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[c]	0.05-0.2 0.2-1	55	10.5 mg + 14 mg	98% in 18 months 100% in 24 months
Talc (tremolite)[d]	0.1-0.2	50	25 mg	{16% in 6 months {44% in 6 months
Synthetic chrysotile[e]	0.02-0.04	55	14 mg + 15 mg	22% in 12 months 53% in 24 months
Ceramic aluminum silicate[f]	2.0	80	10.5 mg	72% in 18 months 45% in 18 months
Glass[g]	1	75	10.5 mg	67% in 12 months 100% in 18 months
Brucite	2-4	15	10.5 mg	17% in 12 months 73% in 24 months
Silicon carbide[h]	0.5-3.0	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, 1 coated with starch binder; 1, coated with resin.
[h]Silicon carbide whiskers from Carborundum Company.
[i]Prepared by reacting sodium silicate with $MgCl_2$ 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-00849-01) 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 99.1%. The average diameter of the crystals was 0.03 to

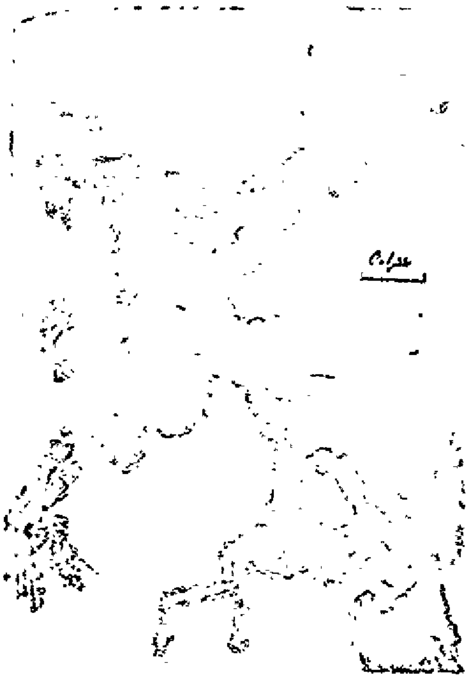

FIGURE 1. Tubular crystals of synthetic chrysotile ...pared at Mellon Institute, Pittsburgh, Pennsyl... ...a.

...ith a length of 1 μ or less, although a few ...s were up to 5 μ in length. Both lots gave ...n diffraction patterns typical of chrysotile ...gures 1 and 2).

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

The ceramic aluminum silicate fibers (Fiber...x from Carborundum Company) had an ...age diameter of 2.0 μ. Two batches were ... ...d been hammer-milled to in... ...d content, and the other was ...ated in a glass tissue grinder. ...bide whiskers, also obtained ...orundum Company, had a fiber ...ng between 0.5 and 3 ... and a ... between 100 and 750 ...

... magnesium silicate was used as ...ntrol dust. It was prepared by ...ation 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 depending on the amount suspended in 1 ml of water which could be injected without killing the rats. Most suspensions contained 3.5 mg of dust per milliliter. Several suspensions contained 25 mg of dust per milliliter.

A total of 424 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 spinal-type needle between the vocal chords under direct observation.

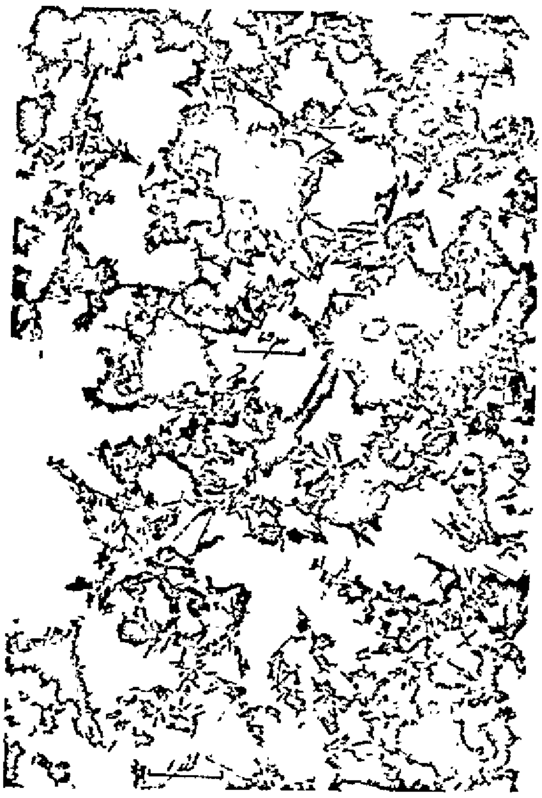

FIGURE 2. Crystals of synthetic chrysotile prepared at the Johns-Manville Research and Engineering Center, Manville, New Jersey. ... these crystals are longer and needle-... The magnification is approximately ... that in Figure 1.

RE 5. A polypoid mass of inflammatory tissue the lumen of a respiratory bronchiole. d macrophages are seen in many alveoli. jected intratracheally with 3.5 mg of ceramic um silicate and killed four days later. Hema- and eosin, 150X.

ophilic fibers were replaced by dense nous tissue.

lungs injected with asbestos dust had, ution to the proliferative inflammation smaller bronchi and bronchioles, similar in the respiratory bronchioles, and al- ducts. These more peripheral polypoid originated from one or more of inating alveoli. Although the former become covered with epithelium, as l in the terminal bronchioles and ages, they did become converted collagen and, as a result, under- derable shrinkage (Figures 3 and 4)

injected with talc showed num- f proliferative inflammation in bronchioles and alveolar ducts e encountered in lungs injected (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 0.5 mg of glass fibers and killed four days later. Hematoxylin and eosin, 150X.

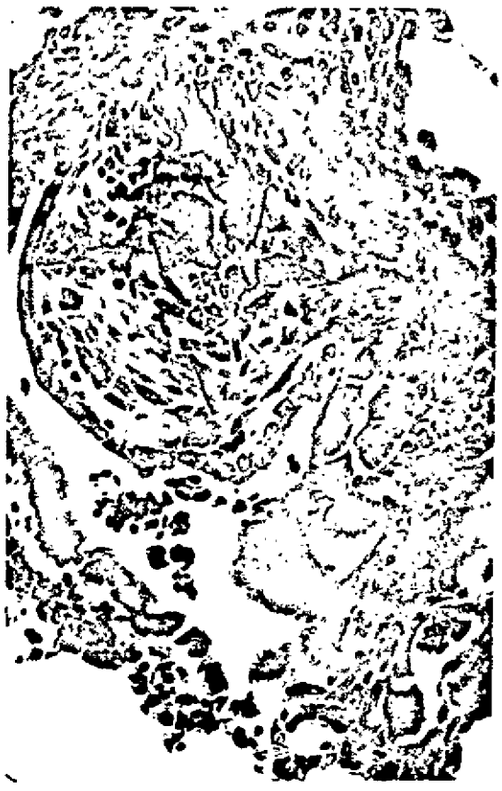

FIGURE 7. A terminal bronchiole containing a polypoid mass of inflammatory tissue enclosing numerous opaque fibers. It is of interest that the inflammatory tissue is already (96 hours) covered by bronchiolar epithelium. Numerous leukocytes are present. Rat injected intratracheally with 3.5 mg of silicon 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 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 seen 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,[7] silicon carbide and glass,[8] it is difficult to explain the production of the proliferative inflammation observed following intratracheal injection of 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 fiber particles, tending to align themselves parallel to the stream, would therefore tend to impinge first on the recessed branching

FIGURE 8. This field is typical of findings in the lungs of rats that had inhaled fibrous glass dust (50 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.

fixing 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.[5,6] 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[7] (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.

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 permane scar and short-lived reversible lesions.

Viewed from another angle, the fera- tive lesions produced by all the fibr dust investigated except those of quartz, sto and talc have the following characte s: 1. Significant collagenization in t ing lung tissue is absent.

2. The anatomic integrity of the a is maintained in spite of the presenc d therein.

3. The lesions are reversible.

These features are those of biologically "i ert" dusts and justify classifying synthe chrysotile, fibrous glass, brucite, silicon carbi whiskers, and ceramic aluminum silicate i this category in spite of the polypoid prolife ative inflammation produced when these dus are injected intratracheally. The proliferativ inflammation is considered to be artifactua dependent on the injection technique.

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Received Jun. 1968