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# Current Status Review
# The role of surfactant in the pulmonary reaction to mineral particles

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A prime objective of investigations into the behaviour of the lung towards inhaled mineral particles concerns their fibrogenic potential. Observations on cellular and biochemical constituents of lavage fluid after administration of dusts, whether compact or fibrous, are often employed as indicators of subsequent fibrogenic capacity, without however establishing a direct connection. Moreover, some components of the pulmonary response may operate in the reverse direction and inhibit fibrosis. Such interference is best seen after intense exposure to quartz when lipid accumulation in alveoli alters the pattern and amount of fibrosis. Changes of a similar nature may follow inhalation of fibres or coal mine dusts, but lipidosis is less pronounced though its effects should not on that account be underestimated.

Particles reaching the alveoli come immediately into contact with the lining layer and rapidly thereafter with macrophages and epithelium. In this connection alveolar macrophages serve a dual purpose, aiming first to clear particles and secondly, if that is inadequate, those particles retained intracellularly initiate the two-stage response which culminates in collagen formation by fibroblasts. Contact between particles and epithelium evokes two possible effects. Submicron particles are able to penetrate the attenuated type I epithelium to reach the interstitium, while larger ones may, directly or indirectly, irritate type II cells, which respond by increased secretion of surfactant. It is to this latter aspect that the present account is mainly directed.

## Surfactant dynamics

### *Composition*

Lung surfactant comprises lipids, proteins and carbohydrates, the latter bound to the other two mainly as glucose and galactose. Lipids constitute 80–90% by weight of surfactant, with phosphatidylcholine forming 70–80% and phosphatidylglycerol (PG) 5–10% of the lipids. Most of the phosphatidylcholine occurs in the saturated dipalmitoyl form (DPPC). Of the proteins, serum albumin and IgG along with secretory IgA form minor components, while surfactant-associated proteins fall into three groups, designated SP-A (26–36 k) the major alveolar form, SP-B and SP-C (5–18 k) (Possmayer 1988).

### *Origin*

DPPC, unlike PG, is considered to be the agent responsible for reducing surface tension and averting alveolar collapse. Labelling studies demonstrate that isolated type II cells respond to stimulation by secreting phospholipids which resemble in constitution and behaviour those lavaged from the lungs

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(Dobbs *et al.* 1982). SP-A may also be localized to type II cells, notably their lamellar bodies, and secreted as a phospholipid–protein complex. However, the latter association may be artifactually induced rather than represent chemical bonding (Shelley *et al.* 1975). According to King (1984), ionic bonds are not involved in the SP-A:lipid association.

Steps in the synthesis of surfactant components were elucidated by Chevalier and Collet (1972) in a pioneering in-vivo study using labelled choline, leucine and galactose for autoradiography at the ultrastructural level. The quantitative features indicated that labelled choline was first taken up by endoplasmic reticulum, then passed to the Golgi complex, followed by recognition in small and later large lamellar bodies which were excreted into the alveoli where labelling finally occurred in the tubular myelin lattices. Protein formation evidently followed a similar pathway, perhaps with minor variation. Evidence assembled by Possmayer (1988) suggests several functions for SP-A. It is apparently needed in the formation of tubular myelin, which is believed to be the source of the lipid monolayer responsible for reduction of alveolar surface tension. For instance, Hook *et al.* (1986) separated lipids and proteins present in effluents lavaged from patients with alveolar lipo-proteinosis and by subsequent admixture were able to reform multilamellated structures indistinguishable from tubular myelin occurring in the alveoli of affected individuals; lipid from normal human lungs produced the same result on addition to protein from patients. Using synthetic lipids and surfactant-associated proteins it also proved possible to reconstitute tubular myelin, the proteins being thought to influence organization of lipid membranes so as to form lattices which possessed a rectangular or hexagonal pattern (Suzuki *et al.* 1989). Moreover, through high affinity binding, SP-A inhibits secretion of phosphatidylcholine by type II cells in culture (Dobbs *et al.* 1987; Rice *et al.* 1987; Kuroki *et al.* 1988); SP-A also encourages type II cells to take up lipid, thereby limiting the alveolar content, while the effects of SP-B and SP-C are enhanced (Possmayer 1990). Glycosylation of SP-A may serve to channel protein through type II cells preparatory to secretion of surfactant (Whitsett *et al.* 1985). That the secretory process is susceptible to neurochemical control is suggested by its stimulation with pilocarpine, a cholinergic agonist (Goldenberg *et al.* 1969), an action which atropine as a cholinergic antagonist is able to prevent (Oyarzún & Clements 1977). Synthesis of surfactant apoprotein by type II cells is evidently under multihormonal regulation, both stimulatory and inhibitory (Whitsett *et al.* 1987).

In contrast to the type II cell, parallel electron microscopic autoradiographic observations directed towards the non-ciliated bronchiolar Clara cells did not reveal incorporation of tritiated choline, although uptake of labelled acetate, leucine and galactose did occur; accordingly, it was suggested that Clara cells participate in the synthesis of protein and possibly cholesterol belonging to the hypophase of the lung lining layer (Petrik & Collet 1974). Surfactant apoproteins, traced immunocytochemically, were synthesized in Clara cells and secreted from granules apparently devoid of lipid while less intense synthesis occurred in type II cells (Walker *et al.* 1986). By means of in-situ hybridization, synthesis of SP-A was confirmed in both type II and Clara cells (Auten *et al.* 1990).

### *Degradation and recycling*

Alveolar macrophages are not only able to ingest surfactant but *in vitro* are also capable of degrading DPPC (Stern *et al.* 1986; Miles *et al.* 1988). Catabolism probably occurs through the agency of macrophage lipoprotein lipases (Okabe *et al.* 1984) and the products are then available for reuse by type II cells. Whether the phospholipase A present in lamellar bodies (Hook & Gilmore 1982), when released into alveoli, plays a part in lysis of accumulated lipid is apparently unde-

termined. A small proportion of surfactant may be conveyed proximally along airways within alveolar macrophages, but its level in the air spaces is now considered to be controlled by reutilization. After injection intratracheally into rabbits, labelled surfactant phospholipids, especially DPPC, entered type II cell lamellar bodies, from which resecretion was possible (Hallman *et al.* 1981). The autoradiographic observations of Chevalier and Collet (1972) had earlier suggested that endoplasmic reticulum rapidly took up labelled choline as migration occurred to the cell surface and then lost it. Further aspects of surfactant turnover are discussed by Wright and Clements (1987).

## Changes induced by mineral particles

Although unspecified lipid and beta/gamma globulin, not further identified, were recognized along with collagen as components of silicotic nodules (Vigliani & Pernis 1958), the biochemical composition of lung tissue as a whole received no attention, presumably because a response from type II cells was not then entertained. This non-fibrotic element may be considered in relation to particle type.

### *Silica*

Accelerated (so-called acute) silicosis affects such workers as sandblasters who are exposed to high concentrations of quartz dust (Buechner & Ansari 1969; Surratt *et al.* 1977) and is characterized by irregular rather than nodular fibrosis accompanied elsewhere in the parenchyma by lipo-proteinosis. The unusual reaction detected in SPF rats after inhalation of quartz (Heppleston 1967), when subjected to combined histochemical and preparatory biochemical analysis, revealed a dissociation between focal fibrotic features and widespread consolidation of alveoli by material which possessed the characteristics of lipo-proteinosis with the emphasis lying on the massive lipidosis (Heppleston *et al.* 1970). Hypertrophy and hyperplasia of type II cells, readily identified under the light microscope by apical alkaline phosphatase activity, were seen in the acute phase while the static phase persisted until termination of the experiment 19 months post exposure. At the ultrastructural level, alveoli were occupied by quadratic lattices, such as is produced by lipid in the liquid-crystalline phase, and amorphous material devoid of cells and in which lay isolated particles of quartz (Heppleston & Young 1972). Moreover, the alveolar framework was preserved though as the condition progressed type I epithelium was sometimes breached, the earlier hyperplasia of type II cells subsided and the disorder entered a quiescent state. A remarkable similarity existed between the disease in rats and human alveolar lipo-proteinosis. Further biochemical analysis disclosed a massive increase in total lung lipid of which phospholipid and especially DPPC formed major constitutents (Heppleston *et al.* 1974). In metabolic terms the synthetic rate of DPPC in the active phase of the experimental disorder proved to be trebled and the rate of disposal doubled, a disparity sufficient to account for the development of lipo-proteinosis over an extended period. The capacity of lung to recycle phospholipids was evidently exceeded. Gradual accumulation of lipid eventually prevented contact between quartz and cellular elements, particularly macrophages and type II epithelium; in consequence, the particulate stimulus to release of the macrophage fibrogenic factor (fibrosin) and to surfactant secretion was abolished, thereby accounting for the paucity of fibrosis and the static state of the lung lesion. The lipid response occurred without alterations in the levels of plasma fatty acids or in the proportions of individual acids, so serving to emphasize the local origin of the lung changes.

Knowledge of biosynthesis of surfactant phospholipid and protein under the influence of silica has been expanded by observations from Hook's laboratory. The proportion of hypertrophic type II cells, isolated from the

lungs of rats injected with silica, was much increased and accompanied by hyperplasia (Miller *et al.* 1987). Compared to controls, these hypertrophic cells contained larger and more numerous lamellar bodies, their content of phospholipid, protein and total RNA being elevated along with incorporation of phospholipid precursors into DPPC (Miller & Hook 1988a). Furthermore, the intracellular surfactant rose proportionately more than the extracellular form, while all subcellular components shared in the elevated protein and phospholipid levels (Dethloff *et al.* 1986a; Miller & Hook 1988b). The extracellular material from silica-treated rats showed increased amounts of soluble proteins whose composition resembled that from normal rats, although several serum proteins were lacking and proteins not identified in untreated animals were present; accordingly, damage to the blood/air barrier was considered not to explain the changes (Dethloff *et al.* 1986b). On the other hand, the phospholipid composition of surfactant, enormously increased under the influence of silica, resembled that from control lungs (Dethloff *et al.* 1986a). The metabolic imbalance detected earlier (Heppleston *et al.* 1974) was confirmed, being initiated by intracellular formation exceeding the secretion rate into alveoli, which in turn was greater than the rate of clearance (Dethloff *et al.* 1989). It is now known that the augmented synthesis of DPPC by hypertrophic type II cells, formed in response to silica, occurs through stimulation of the CDP-choline pathway (Miller & Hook 1989).

Attention has lately focused on the effect of silica in regard to the major surfactant protein. Injected into rats, silica not only caused a great increase of lung lavage phospholipid but also of SP-A; a parallel elevation of phospholipid and SP-A also occurred along with their synthetic enzymes in hypertrophic type II cells (Kawada *et al.* 1989). Dissociation between the quantities of SP-A and SP-AmRNA suggested to these authors that raised rates of synthesis might not account for the whole of silica-induced lipidosis and proteinosis, but turnover studies in affected lungs after inhalation were not attempted. However, increased synthesis of SP-A by type II cells, rendered hypertrophic by silica injection, was attributed to a corresponding rise in the cellular levels of SP-AmRNA (Miller *et al.* 1990).

According to Suwabe *et al.* (1991) neither surfactant hypersecretion by type II cells nor its regulation by SP-A was likely to be concerned in the genesis of silica-induced alveolar lipo-proteinosis. These conclusions relied on type II cells isolated from silica-injected rats and contrast with the weight of evidence demonstrating excess formation of pulmonary DPPC under such conditions.

### *Asbestos*

When the response to inhaled chrysotile or amosite was examined in rats, the level of lavaged surfactant was persistently raised and asbestosis sometimes became a modest feature but the relationship could not be defined (Tetley *et al.* 1976; Tetley & Richards 1981). Type II cells also increased and surface tension forces of the alveolar lining material were markedly reduced (McDermott *et al.* 1977), as had earlier been observed when quartz was employed (Heppleston *et al.* 1975). Increased synthesis of DPPC accounted for the changes in surfactant level after chrysotile inhalation, but phospholipase A activity in the 'free cell' population was unaltered (Tetley *et al.* 1977) so, as with silica, surfactant accumulated because of increased synthesis combined with failure to achieve corresponding clearance or recycling. The role of SP-A in the response to asbestos remains to be defined.

Type II cell hyperplasia and hypertrophy proved to be pronounced features, along with increased alveolar and interstitial macrophage populations, after brief or subchronic exposure to chrysotile (Barry *et al.* 1983; Chang *et al.* 1988). These same cells incorporated tritiated thymidine, especially in the vicinity of the first division of respiratory airways, less than a day after 5 h exposure to

chrysotile, but there were no such changes in larger bronchioles or bronchi (Brody & Overby 1989; McGavran & Brody 1989). Chronic inhalation exposure with prolonged survival thereafter established, by morphometric and three-dimensional analysis, that affected type II cells had developed cisternal dilatations in the rough endoplasmic reticulum in direct proportion to the local density of asbestos fibres (Pinkerton *et al.* 1990). Moreover, this study also identified in type II cells, located near the proximal divisions of respiratory passages, large lamellated inclusions to which normal lamellar bodies were fused, leaving little doubt that the changes occurred in response to closely situated fibres, which nevertheless lay outside these cells.

### *Carbon and coal mine dusts*

Exceptionally, coal workers develop lipoproteinosis, but the lipid reaction is more evident experimentally. Exposed to carbon black, rat lung washings showed increases in phospholipids and lecithin although total lipid was unchanged (Rhoades 1972). Dust-laden macrophages may aggregate focally in relation to respiratory air passages or be more diffusely distributed in surfactant according to rank of coal and even similarly exposed animals may show variation in lipid response with corresponding effect on macrophage location (Civil *et al.* 1975).

### *Other particles*

Non-fibrogenic minerals such as titanium dioxide may lead to lipo-proteinosis after inhalation in relatively high concentration (Lee *et al.* 1986). Pulmonary phospholipidosis, notably DPPC, was a feature in rats inhaling diesel particulates (Eskelson *et al.* 1987) and volcanic ash had a similar propensity though less so than quartz whether by injection or inhalation (Sanders *et al.* 1982; Martin *et al.* 1983; Wehner *et al.* 1986). Lipidosis, particularly affecting levels of DPPC, also followed inhalation exposure to metallic nickel dust (Casarett-Bruce *et al.* 1981; Johansson & Camner 1986) and the effect was potentiated in combination with cobalt (Johansson *et al.* 1991). Aluminium in particulate form has been blamed for the development of alveolar lipo-proteinosis (Miller *et al.* 1984), though the association could be coincidental. It is pertinent to note that inhalation of aluminium lactate inhibited production of total lipid and DPPC by quartz, as measured in lavage fluid from treated sheep (Bégin *et al.* 1989). Phospholipidosis was also a feature of bleomycin-induced fibrosis of the rat lung (Thrall *et al.* 1987) and biophysical changes in lung surfactant may be implicated in the pathogenesis of byssinosis (DeLucca *et al.* 1991).

Irradiation likewise produced a rise in the level of DPPC in lung tissue and lavage fluid (Gross 1978; Rubin *et al.* 1980), while inhaled alpha particles led to profound ultrastructural changes in murine type II cells suggesting excessive functional activity to which alveolar macrophages responded (Heppleston & Young 1985).

The diversity of mineral particles and other agencies which lead to lipid accumulation in the lung attests to the non-specific nature of the reaction and indicates that it is not confined to dusts which are powerfully fibrogenic.

### *Regulation of Type II cell proliferation*

Because augmented type II cell function and hyperplasia affect surfactant production, the means by which the population is controlled assume importance. In this process other cellular elements appear to be involved, since the labelling index of type II cells rose after instillation of colloidal carbon into mice, following which macrophages and polymorphs migrated into the lung (Shami *et al.* 1986). Medium conditioned by macrophages stimulated DNA synthesis in, and increased the number of, cultured type II cells, as did co-culture, suggesting that products of macrophages exerted a regulatory role on proliferation of the epithelial cells

(Leslie *et al.* 1985). Bronchoalveolar lavage fluid exerted a similar effect on type II cells in primary culture (Leslie *et al.* 1989). Growth factors have been postulated (Leslie *et al.* 1990), especially from activated macrophages when cell counting and pulse labelling were used to measure type II cell proliferation (Brandes & Finkelstein 1989).

Some conflict of evidence has however transpired, since hypertrophic type II cells, isolated from rats after silica instillation, exhibited elevated thymidine incorporation and labelling index without increase in cell number, though the so-called mitogenic factor in lavage fluid was not increased in comparison with normal animals (Panos *et al.* 1990). It has now been demonstrated that thymidine incorporation cannot be used as a measure of cell division in primary cultures of type II cells, since on its own the technique demonstrates initiation without completion of scheduled DNA synthesis (Clement *et al.* 1990). Estimation of such synthesis *in vitro* should therefore be accompanied by direct assessment of cell proliferation using stathmokinetic procedures.

## Functional consequences

### *Phagocytosis and metabolism*

As the most pronounced example of surfactant accumulation, alveolar lipo-proteinosis affords the opportunity to gauge its effects on macrophage behaviour. In the developed state alveolar macrophages are scanty but in the earlier phase are better preserved, though even then they are distended by lipid and PAS-positive material, lying in large secondary lysosomes which also contain lamellar fragments, similar to that consolidating extensive tracts of parenchyma.

Functional observations on macrophages lavaged from affected humans revealed poor survival in culture with diminished proliferative capacity (Golde *et al.* 1976). Moreover, such macrophages exhibited both impaired chemotaxis and fungicidal ability even though phagocytosis proceeded normally, while blood monocytes from normal subjects cultured in the presence of lavage fluid from patients acquired morphological features resembling cells derived directly. Accordingly, the impaired fungicidal capacity was attributed to ingestion of surfactant, a phenomenon which, extended to bacteria, could account for the prevalence of opportunistic infections among individuals with alveolar lipo-proteinosis (Carre *et al.* 1990) sometimes leading to fatalities. Likewise, macrophages lavaged from this condition proved to be defective in their ability to kill *Staphylococcus aureus* (Harris 1979), while in other cases impairment of phagocytosis has been demonstrated possibly with defective clearance of surfactant-laden macrophages (Nugent & Pesanti 1983). However, not all accounts concur with this conclusion. Divergencies may depend in part on the amount of surfactant taken up, overloading changing an initial stimulation to depression of metabolic and phagocytic activities (Wiernik *et al.* 1987). Rat lung surfactant enhanced both phagocytosis and killing of staphylococci by alveolar macrophages, whereas human surfactant had no effect on the ability of such cells to destroy the organism, a difference for which interspecies variation was invoked (O'Neill *et al.* 1984; Jonsson *et al.* 1986). Long chain fatty acids evidently determined the antipneumococcal activity of lipid fractions from rat surfactant (Coonrod *et al.* 1984). However, the phagocytic ability of alveolar macrophages towards *S. aureus* was augmented by SP-A but not by surfactant lipids (Iwaarden *et al.* 1990), the protein being bound and ingested specifically via a mannose-dependent mechanism apparently with ensuing activation of macrophages (Manz-Keinke *et al.* 1991). A different relationship between lipid and infection was encountered in rats infected with *Pneumocystis carinii*, the resultant pneumonia leading to deficiency of phospholipid without increase of phospholipase $A_2$ activity in surfactant (Sheehan *et al.* 1986). Presumably the infection destroyed type II cells or impaired their function sufficiently to cause the observed changes.

Participation of arachidonic acid metabolites in the pulmonary reaction to mineral particles constitutes a relatively recent field of enquiry. These metabolites, collectively called eicosanoids, are derived from the arachidonic acid (AA) of membrane phospholipids by cyclooxygenase and lipoxygenase enzymes, whose activities are provoked through a variety of cellular stimuli. In consequence, compounds such as $PGE_2$ and $TXA_2$ are formed via the cyclooxygenase route and $LTB_4$ via the lipoxygenase pathway and the possibility has been raised that these metabolites may exert a controlling role in the development of pulmonary fibrosis. The alveolar macrophage is a source of such eicosanoids, whose release may be determined by ingestion of mineral particles.

Particulate material in the form of zymosan or the ionophore A23187 led to phospholipid turnover in human alveolar macrophages and to release of the major AA metabolites $TXB_2$, $LTB_4$ and 5-HETE (Yoss *et al.* 1990). When crystalline silica was applied to bovine alveolar macrophages, 5-HETE and $LTB_4$ were the principal metabolites identified, but in contrast cyclooxygenase products such as $PGE_2$ and $TXB_2$ diminished as increasing concentrations of silica were employed, changes which preceded cell damage as indicated by escape of LDH (Englen *et al.* 1989). Moreover, silicas of different origins provoked release of AA metabolites to different degrees, though a shift from cyclooxygenase to lipoxygenase products was again encountered as the particulate dose was increased (Englen *et al.* 1990). Metabolites from both pathways were secreted by rat alveolar macrophages treated with chrysotile, as was also the case after phagocytosis of nontoxic carbonyl iron beads, thereby suggesting that the response was non-specific (Kouzan *et al.* 1985). Porcine alveolar macrophages gave a weaker response to chrysotile than intravascular macrophages, which released predominantly lipoxygenase metabolites (Bertram *et al.* 1989), thus conforming with the effects reported for silica. After inhaling coal mine dust, lung macrophages lavaged from rats were increased in number and exhibited alterations in AA metabolism whereby $TXA_2$ and $LTB_4$ were raised but $PGE_2$ diminished (Kuhn *et al.* 1990). It is apparent that responses by alveolar macrophages to particulate minerals present a consistent pattern in this respect. The proposition of a correlation between the ability of particles to release 5-lipoxygenase metabolites from alveolar macrophages and fibrogenic capacity on the basis of in-vitro observations (Englen *et al.* 1990) exposes a considerable gap in knowledge, especially since a direct mechanism has been established (see *Fibrogenesis* below).

How eicosanoids and surfactant interact is not known but human and experimental observations suggest that AA metabolites, whose production silica stimulates *in vitro*, are prevented from participating in pulmonary fibrogenesis by surfactant material. As with human exposure so also in rats the pulmonary reaction to inhaled quartz is accompanied by the presence of extracellular particles in the accumulated surfactant and only in a few restricted sites did rat alveolar walls show evidence of fibrosis which was minimal in degree and interstitial in character (Heppleston *et al.* 1970). Surfactant secretion provoked by asbestos or coal mine dust may not be so prominent as that which silica induces, but the effect on any eicosanoid pro-inflammatory activity is likely to be inhibitory and it is equally difficult to envisage a direct role for these metabolites in pulmonary fibrosis by mineral fibres or mixed dust.

### *Recruitment*

Access of macrophages to the sites of dust aggregation may be considered from local and systemic aspects.

Chemotaxis, demonstrated after chrysotile inhalation, evidently operated by activation of complement proteins on alveolar surfaces (Warheit *et al.* 1986). Accumulation of macrophages was apparent after 3 h exposure and continued for 24 h before declining,

but complement depletion reduced the response. Crocidolite as well as chrysotile generated chemotactic activity for alveolar macrophages in rat serum or lavaged proteins, with complement activation again constituting the likely mechanism for cellular accumulation at sites of fibre collection (Warheit *et al.* 1988). Complement proteins were probably derived through increased transudation of serum, in which AA metabolites could be concerned, and thus ensured a continued supply of macrophages. However, the position is rendered complex by the depressed chemotactic response of alveolar macrophages when bearing chrysotile fibres (Warheit *et al.* 1984). This phenomenon was also noted in macrophages lavaged from rats after inhalation of quartz, coal mine dust or chrysotile at concentrations remote from human limits even though the dust burden was insufficient to account solely for inhibition of chemotaxis (Donaldson *et al.* 1990). None of these studies was directed specifically to the possible effects of surfactant phospholipids and SP-A on chemotaxis under dust provocation. To rectify the omission ideally requires the deployment of inhalation techniques at particle concentrations comparable to those permitted for humans. Excessively high airborne concentrations or injection procedures produce unnatural results, notably in the access of neutrophils whose exudation implies a degree of vascular damage not seen after human exposure where the dominant cell is the macrophage alveolar or interstitial. It is worth noting that lipids are tactic for alveolar macrophages (Tainer *et al.* 1975) and that this activity resides in lipid-laden lung lining material (Schwartz & Christman 1979). The relevance to mineral particles of the augmented stimulation of alveolar macrophage migration by surfactant protein (Hoffman *et al.* 1987) remains uncertain.

By contrast, attempts have been made to elucidate systemic production of macrophages by means of surfactant material and it is to this phenomenon that the term recruitment should be confined. Dissolution of macrophages under a burden of toxic dust requires their replenishment to reingest liberated particles, a process which needs to be self-sustaining. Local proliferation in the lung after quartz inhalation appears to play little part according to in-vivo observations (Brightwell & Heppleston 1977). Systemic recruitment of monocytic cells from the marrow provides the alternative means of cellular refurbishment. Simple and complex lipids as well as quartz given intraperitoneally or intratracheally stimulated activity of the mononuclear phagocytic system as judged by the carbon clearance technique (Conning & Heppleston 1966). Supplementing functional observations by measurement of monocytic proliferation, phagocytic behaviour of marrow mononuclear cells was combined with determination of their size and kinetics under stimulation by lipids extracted from the lungs of rats which had developed alveolar lipo-proteinosis after silica inhalation (Civil & Heppleston 1979). Using litter mate pairs for kinetic analysis by single and double labelling autoradiography, marrow promonocyte kinetics were so altered by intravenous administration of lipid that both the duration of DNA synthesis and the cell cycle time were reduced and the rate of entry into DNA synthesis increased. Lipid produced in excess by type II cells after quartz inhalation is thus able to induce proliferation in marrow promonocytes, probably by a sequence of lipid transport via the circulation from lung to marrow, provocation of monocytopoiesis, release of monocytes and their emergence into the lung to meet local requirements. The mechanism represents a positive lipid feedback and its intensity will depend on the degree of particle activity in the lung, with the strength of the stimulus gradually subsiding as quartz becomes sequestered among connective tissue fibres or in surfactant. Indirect evidence points in the same direction by suggesting that lung surfactant, which alveolar macrophages ingest *in vivo*, led *in vitro* to release by these cells of a factor capable of increasing marrow monocytopoiesis, an effect which

may be prolonged by phagocytosis (Sluiter *et al.* 1988).

*Cytotoxicity*

Toxicity may be considered in relation to alveolar epithelium and to macrophages, with both of which inhaled particles come rapidly into contact.

Rat type II cells, having formed tight junctions in culture, were used to measure paracellular permeability (Merchant & Hunninghake 1989). Silica caused a fourfold increase in permeability without cytolysis, but prior treatment of silica with surfactant blocked this increase in a dose-dependent manner, an effect attributable to DPPC. Depending on relative concentrations, particulate stimulation of type II cells may possibly be accompanied by increased epithelial permeability to plasma proteins, a response which might be selective according to molecular size. Other fibrogenic compact particles and fibrous minerals could lead to similar changes.

Cytotoxicity to macrophages *in vitro*, measured by LDH release, was much reduced if silica particles were pretreated with lavaged surfactant even though phagocytosis was comparable with controls (Emerson & Davis 1983), which could explain their longer survival *in vivo*. However, quartz toxicity to the rat alveolar macrophage, having been suppressed by surfactant *in vitro*, was restored after exposure of treated dust to phospholipase $A_2$, one of the cell's hydrolytic enzymes (Wallace *et al.* 1988). New light on the interplay of surfactant and particles has been generated by Holian's group. Relying on superoxide anion production as an indicator of toxicity to guinea-pig alveolar macrophages, immunoglobulin G as a surfactant protein enhanced the response to chrysotile but not to crocidolite (Scheule & Holian 1989). Cell stimulation by chrysotile appeared to be both specific and irreversible, though not wholly dependent on surface charge, and it was proposed that IgG-coated fibre acted on the macrophage by cross-linking its Fc receptors. Failure with crocidolite, on the other hand, might represent inability either in cell binding or fibre adsorption of IgG or inappropriate orientation of bound IgG. When, however, the comparison was made in respect of specific surface areas, the density of adsorbed IgG was similar, though the area of chrysotile was treble that of crocidolite; hence the distinction in reaction between the two fibres did not depend on differential adsorption of IgG (Scheule & Holian 1990). Surface charge did enter into the reaction by virtue of preferential adsorption of more basic proteins by crocidolite and more acidic ones by chrysotile. Of particular significance was the demonstration that in the presence of different proteins one, even as a minor constituent, could be selectively adsorbed by asbestos provided the electrostatic charges were compatible. To overcome the competitive adsorption, pretreatment with IgG conferred on crocidolite the ability to stimulate alveolar macrophages while enhancing that of chrysotile. Pronounced depression of superoxide anion production occurred when chrysotile was pretreated with lung surfactant, an effect for which phospholipids other than DPPC were responsible (Jabbour *et al.* 1991). The relative concentrations of individual phospholipids adsorbed may therefore determine the overall in-vitro reaction and perhaps reflect more closely the in-vivo situation. Turning attention to human alveolar macrophages, Perkins *et al.* (1991) observed that both crocidolite and chrysotile, but not silica or aluminium beads, stimulated production of the superoxide anion. However, preincubation with IgG not only enhanced anion formation by both types of asbestos but also led to its release by silica and aluminium. A striking contrast emerged after preincubation with a mixture of IgG, albumin and DPPC, the enhancement by asbestos being unaffected while the response to silica and aluminium was abolished.

From these studies it appears that, on the basis of superoxide anion formation, differences exist in the reactions of alveolar mac-

rophages from guinea-pigs and from humans and, of particular importance, that fibrous and compact particulates (whether fibrogenic or not) contrast in behaviour when exposed *in vitro* to surfactant constituents which include both proteins and the phospholipid DPPC. It is also surprising that silica and aluminium, with widely divergent fibrogenic capacity *in vivo*, should react similarly *in vitro*. If superoxide anion operates in asbestos but not in silica-induced disease a paradox remains since silica is at least as fibrogenic as asbestos *in vivo*. Furthermore, other evidence (see *Fibrogenesis* below) reveals a response from macrophages common to fibrous and compact dusts in the generation of collagen, a feature which conflicts with the claim for uniqueness on the part of asbestos (Perkins *et al.* 1991). The emergence of such disparities casts doubt over the role of reactive oxygen species in mineral fibrogenesis, one that may arise from contrasts between short and long-term observations and between the relevance of in-vitro findings to in-vivo behaviour.

*Fibrogenesis*

A consensus exists that the alveolar macrophage is central to mineral fibrogenesis, but most attempts to explain the connection rely on indirect evidence such as is derived from brief cytotoxicity studies. The latter simplify test conditions but fail to reproduce the circumstances which obtain in the intact animal and they also neglect the prime cell responsible for collagen formation, the fibroblast. To separate the effects of macrophage phagocytosis and of fibrosis in silicotic disease, an in-vitro procedure was at first essential and its two stages led to the discovery of the macrophage fibrogenic factor or fibrosin (Heppleston & Styles 1967). Confirmatory observations from other laboratories and the outstanding contributions of Kulonen and his colleagues in elucidating the mechanisms by which quartz acts have recently been outlined (Heppleston 1991). Of particular significance was the demonstration that the factor could be extracted from silicotic lung and purification permitted its characterization as an acidic protein of molecular weight 16 000, whose amino acid composition was determined and which was effective on fibroblasts at a concentration of $10^{-10}$ M in a dose-dependent manner (Aalto *et al.* 1989). The requirement to confirm in-vitro findings by in-vivo observations was thus fulfilled.

The initial reaction with mineral particles occurs at the macrophage surface. Both chrysotile and crocidolite increase membrane rigidity without necessarily involving lipid peroxidation (Gendek & Brody 1990), while fibronectin may serve to bind amphibole to mammalian cell lines (Brown *et al.* 1991). *In vitro*, protein and lipid fractions of the lavaged lining layer inhibited not only the binding of both positively and negatively charged particles but also their phagocytosis (Khan *et al.* 1990). The implication for mineral generation of fibrosin needs examination *in vivo*.

A sequence of events may be traced connecting minerals with both fibrosis and the modification to which surfactant may lead. Deposited slowly and in low concentration, silica comes into contact with the lining layer but is soon engulfed by macrophages, after which removal of adsorbed lipid by enzymatic action allows formation of the fibrogenic factor to proceed. Concomitant damage to type I epithelium by inhaled quartz affords access to interstitial fibroblasts and exposure to fibrosin with gradual accumulation of collagen at the typical sites of dust aggregation, that is in relation to respiratory bronchioles. To this process proliferation of fibroblasts may play a subsidiary role. Reference has already been made to participation of the macrophage in type II cell hyperplasia. When irritant particles reach the lung in higher concentration and over a shorter period, surfactant secretion becomes excessive, some being taken up by macrophages, but most remaining extracellularly. In consequence, macrophages con-

taining few particles are distended and isolated; many disintegrate and leave quartz free in the surfactant now filling alveoli. Particles are thereby dispersed in or separated from macrophages and they in turn from fibroblasts, the combined effect being to limit the potential for production of the fibrogenic factor so that fibrosis becomes inhibited and disorganized, as experiments confirmed (Heppleston 1986). A simpler explanation than is postulated by in-vitro observations on cytotoxicity is thus available to account for the interaction of particles, macrophages and surfactant. Particles less irritant than quartz, such as asbestos or coal mine dust, evoke a correspondingly weaker stimulation of type II cells but even in small amounts surfactant retains the ability to interfere with the fibrogenic process, from which two consequences may flow, notably in connection with coal workers' pneumoconiosis.

Like highly siliceous dusts, inhaled coal mine dusts customarily lead to focal aggregations in relation to respiratory air passages in both humans and animals. However, interstitial fibrosis with dust impregnation, superimposed on characteristic dust foci, is sometimes a feature of human exposure in coal mines and unexplained interstitial fibrosis was encountered in other series of coal workers' pneumoconiosis (see Heppleston 1989). From the experimental perspective, some coal mine dusts elicited focal lesions while others led to less defined changes where macrophages were foamy, their particle burden light, and where they were separated by amorphous material containing few particles. Mine dust from a particular colliery caused such changes but seam dust from the same pit led to the more usual focal aggregations (Civil *et al.* 1975), a distinction for which clay minerals, largely confined to mine dusts, may be responsible in view of the ability of the silicate components muscovite and illite to induce lipo-proteinosis (Martin *et al.* 1977). Whether fibrosis occurs in discrete form or more diffusely is evidently influenced by the prominence of the lipid reaction to deposition of particles and may thus depend on the degree to which they are prevented from aggregating and achieving cellular contact. A second factor, that of particle size, may also enter into the development of diffuse interstitial fibrosis. Submicron particles, compact or fibrous, possess the ability to gain the interstitium by penetration of type I epithelium (Heppleston & Young 1973; Brody *et al.* 1981, 1984) and there initiate a relatively diffuse fibrosis, whereas micron-sized particles are incorporated from alveoli where aggregation of laden macrophages into foci has taken place. A question yet to be addressed concerns the possible role of surfactant in determining the diffuse and patchy distribution of fibrosis seen after asbestos exposure.

Prevalence of pneumoconiosis in coal workers constitutes the other area in which surfactant may intervene. Mean long-term dust concentration to which miners were exposed generally determined the prevalence of disease, but this pattern was subject to variation among different coal fields. Substantial disparities between observed and expected values for pneumoconiosis progression occurred at particular collieries and the environmental data provided no explanation, high progression being sometimes associated with low dust exposure or vice versa, with opposite extremes occasionally being found at different times in the same mine (see Heppleston 1988). On epidemiological grounds quartz and ash contents of coal mine dust sometimes showed wide disparity with prevalence, while experimentally concentration appeared more important than composition when coal mine dusts were tested for fibrogenic capacity *in vitro* (Heppleston *et al.* 1984). If lipid participation is a variable, perhaps related to dust composition, which may not be constant over a working life, the fibrotic response could be affected. Lipidosis and fibrosis do not necessarily exhibit a strictly quantitative inverse relationship, since small amounts of lipid might suffice to separate particles from cells. The nature and proportions of non-coal

components may affect the degree of lipid production and be reflected as disparities of prevalence between collieries. Moreover, removal of lipid by degradation or reuse would obscure its former operation.

Overall, however, fibrosis is inhibited by surfactant accumulation not only experimentally but also in human lung disease unassociated with mineral dusts. Inflamed or fibrotic lung tissue, obtained immediately post-mortem, showed a significant decrease in phosphatidylcholine content with total lipids being reduced in the presence of fibrosis (von Wichert 1971). Patients afflicted with idiopathic pulmonary fibrosis, diagnosed by biopsy, had significantly less phospholipid, particularly the PG component, in bronchoalveolar lavage than did healthy volunteers, the severity of the reduction being related to the more advanced degrees of fibrosis (Robinson *et al.* 1988). Furthermore, the SP-A content of lavage fluid was also reduced in patients with idiopathic pulmonary fibrosis or other fibrotic states, the degree of reduction correlating with the subsequent clinical course (McCormack *et al.* 1991).

*The immune response*

Immunological changes, whether humoral or cellular, occur inconstantly in humans exposed to fibrogenic dusts and many individuals show no significant deviations. Such was the position in respect of circulating immune complexes, ANA and RF in asbestos workers (Zone & Rom 1985) and the idea of immune imbalance among exposed individuals had to be treated with caution (Sprince *et al.* 1991). Alveolar macrophages which have ingested compact or fibrous particles may nevertheless, as an epiphenomenon, facilitate a cell-mediated immune response through T-cell proliferation and IL-1 release. Secondary stimulation of the immune system, perhaps involving denaturation of collagen, may also occur, though not universally, and represent a non-specific event. For instance, alveolar macrophages and neutrophils in lavage fluid, typifying the alveolitis elicited by intratracheal injection of quartz or coal mine dust, were considered to enhance lymphocytic proliferation with liberation of IL-1, a feature for which wide applications were entertained in respect of pulmonary inflammation and fibrosis, however produced (Kusaka *et al.* 1990a, b). Negating this view was the observation that neither T-cells nor the cells they influence affected collagen deposition in silica-treated mice (Hubbard 1989). Inhibition of lymphocyte response to mitogens was preceded by enhancement, which could again reflect an initial alveolitis induced in sheep by injection of asbestos (Rola-Pleszczynski *et al.* 1984). Immune intervention is not a necessary requirement for chronicity in mineral fibrogenesis, since particles themselves provide a self-propagating state.

Early enquiries demonstrated suppression of response on the part of peripheral blood lymphocytes to mitogens unrelated to cytotoxicity in the presence of bronchoalveolar lavage under conditions of culture (Ansfield *et al.* 1979, 1980) with phosphatidylcholine and PG playing the major role (Ansfield & Benson 1980). Immune regulation by surfactant was expressed by enhanced cytotoxicity of human alveolar macrophages and monocytes for tumour cells, this effect being exhibited by some, but not all, phospholipids (Baughman *et al.* 1987). Whole surfactant and individual lipids from man, pig and rabbit suppressed proliferation of peripheral blood lymphocytes, both B and T cells, in response to mitogens, suggesting that these lipids possessed a down immunoregulatory role (Wilsher *et al.* 1988). Moreover, alveolar macrophages, which constitute the predominant cell type in the reaction to inhaled dust, have been implicated in down regulation of local lymphoid cells, probably related to macrophage insensitivity to IL-1 stimulation (Holt 1986). Surfactant could thus exert a suppressive effect on such immunological reactions as may follow dust exposure. Experiments devoted to mineral reactions which do not take into account the impact of surfactant are unlikely to reflect the com-

plete in-vivo state in this and the other functional aspects.

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