to be tenable (*27,28*), has been displaced. The surface hydroxyl (silanol) groups are thought to be the adsorption sites, since their destruction at high temperature (converting them into siloxane groups) renders the particles much less active biologically (*29*). Another concept adopts the electron theory of catalysis, whereby electrons are transferred between silica particles and membranes. Measurements of the electron trap structure and configuration of silanol groups have therefore been proposed as indicators of potential cytotoxicity and possibly of fibrogenicity (*29*), but precise correlations are awaited. Surface area as such may not offer a complete explanation since increased particle number per unit weight greatly augmented the hemolytic activity of quartz whereas total surface area was comparatively unimportant (*30*), an aspect also relevant to fibrogenesis.

Damage to membranes is reflected in biochemical changes. Macrophages secrete many active agents (*31*), and those that have been closely examined after ingestion of silica include lysosomal acid hydrolases (such as acid phosphatase and β-glucuronidase) and cytoplasmic lactate dehydrogenase (LDH). Macrophages take particles into phagosomes by invagination of the surface membrane with the formation of phagolysosomes, which at first retain their enzyme complement but soon rupture to release the enzymes into the cytoplasm and then into the extracellular environment along with LDH; diamond and aluminum-coated silica dusts employed as controls induced no such changes in peritoneal macrophages (*32,33*). The action of silica is nonselective (cf. asbestos), while the inhibitory action of aluminum compounds on cytotoxicity probably depends on the substitution of Al ions for Si ions in the SiO_2 lattice. PNO was taken into phagosomes, and, if silica ingestion followed in polymer-free medium, the two compounds came to lie in the same phagolysosome and enzyme leakage was much reduced irrespective of whether animals or macrophages were pretreated (*32, 34*). Ultrastructurally, macrophages reacted similarly to silica *in vitro* or *in vivo* (*35–37*), the smaller dust burden achieved by inhalation of quartz permitting a slower cellular reaction and better visualization of the ensuing toxic effects such as diffusion of acid phosphatase into the cytoplasm. Enzyme release has been applied quantitatively *in vitro* in order to grade the cytotoxicity of particles. Measuring reduction of triphenyltetrazolium chloride (TTC), tridymite and cristobalite proved more toxic than quartz or amorphous silica (*38*), but quartz and cristobalite have been considered equally toxic (*39*). It cannot, however, be assumed that quartz is a standard compound, since the source apparently affects cytotoxicity to peritoneal and alveolar macrophages as well as *in vivo* fibrogenicity, with which quantitative enzyme assays do not always show a close and consistent correspondence (*40–42*). These differences are believed to depend on the presence of amorphous silica, incorporation of foreign ions such as Al, temperature influences during cooling of the magmatite and possibly mode of mechanical disintegration. Toxicity of quartz is much diminished by heating without detectable surface change on infrared spectroscopy. Disparities were also apparent in the protection against silica afforded to cell membranes *in vitro* and against fibrogenicity *in vivo* by certain water-soluble polymers, some compounds protective to cultured cells being feeble inhibitors of collagen synthesis in animals (*43*). Density differences in the tetrahedrally coordinated forms of crystalline silica may affect the accessibility of surface reactive groups.

A simple though rather laborious test of toxicity relies on assessment of cell viability by dye exclusion, with the use of trypan blue or eosin. Dye penetration into the cytoplasm implies pathological permeability of the plasma membrane and along with retraction of processes and damage to organelles presages cell death. It thus offers a conclusive endpoint, and the proportion of dying cells can be estimated microscopically. Silica toxicity is, however, delayed in the presence of serum, which has to be removed by enzymatic degradation after particle incorporation into phagolysosomes. There appear to be few direct comparisons of the value of dye exclusion and enzyme release, bearing in mind that LDH levels in macrophages are affected by elicitation and cultural conditions (*44*).

Fibrogenesis

The degree of pulmonary fibrosis paralleled the quantity of dust administered intratracheally both in regard to quartz (*45*) and to tridymite (*46*). For the typical nodules to develop in man, the lung dust usually had at least 18% quartz (*47*), while in rats definite fibrosis occurred only when the airborne and lung dusts contained 20% or more of quartz (*48*). Inception of experimental fibrosis by dust mixtures containing a

lower proportion of quartz was obtained with clouds of exceptionally high concentration (*42*). In smaller amounts, quartz appears to be sufficiently isolated from contact with cells or their organelles as to preclude its typical pathological changes, though another possibility is considered in discussing coal. The physical form of silica may also affect the pulmonary response. Judged by the speed and degree of fibrosis in rats after intratracheal doses standardized as accurately as possible, the fibrotic reaction was least with amorphous silica and increased via quartz and cristobalite to a maximum with tridymite, despite similar solubilities for all forms (*49*). On the other hand, no differences were found in the peritoneal granuloma masses caused by these varieties of silica or in the responses to PNO (*50*). The high-pressure, high-temperature forms of silica proved peculiar, in that coesite (tetrahedrally coordinated) possessed only a small fraction of the fibrogenic potency of quartz, while stishovite (octahedrally coordinated) behaved as an inert dust (*51,52*). To determine the influence of particle size and surface area, flint, which contains both quartz and cristobalite, was used in a range of sizes (*53*). At constant weight, pulmonary fibrosis developed more rapidly and was more severe as particle size diminished, while at constant surface area the severity and rate of fibrosis were maximal for particles of 1–2 μm. These observations tended to implicate both particle size and surface in fibrogenesis, but surface area has been considered irrelevant (as also in regard to hemolysis), whereas particle size or the amount of silica retained was dominant (*54*). Both these studies, however, suffer from the defects of the tracheal route for injection of silica and the assessment of fibrosis histologically.

Pulmonary fibrosis may be prevented by concurrent exposure to aerosols of quartz and soluble compounds of aluminum, especially the hydroxide, hydroxychloride or chlorhydroxyallantoinate, but resolution of established silicotic lesions proved less tractable, and withdrawal of aluminum prophylaxis led to resumption of quartz fibrosis (*55*). Other materials, notably metallic aluminum and iron compounds, afforded variable protection and the results have been summarized (*56*). Aluminum may be beneficial because it is slowly released in soluble form to react with the silica surface. Long-term prophylaxis of silicosis by inhalation of metallic aluminum dust in gold miners was claimed to be entirely successful, with no ill effects from the aluminum dust itself being observed (*57*), but dust suppression measures applied concurrently could well take the credit. Metallic compounds and PNO thus appear to act on the quartz particles, the polymer protecting macrophages *in vitro* and inhibiting fibrogenesis whether given by injection or inhalation, as Schlipköter and Brockhaus (*12,13*) first demonstrated and as others have confirmed. Concentration on membrane damage, especially to the macrophage, has tended to divert attention from the possibility of other intracellular reactions and from the fibroblast as the collagen producer. The evidence adduced serves to reinforce the suspicion that, because of their temporal relationship, phagocytosis of siliceous particles is connected with the subsequent fibrosis.

The nature of this connection was revealed only when the two processes were allowed to proceed independently, a distinction demanding cell culture (*58,59*). Using a single synthetic medium supplemented to promote both cell survival and hydroxyproline (HOP) formation, peritoneal macrophages from rats were first incubated with quartz particles, surviving cells disintegrated by repeated freeze-thawing and the suspension separated into deposit and supernatant. The latter then replaced the medium of independently grown chick embryo fibroblasts, whose HOP and DNA contents were estimated 2 or 4 days later. A variety of control procedures was run in parallel and several conclusions emerged. The extract from the macrophage–quartz reaction repeatedly led to a highly significant elevation of HOP formation by fibroblasts, whereas particulate quartz or silica dissolved in the medium were without effect when applied directly to fibroblasts. Furthermore, extracts from untreated macrophages had no comparable effect and disintegrated cells did not react with quartz. The latter observation suggested that simply damaging the cell membranes was inadequate to account for the fibrogenic effect of quartz and hence that an essential reaction took place with other cell constituents. Pretreatment of macrophages with PNO abolished the quartz effect, which thus appeared to have two components, an initial attack on membranes and a subsequent intracellular reaction leading to the formation or release of a macrophage fibrogenic factor (MFF). In contrast to the supernatant, the residue from macrophage–quartz cultures inhibited collagen production. Throughout, DNA levels of fibroblasts were unchanged, so indicating that the active agent stimulated functional activity but not proliferation. Titanium dioxide, a white powder of fine dimensions and without fibrogenic capacity *in vivo*, was employed in parallel with quartz but lacked stimulatory action. Inhaled particles are taken up by alveolar macrophages, which differ in several physiological respects from cells of peritoneal origin, but when subjected to the same procedure also produced MFF.

Confirmatory evidence has come from a number of sources, employing the same basic system but adopting technical variations and refinements (*60*), with Kulonen and his colleagues making notable contributions towards characterization of the MFF. They have shown the factor to be a soluble homogeneous, acidic protein of molecular weight 14,300 (*61,62*), against which a neutralizing antiserum could be raised (*63*). The MFF may stabilize fibroblast RNA possibly by suppression of macrophage RNase, though collagen formation may be specifically activated (*63*). The 700 to 5000*g* sediment of normal disintegrated macrophages reacted with quartz to produce the factor (*64*), thereby stressing the intracellular site of reaction, identification of which remains to be established along with final characterization of the MFF for which mass production of macrophages is needed. Quartz, after being taken into phagosomes,

was liberated into the cytoplasm before being enclosed by secondary membranes that could be of Golgi origin and free ribosomes were much increased (*65*); the MFF might be formed in these secondary lysosomes. The target cell for the MFF *in vivo* can hardly be other than the interstitial fibroblast, exposed by silica-induced damage to the Type I alveolar epithelium as revealed ultrastructurally (*66*).

In vitro observations have proceeded sufficiently to enquire how far they bear on the *in vivo* reaction to quartz. Silica-treated macrophages promoted collagen formation *in vivo* (*67*), while rheumatoid synovial extracts reacted with macrophages to form a supernatant which stimulated collagen formation (*64,67*). Extract of mouse liver after tetrachloride necrosis was also active, and the effect may be mediated by Kupffer cells which belong to the mononuclear phagocytic system (*67–69*). Human monocytes and macrophages as well as lines of human histiocytic lymphoma cells and transformed mouse macrophages proved effective targets for generation of the MFF by silica (*70*). The evidence now available thus suggests a wider relevance for the MFF, encouraging the belief that it may be applicable, no doubt with modifications, to the process of fibrosis in general, where a variety of provocative agents may operate. A scheme that covers the known and suspected interactions has been outlined (*60*).

Another approach to the pathogenesis of silicosis deployed diffusion chambers, by which silica within could be separated from cells without after insertion into the peritoneal cavity or subcutaneously. With membranes of pore size 0.3 to 0.5 μm (*71*) or < 0.1 μm (*72*), no reaction occurred around chambers containing quartz or tridymite and the results were opposed to the silica solubility theory of fibrogenesis. However, using four forms of silica, with a Stokes diameter of 0.5 to 2 μm, as well as a dried silica gel of particle size 20 to 30 nm (i.e., less than the pore size of < 0.1 μm), colloidal silicic acid did not escape from the chambers in a concentration or at a rate sufficiently high to have a fibrotic effect (*73*). All five forms of silica were fibrogenic when brought into direct contact with peritoneal tissues. When macrophages and silica were enclosed together and the chambers implanted intraperitoneally, no surrounding fibrosis developed (*74*). The claim of dose-related fibrotic response (*75*) suffered from the fact that the pore size was 0.8 μm, which not only permitted escape of the finer silica particles but also allowed cell–cell contact from either side. A membrane with cylindrical pores of a fairly uniform 0.05 μm diameter prevented direct cell contact (*76*). Quartz particles of respirable size enclosed with mouse peritoneal macrophages induced less fibrosis in the peritoneum of syngeneic animals than cells alone, while with silica on its own the changes were similar to control chambers either empty or medium-filled. This failure to excite fibrosis with a macrophage–silica combination again reflects the inadequacy of the diffusion chamber technique to elucidate the sequence of *in vivo* reactions to silica, since the finite number of cells was soon eliminated, and in the absence of recruitment no further interaction was possible.

Lipid Participation

Inhaled silica reacts not only with alveolar macrophages but also with alveolar epithelium, especially the Type II cell to which the weight of evidence ascribes the production of lipids notably dipalmitoyl lecithin (DPL) via the osmiophilic lamellar bodies. Although lipid accumulation has been recognized as a component of the silicotic response, its significance remained obscure. Specific pathogen-free rats exposed to quartz inhalation developed widespread alveolar consolidation by amorphous material with hyperplasia and enzyme hyperactivity of Type II cells, macrophages gradually disappearing though quartz particles remained in the alveolar material (*77*). Electron microscopy showed the latter to contain quadratic lattices and lamellae, typical of phospholipid in the liquid–crystalline phase, along with extruded lamellar bodies that were often fragmented (*66*). In these respects, the experimental disease closely resembled the human counterpart, to both of which the term lipoproteinosis should be applied so as to stress the essentially lipid nature of the reaction. Protein constitutes only a minor and possibly incidental element, while its binding to lipid appears to be artifactual (*78*). Biochemically, phospholipids were much more affected than neutral lipids, with DPL showing the maximum elevation. Metabolic studies with labelled palmitate in the early stages of the disorder established that, although the rate of DPL decay was raised, its rate of production was greater, so that the net effect was a steady accumulation in the alveoli (*79,80*). In consequence of the paucity of macrophages and the dispersion of quartz particles in the alveolar lipid, the macrophage–quartz reaction was prevented and fibrotic nodules failed to develop, while the stimulus to Type II cells subsided and the consolidation remained, possibly aided by loss of phospholipase activation in macrophages by quartz (*81*). The biophysical features of rat lungs consolidated by lipo-proteinosis were consistent with the accumulation of surface-active agents (*82*) as was also the case after brief exposure to quartz inhalation (*83*). The rate at which quartz particles were deposited in the alveoli appeared to determine whether fibrosis or lipidosis predominated; prolonged exposure and low concentration favored fibrosis, while relatively short exposure to high concentration encouraged lipidosis (*59*). The latter situation obtained in men engaged in sandblasting (an occupation proscribed in Britain), the response to which comprises a mixture of atypical fibrosis combined with lipidosis in the adjacent parenchyma.

Silica toxicity would soon deplete the normal complement of alveolar macrophages and abolish generation of the MFF, so a mechanism should exist to ensure their continuing availability. As a component of the mononuclear phagocytic system, macrophages are ultimately derived

from the marrow, though under normal conditions *in vitro* evidence suggested that division of precursors pausing interstitially served to replace macrophages lost by disintegration or bronchial excretion (*84,85*). However, cytodynamic observations after inhalation of quartz by mice afforded no indication of cell proliferation in relation to dust aggregates, a phenomenon explicable by rapid emigration of monocytes into the alveoli (*86*). Since local proliferation did not offer a satisfactory basis for macrophage maintenance in the presence of an irritant, the alternative was systematic recruitment. Functional stimulation of the mononuclear phagocytic system for long periods followed intraperitoneal and intratracheal administration of silica, and lipids also elevated the phagocytic index (*87*). Endogenous lipid, derived from the lungs of rats with alveolar lipoproteinosis and evidently contributed by Type II cells and alveolar macrophages, was therefore utilized parenterally in other rats and the cell kinetics of the marrow mononuclear series studied (*88*). The duration of DNA synthesis and the cell-cycle time of promonocytes was reduced and their rate of entry into DNA synthesis increased in lipid-treated as compared with untreated rats. Accordingly it may be proposed that a powerful pulmonary irritant such as quartz, in stimulating local lipid formation, provides a positive feedback to the marrow and leads to proliferation of the monocytic series. It is thus possible to comprehend how the population of alveolar macrophages is maintained at a level much in excess of normal requirements and also how the feedback of lipid may subside as quartz particles increasingly become isolated in the alveolar material from contact with the Type II cells. The means by which monocytes travel from capillaries to the sites of need within alveoli has yet to be established with finality, but inferential evidence suggests that chemotactants and augmented vascular permeability could well be concerned (*60*). A role has been suggested for the products, especially lipids, of macrophage breakdown under the influence of quartz in the recruitment of phagocytes, predominantly neutrophils (*89*). Such a view overlooks the involvement of lipid feedback from Type II cell products, and they could have been the active agents, since lung macrophages ingest surfactant.

Coal

Although coal worker's pneumoconiosis (CWP) and silicosis are embraced by the term pneumoconiosis, they are distinct entities and should not be confused by applying the designation of silicosis or anthracosilicosis to the disease in coal workers.

Morphology

Since the collection of inhaled compact particles in the lung follows a similar pattern irrespective of the nature of the dust, the simple lesion produced by coal is situated at the same site as the silicotic nodule, that is in relation to respiratory bronchioles especially those of first and second orders. Alveoli opening into or abutting on respiratory bronchioles become consolidated by tightly packed masses of coal-laden macrophages, among which reticulin fibers are gradually laid down and in some cases collagen also forms perhaps with a few fibroblasts. The respiratory bronchioles are transformed into more or less smooth tubes encased in a sheath of dust-consolidated parenchyma. In many, though not all, cases these same bronchioles undergo dilatation of varying degree, so giving rise to proximal acinar emphysema (*90–92*). Because of their location in relation to the apex of the lung acinus, the aggregates of coal are customarily seen as discrete lesions of which there may be up to five, measuring up to 5 or 6 mm across, in a secondary lobule and which in transverse section possess a stellate appearance often with enlarged air spaces in and around them. The emphysema is sometimes sufficiently severe that neighboring lesions coalesce and the originally circumscribed form of the dust aggregates may be obscured. In contrast to the silicotic nodule, the simple dust lesion of coal workers exhibits a preponderance of dust over connective tissue, which is irregularly disposed, together with the occurrence in some cases of a particular form of emphysema. There is no suggestion of bronchiolar stenosis, though sometimes the lesions are more cellular and the coal-bearing phagocytes less densely packed.

Cytotoxicity

The ingestion by guinea pig alveolar macrophages of inhaled coal dust containing 2% quartz led to the formation of phagosomes, but the cell structure was for the most part well preserved and cell death and debris were rarely seen (*93*), while coal dust taken up *in vitro* had little effect on dehydrogenase activity (*38*). However, extensive epidemiological investigations pursued for many years at British and German collieries have raised issues concerning the pathogenicity of airborne dusts from coal mines of different rank. The initial suggestion (*94*) that exposure to high rank (anthracite) dusts resulted in a higher prevalence of pneumoconiosis than dusts of lower rank was subsequently explained on the basis of the mass concentration of respirable dust (*95*) and the cumulative effect of dust exposure, although other factors may be concerned in individual mines (*96*). Nevertheless, disparities have been observed between the attack rate or incidence of pneumoconiosis and the mineral content, especially quartz, of the airborne dust (*97–99*), and high progression was sometimes apparently associated with low dust concentration or vice versa (*100*). This aspect has recently been emphasized by estimating the probability of developing radiological category 2 or higher simple pneumoconiosis over a working life (*101*). The results showed wide and unexplained colliery-associated variations which were not explicable on the basis of quartz content of the respirable dust in estimated cumulated exposures having

an average quartz level of 5% and rarely exceeding 10% of the mixed dust. Only when the level of quartz exposure was higher were unusual radiological changes observed, as might be expected from Nagelschmidt's (*47*) correlation of lung pathology and mineral content.

The preoccupation with the role of quartz in the genesis of CWP nevertheless continues in studies of cell toxicity as well as of fibrogenesis. The noxiousness of respirable coal mine dusts, as judged by the TTC test could not, however, be correlated merely with their quartz or mineral content (*99,102,103*). Study of the surface properties of quartz in coal mine dusts by scanning Auger spectroscopy and thermoluminesce suggested that, though the quartz surface may be contaminated, areas with the electronic structure of silica remained (*104*). However, the method of air sampling may influence the estimate of mineral in the dust (*105*) and the issue of mine dust toxicity cannot be regarded as settled, despite experiments showing inhibition of quartz toxicity by anthracite (*106*). Using the P388D_1 cell line (*107*) toxicity was not defined solely by the quartz content of the dusts, some being less harmful than the titanium dioxide control. For high rank dusts, the kaolin and mica contents related better to toxicity than quartz, while for low ranks these mineral constituents were not so related, and the toxicity varied widely with similar quartz contents; the possibility of lipid interference *in vivo* was not considered. Hemolysis by dust of low rank mines did not correlate with its total or individual components, while lysis by dust from high rank pits increased with the amount of noncoal mineral and its quartz but not kaolin or mica concentrations (*107*). Hemolysis and toxicity were thus poorly correlated and dust hazards could not be assessed on the basis of one test system applied at different times. Although noncoal mineral content appeared important in determining toxicity, the role of quartz was obscure, while the relationship between mineral content and toxicity varied markedly between high and low rank collieries. Another approach (*108*) relied on *in vitro* assessment of L cell growth, a leachate of respirable particles from a high prevalence mines depressing and that from a low prevalence mine stimulating cell growth; total protein is not, however, adequate to determine cell proliferation, for which DNA measurement is required, and the important criterion is HOP formation.

Fibrogenesis

The structure of the typical coal dust lesion differs so strikingly from that of the silicotic nodule as to suggest an alternative or much modified mechanism for the genesis of the comparatively minor amount of connective tissue. The danger from an inhaled dust has customarily been held to depend on its capacity to induce pulmonary fibrosis. Since this feature is pronounced after exposure to silica, pneumoconiosis developing in coal workers was generally attributed to the siliceous element in the mixed mine dust. However, the proportion of quartz in respirable coal mine dust is small, sometimes less than 1% (*1*), while the quartz content of lung dust in simple CWP averaged 2.2% in one series (*109*) though in another it was a little higher, even though the mean amount of quartz in the lungs was only about 0.2 g (*110*). Certain cases apparently had a quartz content high enough to produce lesions of silicotic type (*110*), yet characteristic nodules did not occur, but the amount of quartz in the lung dust might be very small (*111*). Some lesions show a greater cellularity and a looser arrangement of dust-bearing phagocytes but the mineral content of individual lesions is not known. Previous experience had established the essential microanatomical and histological similarity of the simple dust lesions developing in anthracite, steam or bituminous coal workers from Britain, especially South Wales, or the U.S.A. (*112*). Four decades ago Gough (*113*) demonstrated that coal trimmers, now replaced by mechanical loading, inhaled coal dust virtually uncontaminated by minerals from the rock strata, yet they developed a disease identical with that of coal workers who were exposed to the mixed dust. Not only may coal workers develop simple pneumoconiosis in the presence of very little or possibly no quartz, but indistinguishable lesions (color apart) occur in hematite workers, and their lungs contained about 1.5 g quartz on average though in one none was detected (*114*). Furthermore, lesions resembling those in coal workers have been found in carbon electrode (*115*) and carbon black (*116*) workers, whose lung dust was almost entirely devoid of quartz, while the same pathological changes affected a graphite worker from whose lung no quartz was recovered (*117*). Exposure to nepheline dust, a feldspar composed of silicates but containing no free silica, led to a pneumoconiosis resembling that of coal workers in all but color (*118*). These pathological and chemical studies on human material combine to minimize and even eliminate a necessary role for quartz in the genesis of simple CWP, though prolonged residence in the lung may permit some solution of quartz and lymphatic removal from established lesions cannot be eliminated. The mineral component of airborne dust from coal mines may nevertheless participate in the pathological response, but its effect appears neither dominant nor specific.

The evolution of the coal dust lesion evidently proceeds in several stages (*10,90,112*). Laden macrophages congregate in alveoli at the apex of the acinus and as they or their contents make contact with the attenuated Type I epithelium it may become disorganized and expose the interstitium. The reticulin fibers then lie adjacent to dust cells, which thus appear to be interstitial as the epithelium reforms over them, though some new reticulin may form. The sequence is repeated until whole alveoli are overrun and a cylinder of consolidated parenchyma encloses the respiratory bronchioles. A dual effect may then follow in some lesions, diminished recoil on expiration and augmented traction on inspiration, which together afford a mechanical

explanation for the development of dust-related, proximal acinar emphysema (*119*). Lesions exhibiting the emphysematous change generally show loss of smooth muscle from the respiratory bronchioles, presumably the consequence of incarceration and atrophy, so that expiratory narrowing and shortening are reduced or abolished, but where smooth muscle persists respiratory bronchioles remain more or less normal in size. Consolidation exaggerates the inspiratory traction on these airways by adding to the normal force that which was expended on the consolidated alveoli. The characteristic emphysema can hardly be attributed to elastic tissue loss, whether by elastase digestion or other means, since irrespective of the occurrence of emphysema elastic tissue within the dust aggregates is fragmented. It does not seem reasonable to invoke local deficiency of α_1 antiprotease, since until the late stages there is no disruption of alveolar walls, the spaces simply being occupied by dust cells and connective tissue. Loss of surfactant secreting Type II cells might assist in condensing the dust lesions. The simple dust lesion of coal workers and others exposed to a similar hazard may best be interpreted as a nonspecific reaction to excessive accumulation of dust which becomes immobilized by a minimal degree of fibrosis.

Experimental studies on the origin of the connective tissue in coal dust lesions have nevertheless continued to concentrate on the quartz component. Prolonged exposure of rats to a high concentration of 10% quartz mixed with coal-mine dust led to more collagen formation in the lungs than coal-mine dust alone (*120*). Coal–quartz (4%–30% range) mixtures again inhaled by rats in abnormally high concentrations led to a proportionate increase in collagen production, the mixture with the lowest concentration of quartz behaving almost as quartz-free coal dust and the higher concentration inducing silicotic type lesions (*42*), as might have been expected. Natural dusts from coal mines containing 5% or 15% quartz were no more fibrogenic than coal largely free from quartz, but artificial mixtures of this latter coal with the same proportions of quartz were fibrogenic, and it was concluded that under natural conditions inhibitory substances, possibly aluminum compounds, combined with the surface of quartz particles (*121*), but ineffective mixing may also have been responsible for the difference. The behavior of coal mine dusts has been assessed in the regional lymph nodes after IP injection and a two-phase hypothesis proposed (*122*). Penetration to the nodes was considered to be determined by cytotoxicity but unrelated to quartz content and unaffected by PNO, whereas fibrogenecity did depend on quartz content and was inhibited by PNO. If, however, penetration of quartz is determined by other constituent minerals whose concentration varies independently, the proportion of quartz in the primary and secondary locations could differ so that the nodal response may not correspond closely with that in the primary site, as some findings (*123*) tend to indicate. It seems peculiar that in nodes "quartz typical" areas of fibrosis should occur separately from the remainder of the dust reaction. Further analyses are intended to coordinate the human and experimental aspects of the responses to dusts (*105*).

PNO reduced the fibrogenic response to natural mine dusts, dusts extracted from colliers' lungs or artificial mixtures of coal and quartz given intraperitoneally or intratracheally (*124*), but long-term treatment of rats and rhesus monkeys inhaling coal–quartz (40%) mixtures showed in most experiments no substantial therapeutic benefit, nor was any reliable effect obtained prophylactically (*125,126*). These findings contrast with those in experimental silicosis, despite the high quartz content of the dusts used, and offer little prospect of human application. Aerosols of soluble aluminum compounds exerted a prophylactic and a therapeutic action on rats exposed to coal–quartz (17%) mixtures in high concentration, but the curative effect diminished as the silica content was lowered (*55*). The beneficial action of coal in mixtures was attributed to coating of the quartz surface by soluble substance(s) derived from the mineral matter in coal. On substituting titanium dioxide for coal there was no inhibition of the harmful effect of quartz, a finding which was held to exclude a dilution effect (*127*), but electron microscopically titanium dioxide particles exist as large aggregates that are resistant to disaggregation by sonication and which are thus unable to isolate admixed quartz particles from cellular contact (*128*). In any case, contamination of quartz surfaces in natural mine dusts was probably incomplete (*104*). Rabbits exposed underground in a Welsh steam coal mine failed to develop fibrosis in the focal aggregates of dust, while rats exposed to airborne dusts from British coal mines of widely differing ranks also failed to acquire fibrous lesions (*129,130*). The noncoal minerals of mine dust or the products of cell break down under their load may react with macrophages to produce the MFF in low concentration. The application of respirable airborne dust from coal mines and artificial coal–quartz mixtures to the macrophage–fibroblast test system for collagen formation is being made, and the prospect of replacing the indirect cytotoxicity test with a direct fibrogenicity assessment merits exploration.

Lipid Involvement

Comparatively little is known about the interaction of coal mine dust with Type II cells. The levels of total phospholipid and lecithin were raised in lung washings from rats after inhalation of carbon dust (*131*). Although biochemical studies comparable to those on silica are lacking, a limited degree of phospholipid accumulation in alveoli was observed electron microscopically after mine dust inhalation by rats (*66*), and the degree of alveolar lipo-proteinosis varied with different ranks of coal (*129,130*). By interference with the macrophage–particle reaction, lipid accumulation could have a bearing

on the apparent anomalies in prevalence of CWP at different mines; it is a factor of which no account has been taken.

Feedback of lipid from lung to marrow may again regulate macrophage recruitment and, although an excess of these cells is required for disposal of coal dust, their loss is less evident than after uptake of silica and a correspondingly lower level of lipid activity might be expected.

Asbestos

Although asbestosis was recognized as long ago as 1907 (*132*) and descriptions of the human disease followed (*133,134*), the magnitude of the risk from asbestos exposure only became apparent in 1930 (*135*), since when the health hazard has loomed large (*136*).

Penetration of fibers into the respiratory tract constitutes a complex problem (*137–139*). Fibers up to 200 μm may be respirable provided the diameter does not exceed 3 μm. Among mechanisms concerned in fiber deposition, sedimentation and interception probably cause fibers to alight more proximally in the acinus than compact particles. Chrysotile fibers, being curved, present a larger collision area and tend to penetrate less deeply than the straight amphiboles.

Morphology

Compared with the simple dust lesions of silicosis and CWP, fiber aggregation and asbestotic fibrosis are less well defined. Though initially affecting alveoli around respiratory bronchioles in a fairly circumscribed manner, parenchymal extension gradually leads to fibrous areas adjacent to or enclosing nonfibrosed lung tissue which commonly dilates to form cystic spaces. This honeycomb pattern is a characteristic feature but the lung is irregularly affected and large tracts are often spared. In many cases the distribution of the fibrocystic change is basal or posterior and sometimes the subpleural zone is involved. Occasionally the fibrosis assumes a solid character. Fibers lie both in fibrosed lung and in alveoli, a minority acquiring a ferroprotein coating to become asbestos bodies which are often particularly evident in alveoli. It is generally considered that coated fibers are no longer harmful (*140*). The formation of asbestos bodies evidently depends on the activity of macrophages, which probably leave small accretions of cytoplasm on fibers they have failed to ingest completely; shrinkage of a succession of such deposits is likely to give the body its beaded appearance though an evenly formed coating could later fragment.

The uneven distribution of asbestotic fibrosis makes its quantitation rather imprecise and the choice of particular areas of lung for routine histological study is unrealistic. Short of strictly morphometric methods, the severity of lung involvement may be graded on a simple visual four-point scale. Fibrosis showed an overall correlation with the content of particles, both coated and uncoated fiber numbers rising as the extent of the fibrosis increased from nil, through mild, to moderate grades (*141*). Uncoated fibers formed about 70% of those seen optically and comprised 12 to 30% of the total count determined electron microscopically. The situation differed in cases with severe asbestosis, where no relationship was evident between either the concentration or the proportion of uncoated fibers and the form the lesions took, whether in areas of zero, focal, cystic or solid fibrosis. The time the mineral had resided in the lung was also unrelated to the severity of the asbestosis. There was a striking paucity of chrysotile in the extracted dust. These variable features of the pulmonary response, including the presence of fibers in nonfibrosed lung, raised the question of complicating factors such as the development of chronic inflammatory states as a consequence of the presence of particles or the fibrosis to which they gave rise; tuberculosis is now an uncommon occurrence in asbestosis. Although retention in the peripheral lower lobes of the lung has been stressed (*142*), no attempt was made to relate fiber concentration to the type and degree of pathological change. Particles of serpentine or amphibole in lungs of individuals with asbestosis or mesothelioma were mostly < 5 μm long and < 0.5 μm diameter, chrysotile having the shortest and finest fibrils and amosite the longest and widest with crocidolite occupying an intermediate position, but in all cases the majority of fibers had an aspect ratio over 10:1 (*143*). In individual lung specimens from Finland anthophyllite fibers had a mean length generally < 10 μm and a diameter < 0.6 μm (*138*).

Cytotoxicity

Chrysotile like silica proved strongly hemolytic but the amphiboles were relatively inactive (*16,144*). The magnesium content of the fiber bore a linear relationship with the degree of lysis and since the silica content was more or less constant its contribution to hemolysis could be eliminated (*16*). Magnesium hydroxide proved highly lytic and removal of the brucite outer layer of chrysotile by acid leaching reduced its activity (*145*). Silica and glass fiber do not, however, require magnesium for hemolysis. As with silica, the lytic potential was not related directly to the surface area of asbestos minerals. Chrysotile hemolysis could be prevented by sodium ethylenediaminetetraacetate (EDTA), which is a nonspecific chelator of metal ions, and was even more effective against brucite. The sialic component of membranes imparts a negative charge to which the positively charged chrysotile is attracted. Removal of sialic acid by neuraminidase increased erythrocyte resistance to lysis by chrysotile, while prior addition of sialic acid to the fiber had a similar effect. PNO, on the other hand, exerted little protective effect on hemolysis by chrysotile, there being no possibility of hydrogen bond-

ing as suggested for silica. The hemolytic activity of amphiboles was enhanced in the presence of serum, but chrysotile and silica were unaffected (*146*), suggesting that *in vitro* and *in vivo* effects may not be comparable. On the other hand, adsorption of negatively charged albumin onto chrysotile inhibited lysis, though as might be expected it was without effect on silica (*22*).

Measurement of zeta potential showed that serpentine and amphibole asbestos had surface charges of the same magnitude but opposite polarity; leaching reduced this difference of potential, so that hemolysis by chrysotile diminished while that by crocidolite increased, thereby reconciling theoretically the short-term (*in vitro*) differences and the long-term (*in vivo*) similarities of the classes of asbestos (*147–149*). Contact with the alveolar lining layer occurs as soon as fibers are deposited. After treatment with DPL the hemolytic activity of both groups of asbestos but especially the amphiboles was diminished in proportion to the zeta potential, thus suggesting a difference between *in vitro* and *in vivo* behavior. In other hands, adsorption of DPL liposomes and red cell membranes onto chrysotile decreased its surface charge and diminished hemolysis, but the weakly lytic amphiboles did not have the same pattern of reaction with phospholipids (*21,150,151*). Chrysotile hemolysis has been attributed to clustering of membrane sialoglycoproteins, so as to leave channels for osmotic attraction of ions but not large molecules (*16,152*). However, removal of sialic acid from erythrocytes did not effect lysis by chrysotile (*153*) and the likely mechanism is electrostatic attraction and phospholipid extraction (*150,151*).

Relying on loss of peritoneal cells or dye exclusion, amosite and crocidolite proved much less harmful than chrysotile, which was as toxic as amorphous silica, while long fibers were considered to be more deleterious than short ones (*41,154,155*). Toxicity to stable cell lines as well as to peritoneal macrophages was maximal for fibers $\geq$ 10 μm long and $\leq$ 1.4 μm diameter (*156–158*); other reports confirmed the importance of fiber length (*159–161*).

The biochemical consequences of asbestos ingestion by macrophages vary with fiber type, acid phosphatase being released into the medium on treatment with chrysotile (or silica), but not with crocidolite, amosite or rutile (a crystalline form of titanium dioxide) (*162*). Chrysotile, in contrast to silica, permitted the selective escape of lysosomal enzymes in a dose-dependent manner, although the total level in cells and medium was unchanged; nonlysosomal LDH remained intracellular but its level rose in parallel with the lower range of chrysotile dosage (*163*). The distinction between lysosomal and cytoplasmic enzyme release is not however absolute, since chrysotile caused a small escape of LDH, while amosite, glass fiber and fibrous Dawsonite released similar amounts of LDH and β-galactosidase, and crocidolite reversed the ratio of the two enzymes (*21,156*). Removal of the magnesium hydroxide layer from the surface of chrysotile fibers altered the *in vitro* biochemical consequences inconsistently, toxicity being reported as augmented (*21,164*), dependent on the proportion of magnesium removed (*145*) or depressed (*165*). A nonhomogeneous loss of magnesium from chrysotile was found in fibers recovered from human lung or from alveolar macrophages after ingestion *in vitro* (*166*). PNO did not affect the ultrastructural response of alveolar macrophages to chrysotile (*167*), nor the cytotoxicity of serpentine or amphibole asbestos (*155*), but the toxicity caused by exposure of the silicate surface of chrysotile was suppressed (*164*). Membrane interactions with asbestos fibers not only differ from those with quartz but also vary between the two main groups and the molecular basis has yet to be established.

Fibrogenicity

Epidemiological evidence implicates all forms of asbestos in the genesis of pulmonary fibrosis in man, but mixed exposures are common. Progression of asbestosis sometimes occurred after cessation of exposure to chrysotile, and radiological evidence of disease occasionally appeared first many years after leaving the mine (*168*). All the main types of UICC asbestos samples induced pulmonary fibrosis by inhalation in rats, but significant differences were evident in the severity of response; in sacrificed animals, fibrosis was most pronounced with Canadian chrysotile and anthophyllite, Rhodesian chrysotile and crocidolite being less active, and amosite the least fibrogenic, whereas in survivors only the difference for amosite persisted (*169*). Progression continued after removal from exposure. These results might have been influenced by the length distributions of the UICC fibers, especially amosite, since reference samples were subject to some variation (*170*). Rhodesian chrysotile proved much more fibrogenic to rats than amosite or crocidolite despite much greater retention of the two amphiboles, and these relationships were the same in respect of the mass and the number of fibers in each type (*171*). While these findings suggested that chrysotile was distinctly more fibrogenic than amphiboles, the proportion of long chrysotile fibers considerably exceeded that of crocidolite or amosite.

In rats and guinea pigs exposed to amosite clouds of equal mass concentration, much more fiber was deposited when the length was $<$ 11 μm than when $>$ 11 μm, but there was no preferential deposition between individual lobes of the lungs. The subsequent pulmonary reaction proved to be mineral with short fibers but prominent with long ones (*172*). It may be suggested that after ingestion short fibers were removed by proximal migration of alveolar macrophages, but long ones could not be so eliminated and remained in the vicinity of respiratory bronchioles to induce fibrosis. IP administration also emphasized the importance of fiber length in the genesis of asbestotic fibrosis (*173*). Using mouse pleura as the target for chrysotile, long fibers led to the formation of much larger granulomata than did short ones (*174*) and similar results were obtained with glass

fiber given intrapleurally and intraperitoneally to rodents (*175*). It is worth recalling that attention had been drawn to the importance of fiber length in asbestosis by Gardner in 1938 (*176*) and Vorwald et al. in 1951 (*140*). Fibrogenicity by asbestos and man-made mineral fiber thus appears to depend to a large extent on fiber shape, but the surface properties of chrysotile with its peculiar outer layer may contribute an additional physicochemical component, and it would be useful to know whether removal of magnesium affects its fibrogenesis by inhalation. The current concern with long fibers overlooks a possible role for the short ones. Since most fibers recovered from the lungs of exposed humans are < 5 μm long (*142,143*) macrophages are capable of ingesting them completely and of assisting their translocation, while toxic effects will be low. Small amounts of short fibers may be cleared via the ciliary escalator, but larger quantities may well accumulate in alveoli related to respiratory bronchioles and there initiate a low grade progressive reaction. The manner of accumulation then resembles that of coal particles, though the fibrogenic potential of fibers may be greater. It is, therefore, unjustifiable to relegate short fibers to an insignificant role in the genesis of asbestotic fibrosis.

The proximate mechanism of fibrosis by mineral fibers has yet to be established. Mechanical disturbance of the macrophage cytoskeleton, nuclear or cytoplasmic, by long and partially ingested fibers, especially during cellular movement, cannot be discounted. Surface properties appear to play a part in the response to chrysotile, but the availability of reactive groups on the surfaces of amphiboles in their natural state is not clear. Cytoplasmic enzyme release seems an adequate basis for fibrosis, since extracts of disintegrated normal macrophages had little or no effect on fibroblasts in the *in vitro* system (*58*). Too exclusive a concentration on macrophages detracts from the significance of fibroblasts and two further mechanisms merit consideration. The comparatively low toxicity of asbestos to macrophages permits longer contact with cell structures than does silica and the fibrogenic response is less prominent. The formation of a MFF might occur at a slower rate and in lower concentration; recent evidence supports this supposition in that chrysotile proved as potent as silica (*61*) and short fibers of amosite were particularly active (*177*). An alternative explanation invokes the phenomenon of anchorage dependence. Fibroblasts in suspension culture achieved a maximum growth on long glass fibers but failed to grow on fibers ≤ 20 μm long (*178*) and it appeared that linear extension offered a stimulus for division of anchored fibroblasts (*179*). To establish the anchorage effect demands the application of cell kinetic techniques to measure fibroblasts proliferation along with the estimation of collagen formation, but macrophage participation is not excluded. Deployment of the diffusion chamber technique, in which UICC chrysotile and peritoneal macrophages were implanted IP, demonstrated greater surrounding fibrosis than with macrophages alone, the response being maximal at 2 to 4 weeks and subsiding to control levels at 4 months (*76*). The initial fibrosis presumably reflected the lesser toxicity of asbestos, which thus had longer to react with the cells than in the case of silica. These results not only stress macrophage involvement in asbestos fibrogenesis and the formation of a diffusible factor, but also re-emphasize the necessity for macrophage recruitment to sustain the response.

Lipid Participation

Lung extracts from rats inhaling chrysotile showed changes in surface properties (*83*) and, as with silica (*66,80*), hyperplasia of Type II alveolar epithelial cells occurred. In these respects, compact and fibrous particles evidently correspond qualitatively, though differing quantitatively, and fiber isolation from cells may be a consequence; amphiboles require further analysis.

Carcinogenicity

Although there is no reliable evidence of increased prevalence of pulmonary carcinoma in men with silicosis (*180*) or CWP (*181,182*), exposure to asbestos carries a peculiar liability as well as to mesothelioma of the pleura and peritoneum. The subject is vast and epidemiological aspects are covered in other accounts (*183,184*).

All types of asbestos fiber are potentially carcinogenic and the combination of cigarette smoking and asbestos exposure exerts a multiplicative effect (*185, 186*). Amphiboles and chrysotile were capable of inducing lung tumors, many malignant, in rats by inhalation exposure, but few mesotheliomata were encountered in each group; chrysotile carried the highest carcinogenicity (*169,171*). As with hemolytic activity and cytotoxicity, magnesium depletion reduced the capacity of chrysotile, given intrapleurally, to induce mesotheliomata (*145*), but the role of magnesium cannot be considered crucial in view of the carcinogenic ability of crocidolite. Asbestosis and the development of lung tumors showed a positive correlation both in man (*187*) and in animals (*169*).

Asbestos fibers retain their carcinogenicity after removal of naturally associated hydrocarbons, and there is no evidence to suggest that carcinogenic metals such as nickel, cobalt or chromium, contaminating asbestos are, in the amounts present, responsible. *In vitro* adsorbed polycyclic aromatic hydrocarbons may nevertheless be conveyed to microsomes or lipid vesicles (*188*). The composition and structure of the asbestos types are so varied that some other characteristic evidently determines the potential for neoplasia, and the most important factor so far identified is that of fiber dimensions, as also obtained in regard to cytotoxicity and to fibrogenicity. The main evidence in regard to mesothelioma derives from application to the pleura of a wide range of fibrous minerals including UICC asbestos, fibrous glass, aluminum oxide and other man-made substances (*189–192*). Serpentine and amphibole asbestos yielded high incidences of pleural mesothelioma, as

did fibrous glass and aluminum oxide whiskers, and it was concluded that the major factor in both carcinogenesis and fibrogenesis was a durable fibrous shape in a narrow size range. Fibers ≤ 1.5 μm diameter (optimum < 0.25 μm) and ≥ 8 μm long possessed the highest probability of tumor production. Since implantation and injection introduce particles by unnatural routes, confirmation is required from inhalation experiments that employ fibers graded by length and with a diameter optimal for penetration to subpleural alveoli. The longer ones and those with a greater collision area may concentrate in the larger airways and lead to bronchogenic carcinoma, while slimmer and shorter ones may reach the pleura and institute a mesothelial reaction. The mass of fiber necessary for carcinogenesis remains a problem, the uncoated fiber content of the lung seeming to be less significant in the genesis of neoplasia than of fibrosis (*141,193*). Mesothelioma may indeed ensue from brief exposure to asbestos, neighborhood or domestic, and after a long latent period.

Comment

Attention has been confined to the primary noxiousness of mineral particles, but secondary factors may cooperate and aggravate the *in vivo* responses. Complicated pneumoconiosis of coal workers and of silicotics sometimes exhibits a tuberculosis component, though nonspecific viral and bacterial infection may participate. Immunological consequences occur in some though not all cases, though a genetic factor is not now believed to affect individual susceptibility to pneumoconiosis.

Differences are apparent in the primary responses, human and animal, to silica, coal and asbestos. *In vitro* reactions between particles and cells are intended to determine whether short-term behavior reliably forecasts the long-term consequences *in vivo*. Disparities have emerged between hemolytic and cytotoxic assays in regard to a range of minerals, both compact and fibrous (*107,144,194,195*). Despite claims to the contrary (*196*), a poor correlation exists between lytic ability and fibrogenicity, especially in respect of mixed dusts. Hemolysis and enzyme release from macrophages reflect membrane damage, but cytoplasmic enzymatic activity appears unable to account for fibrosis (*58*). The correlation between cytotoxicity and fibrogenic response is likewise imprecise, notably with particles exerting a moderate effect *in vivo*, where prediction would be particularly valuable. Cells of natural origin may react differently to some of the stable cell lines, in which for instance quartz induced no damage though all UICC samples of asbestos were toxic (*197*); such lines thus appear to be of dubious value in regard to fibrogenicity. Toxicity is probably best indicated by cell death, which can be quantified, but toxic dusts are not always fibrogenic.

Vital reactions to inhaled particles are not confined to macrophages, but include the alveolar epithelium. Stimulation of Type II cells leads to lipidosis, which may vary in severity according to the particles' nature and may well modify fibrogenesis by isolating them from cells. Furthermore, macrophage recruitment constitutes a systemic feature of the *in vivo* response (*88*), which cannot be duplicated *in vitro* and may account for divergencies, while in culture particles cannot be eliminated.

Fibrous particles known to be carcinogenic by intrapleural application or injection are also toxic to stable cell lines (*161,192,197*), though toxicity is exhibited by particles which are only fibrogenic. Fiber length clearly plays a role in both toxicity and carcinogenicity, but again the *in vitro-in vivo* analogy may be inappropriate, since under natural conditions fibers reach the lung and pleura by other than parenteral means and in much smaller quantity, as well as making immediate contact with the alveolar lining layer. Certain rodents tend to develop neoplasia after parenteral introduction of solid particles and other objects, so that care is needed in applying the results of such studies to human disease. Bronchogenic carcinoma arises from epithelium not the mononuclear phagocytic system and a prerequisite in respect of asbestos appears to be the development of fibrosis. Mesothelioma commonly has a prominent mesenchymal component and similar considerations may apply, although mesothelium could react independently since it is both phagocytic and releases a fibrogenic factor.

On present evidence it therefore seems prudent to reserve judgment on the validity of short-term *in vitro* assessment as a means of identifying particles which may prove deleterious to man by inhalation.

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