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The Pulmonary Response to Fibrous Dusts of Diverse Compositions

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Fibrous quartz, chrysotile asbestos, and tremolite talc dust, all of respirable particle size, injected intratracheally, produced polypoid proliferative inflammations within smaller air-conducting tubes as well as more peripherally. With time, the inflammatory tissue became converted into collagenous scars which often caused permanent deformities of bronchi and bronchioles. After intratracheal injection of a fibrous dust such as synthetic chrysotile, ceramic aluminum silicate, silicon carbide, glass, or brucite, the main pulmonary response was a macrophage reaction with minimal stromal participation. In addition, within 4 days after the injection, there were foci of polypoid proliferative inflammation but limited to the more peripheral respiratory bronchiole and alveolar ducts. Because these polypoid lesions did not collagenize and did not destroy the anatomic integrity of the air spaces, and because the lesions were reversible, the dusts calling forth this type of response must be classed as biologically "inert." Furthermore, the polypoid lesions are believed to be artifactual in the sense that their production is determined by the method of introducing the dust into the lungs, since such lesions are not seen in animals *inhaling* high concentrations of the same dusts.

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Introduction

WITH THE INCREASING production and use of fibrous materials, both the naturally occurring and those industrially produced, the dust created by their fragmentation is becoming more prevalent. We know that one type of naturally occurring fibrous dust, namely asbestos, is biologically active and is capable of causing extensive and fatal scarring of the lungs, and some kinds of this mineral have been associated with the production of cancer.

Inasmuch as it is not known exactly what it is about the asbestos dust particle that is responsible for its pathogenicity, the simplest explanation, which seemed to be attractive to many in the past, was that the pathogenicity of asbestos, and, therefore, of all fibrous dusts, was related to the fibrous shape of the particles. According to this theory, when the fibers are inhaled, their sharp ends traumatize the cells they contact, and fibrosis results from the multiple traumata.

Although some years ago we had investigated the pulmonary response to one industrially produced fibrous dust, namely ceramic aluminum silicate fibers, and found it to be biologically "inert"[1] in recent years the needle-like character of fibrous dust has again been implicated as the pathogenic factor. This has occurred in connection with fibrous glass dust, the pathogenic potential of which has been questioned in spite of the fact that nonfibrous glass dust has been found to be biologically "inert."[2]

This paper is concerned with a study of the pathogenic potential of fibrous dusts not heretofore documented and with the pathologic

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TABLE I

Tabular Protocol of Rats Injected Intratracheally with Dusts

Dust	Fiber Diameter, μ	No. of Rats	Dose	Mortality[a]
Microquartz[b]	1.2	62	25 mg	26% in 6 months 45% in 15 months
Natural chrysotile	0.1 to 0.2 0.2 to 1	55	10.5 mg 11 mg	90% in 18 months 100% in 24 months
Talc (tremolite)[d]	0.1 to 2	50	25 mg	10% in 6 months 44% in 6 months
Synthetic chrysotile[e]	0.02 to 4	55	11 mg 4 mg	22% in 12 months 53% in 24 months
Ceramic aluminum silicate[f]	2.0	80	10.5 mg	2% in 18 months 85% in 18 months
Glass[g]	1	75	10.5 mg	67% in 12 months 100% in 18 months
Brucite	2.5	15	10.5 mg	47% in 12 months 73% in 24 months
Silicon carbide[h]	0.5 to 10	22	3.5 mg	18 rats sacrificed at intervals (no deaths in 6 months)
Amorphous magnesium silicate[i]		10	75 mg	10% in 6 months

[a]Range of mortality in months of different groups.

[b]High nickel, 35 rats; medium nickel, 31 rats; and low nickel, 27 rats.

[c]Ball-milled dust given to 40 rats and hammer-milled dust to 15 rats.

[d]Talc with high and low natural nickel content (given to 25 rats each).

[e]One batch prepared at Mellon Institute, the other at Johns-Manville Research and Engineering Center.

[f]Named Fiberfrax, obtained from Carborundum Company.

[g]Groups of 15 rats given different kinds of fibrous glass: 1, etched glass; 2, uncoated; 3, coated with starch binder; 4, coated with resin.

[h]Silicon carbide whiskers from Carborundum Company.

[i]Prepared by reacting sodium silicate with MgCl and washing precipitate.

effects of these, as well as of previously investigated dusts that have not been reported. This study is part of a more basic investigation being conducted in cooperation with the U.S. Public Health Service (Grant No. 1 R01 UI-00819-01) and industry, the purpose of which is to determine the locus of pathogenicity of asbestos dust.

Method and Materials

A tabular summary of the types of dust studied, the number of rats employed, and the dose of dust administered is given in Table I. Included under any one type of dust may be two or more materials from different sources of slightly different compositions but grouped together because, for the purpose of this study, no significant difference was noted.

For instance, microquartz prepared at the Johns-Manville Research and Engineering Center consisted of resintered, acid-leached glass fibers with an originally high alkali content. The average diameter of the fiber was 1.2 μ. Two batches had been prepared: one with a metallic nickel content of 0.11% and the other, of 3.1%.

The natural chrysotile was also of two kinds. One had been ball-milled and then hammer-milled. In the latter process, besides being reduced to submicronic dimensions, it also acquired an increased nickel content from the nickel-steel alloy of the hammers. The other was comminuted by ball milling only.

The talc dust was of the tremolite variety, and there were two kinds. One had a high natural nickel content and contained fibers with an average diameter of 0.2 μ; the other had a low nickel content and contained fibers with an average diameter of 0.1 μ.

Two batches of synthetic chrysotile were employed. One, prepared at the Mellon Institute, Pittsburgh, Pennsylvania, had a purity of about 90%. The impurities consisted largely of brucite ($Mg(OH)_2$). The diameter of the tubular crystals averaged 0.02 μ and their length varied from 0.08 to 0.17 μ. The other batch was synthesized at the Johns-Manville Research and Engineering Center, Manville, New Jersey. Its purity was 99.4%. The average diameter of the crystals was 0.03 to 0.04

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FIGURE 1. Tubular crystals of synthetic chrysotile prepared at Mellon Institute, Pittsburgh, Pennsylvania.

...e with a length of 1 μ or less, although a few fibers were up to 5 μ in length. Both lots gave x-ray diffraction patterns typical of chrysotile (Figures 1 and 2).

Five different varieties of fibrous glass were injected into rats. These averaged about 1 μ in diameter. One was etched, two were uncoated, one was coated with a textile-type of *binder* (mostly starch), and the last was coated with a phenol-formaldehyde resin type of binder (used largely for insulation).

The ceramic aluminum silicate fibers (Fiberfrax from Carborundum Company) had an average diameter of 2.0 μ. Two batches were used: one had been hammer-milled to increase its nicked content, and the other was briefly comminuted in a glass tissue grinder. The silicon carbide whiskers, also obtained from the Carborundum Company, had a fiber diameter ranging between 0.5 and 3 μ and a length ranging between 100 and 750 μ.

Amorphous magnesium silicate was used as a nonfibrous control dust. It was prepared by reacting a solution of magnesium chloride with a solution of sodium silicate. The resulting precipitate was washed with abundant water, and a standard suspension was prepared.

All dusts were suspended in water, the concentrations dependent on the amount suspended in 1 ml of water which could be injected without killing the rats. Most suspensions contained 5.5 mg of dust per milliliter. Several suspensions contained 2.5 mg of dust per milliliter.

A total of 171 rats was injected intratracheally with these dusts. In some groups the total dose was administered by as many as four injections. The injections were made under light ether anesthesia with the aid of an illuminated laryngeal speculum which allowed the introduction of a spatial type needle between the vocal chords under direct observation.

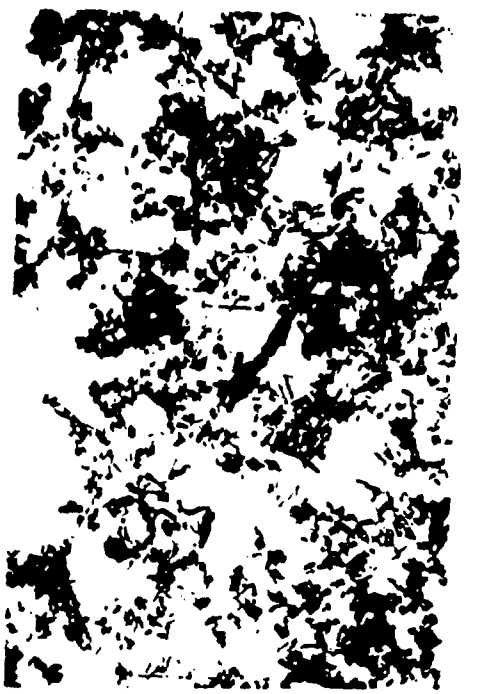

FIGURE 2. Crystals of synthetic chrysotile prepared at the Johns-Manville Research and Engineering Center, Manville, New Jersey. Note that these crystals are longer and needle-like; also the magnification is approximately one-tenth of that in Figure 1.

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In order to study the rat's pulmonary response to the various types of dust, four rats were killed from each group four days after the first intratracheal dust injection. The rest were allowed to live out their lives. The lungs of all animals were distended with 4% formaldehyde solution under a head of 10 to 12 cm of water. Paraffin sections of the lungs were stained routinely with hematoxylin and eosin. Pertinent fields were photographed, and after impregnation with silver the same fields were rephotographed in order to study the relationship between cells and stroma. In order to study the relationship of the dust to the lesions, some sections, cleared unstained, were examined or photographed under dark-field illumination; other sections were subjected to microincineration and were similarly examined or photographed under dark-field illumination.



FIGURE 3 Contracted, densely collagenous scar in the lung of rat injected intratracheally with 15 mg of very finely comminuted chrysotile 24 months previously. The scar probably represents an obliterated bronchiole as indexed by the size of associated blood vessels on its right border. Hematoxylin and eosin. 150X.

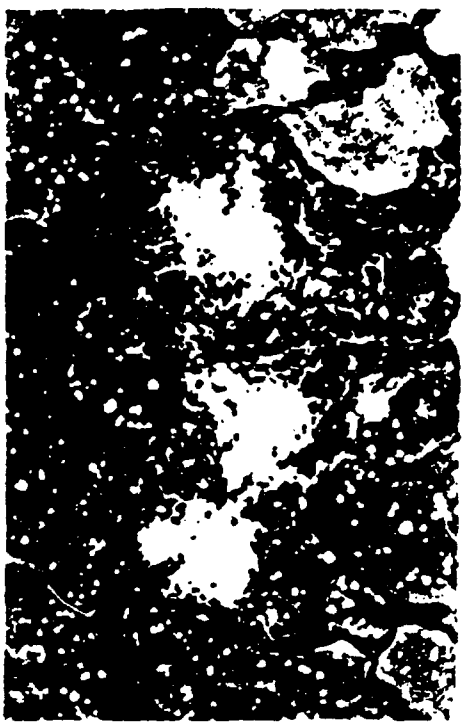

FIGURE 4 Acid-insoluble ash pattern superimposed on the same field as in Figure 3. It shows three dense deposits of chrysotile dust in what probably was originally the lumen of the bronchiole. The "snow" in the background is artifactual. Microincineration. 150X.

Results

Four days after the intratracheal injection the lungs hardened with asbestos, talc, and microquartz showed a proliferative inflammation, involving widely scattered smaller bronchi and bronchioles. This was characterized by polypoid processes of avascular fibroblastic tissue rich in argyrophilic fibers which enmeshed large amounts of the injected dust. These polypoid structures originated from one or several widely separated ulcers in the mucosa and distorted the bronchial lumen, converting it into disconnected circumferential channels that tended to encircle the central stromal plug. These channels quickly became invested with normal-appearing ciliated columnar epithelium. Within a few months, the



FIGURE 5. A polypoid mass of inflammatory tissue occupies the lumen of a respiratory bronchiole. Scattered macrophages are seen in many alveoli. Rat *injected intratracheally* with 3.5 *mg of ceramic* aluminum silicate and killed four days later. Hematoxylin and eosin, 150X

argyrophilic fibers were replaced by dense collagenous tissue

The lungs injected with asbestos dust had, in addition to the proliferative inflammation in the smaller bronchi and bronchioles, similar lesions in the respiratory bronchioles, and alveolar ducts. These more peripheral polypoid structures originated from one or more of the evaginating alveoli. Although the former did not become covered with epithelium, as happened in the terminal bronchioles and larger passages, they did become converted into dense collagen and, as a result, underwent considerable shrinkage (Figures 3 and 4).

The lungs injected with talc showed numerous foci of proliferative inflammation in respiratory bronchioles and alveolar ducts similar to those encountered in lungs injected with asbestos (Figures 3 and 4) ; and, like the latter, considerable shrinkage of the lesions occurred months later when the initially argyrophilic stroma became converted into dense collagen

The main pulmonary response to the dusts of synthetic chrysotile, ceramic aluminum silicate fibers, fibrous glass, brucite, and silicon carbide whiskers was the mobilization of macrophages which, filled with dust, occupied alveoli evaginating off respiratory bronchioles and alveolar ducts along with much extracellular dust. The walls of these alveoli were thickened by a combination of surface cell enlargement and arborescence of the septal argyrophilic stroma. Perhaps the most interesting feature of the pulmonary response was the development of fibroblastic tissue proc-



FIGURE 6. *Polypoid masses of inflammatory* tissue occupy the lumen of a respiratory bronchiole (middle left) and the lumen of an alveolar duct (lower right). The inflammatory tissue is loose and cellular. Transparent fibers and a giant cell are seen in the polyp in the lower right portion of the field. Rat injected intratracheally with 3.5 mg of glass fibers and killed four days later. Hematoxylin and eosin, 150X.

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FIGURE 7. A terminal bronchiole containing a polypoid mass of inflammatory tissue enclosing numerous opaque fibers. It is of interest that the inflammatory tissue is already 96 hours covered by bronchiolar epithelium. Numerous leukocytes are present. Rat injected intratracheally with 3.5 mg of silicon carbide whiskers and killed four days later. Hematoxylin and eosin, 300X.

esses from one or several of the evaginating alveoli of respiratory bronchioles and alveolar ducts. This inflammatory tissue, consisting of argyrophilic stroma, extended in a polypoid manner into the lumen of the parent structure (Figures 5, 6 and 7). Well-developed by the fourth postinjection day, these lesions were less numerous by the fourteenth day and could not be found six months and longer after the injection. Collagenization of these lesions was not observed at any time. Evidence of the dust injections was still present in the form of dust-laden macrophages scattered throughout the section, but these were less numerous, loose, and usually separated from one another, and the walls of the air spaces in which they were found now were thin and delicate. Along with the disappearance of the intraluminal polypoid inflammatory tissue and the reduction in macrophages, the amount of dust in the sections appeared to undergo a parallel reduction.

The lungs of rats injected with amorphous magnesium silicate also showed occasional proliferative polypoid fibroblastic inflammation in respiratory bronchioles and alveolar ducts; like the lesions associated with synthetic chrysotile injections, they were no longer found some months later. In the main, the pulmonary response was a macrophage reaction with minimal stromal reaction. Giant cells were also prominent.

Comments

According to the commonly accepted definition of a fiber (a particle whose length is three times its diameter or longer), synthetic chrysotile certainly is fibrous. In one batch (Mellon Institute), the individual particles when viewed under an electron microscope are tubular crystals, the diameter of which in relation to their length is such that by no stretch of the imagination can they be considered needlelike (Figure 1). Nevertheless, this material, injected intratracheally, has produced proliferative inflammatory lesions similar to those produced by injected brucite. Furthermore, identical lesions have been seen in an occasional animal injected with amorphous magnesium silicate. It appears, therefore, that the proliferative inflammation noted four days after synthetic chrysotile injections may be ascribed to the high local concentrations of magnesium silicate associated with the intratracheal injections.

In view of the proved biologic inertness of ceramic aluminum silicate,[1] silicon carbide,[3,4] and glass,[2] it is difficult to explain the production of the proliferative inflammation observed following intratracheal injection of the needlelike particles on any other basis than that of mechanical trauma. It would seem that the injection under pressure from the syringe causes the fluid to emerge from the needle with high velocity. Also, the fibrous particles, tending to align themselves parallel to the stream, would thereby tend to impinge point first on the mucosa of branching con-

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FIGURE 8. This field is typical of findings in the lungs of rats that had inhaled fibrous glass dust (100 mg/m³) for 232 days, 6 hours per day. It is noted that there is no fibrosis. The alveolar walls are thin and delicate but small clusters of darkly staining alveolar macrophages are present in alveoli clustered about some alveolar ducts. Hematoxylin and eosin, 150X.

ducting tubes, and the possibility of multiple small traumata, amounting to abrasions, becomes a probability.

Nevertheless, we are faced with apparent contradictions. When we first investigated the biologic potential of ceramic aluminum silicate fibers,[1] we did not observe the proliferative inflammatory lesions described above. The reason for this failure lies in the fact that these lesions disappear with time, and we had not examined the lungs during the first two weeks after the intratracheal injection.

Another highly significant contradiction lies in the fact that such polypoid intraluminal proliferative lesions as are found following the intratracheal injection of certain fibrous dusts are not encountered when the same dusts are inhaled, even in high concentrations. Examples of this contradiction are chrysotile asbestos and fibrous glass. Animals have been exposed to high concentrations of chrysotile asbestos dust in inhalation chambers for more than a year without such polypoid-proliferative lesions having been observed.[2,3] We have under study at the present time rats and hamsters that have inhaled coated and uncoated fibrous glass in concentrations approximating 100 mg/m³ for over one year, without detecting any such proliferative lesions[4] (Figures 8, 9 and 10).

We are, therefore, forced to conclude that fibrous dust, when injected intratracheally under pressure, may produce mechanical trauma resulting in inflammatory foci which, however, resolve and disappear with time. These lesions must be considered artifactual.



FIGURE 9. The same field as in Figure 8 after decolorization and silver impregnation showing minimal stromal reaction which is limited to the regions where macrophages are clustered and consists of arborescent reticulin fibers. Gordon and Sweet, 150X.

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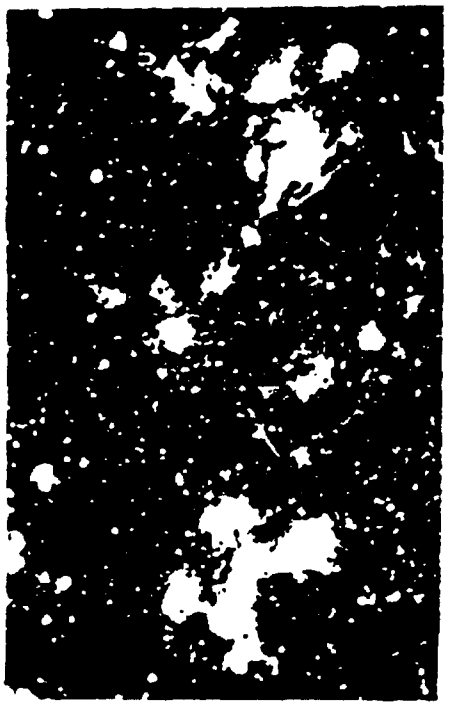

FIGURE 10. This is the acid-insoluble ash pattern superimposed on the same photograph as in Figure 8. The large amount of fibrous glass dust demonstrable and the insignificant tissue reaction to its presence point to the biologic "inertness" of this fibrous dust. The "snow" in the background is artifactual. Microincineration, 150X.

The difference between the proliferative lesions produced by the intratracheal injection of fibrous quartz, asbestos, and talc on the one hand, and those produced by similar injections of synthetic chrysotile, silicon carbide whiskers, fibrous ceramic aluminum silicate, fibrous glass, and brucite on the other hand, is the difference between the deformed bronchi and bronchioles caused by permanent scar and short-lived reversible lesions.

Viewed from another angle, the proliferative lesions produced by all the fibrous dusts investigated except those of quartz, asbestos and talc have the following characteristics:

1. Significant collagenization in the reacting lung tissue is absent.

2. The anatomic integrity of the air spaces is maintained in spite of the presence of dust therein.

3. The lesions are reversible.

These features are those of biologically "inert" dusts and justify classifying synthetic chrysotile, fibrous glass, brucite, silicon carbide whiskers, and ceramic aluminum silicate in this category in spite of the polypoid proliferative inflammation produced when these dusts are injected intratracheally. The proliferative inflammation is considered to be artifactual, dependent on the injection technique.

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