

Although the occurrence of ferruginous bodies and of naked fibers in the lungs of city dwellers has not been associated with disease ascribable to the inhalation of fibrous dust particles, the suspicion has been publicized that pulmonary fibrosis and cancer may develop in the lungs of city dwellers from the inhalation of this fibrous dust presumed to be asbestos.^{4,5}

While studying the sediment derived from the digestion of lung tissue, we were impressed with the large number of naked transparent fibers, most of which were less than 8μ in diameter and less than 50μ long. These had no distinguishing features that enabled us to differentiate asbestos from nonasbestos fibers at the magnifications employed ($\times 450$). Subsequently, however, we have been able to do this readily with the electron microscope.⁶ Having shown that at least some of the fibers inhaled by urban dwellers are probably asbestos, we sought to determine how much fibrous dust was inhaled by these people during their lifetime and what was the size distribution of the fibers. We also wished to study the relationship of the fibrous dust (1) to the total dust inhaled, (2) to the number of ferruginous bodies found, and (3) to any possible pulmonary disease present.

To answer these questions, we first had to develop a method for quantitatively separating the transparent fibers from the carbonaceous material. We then needed to devise a procedure for counting the fibers so as to obtain reproducible results. We also found it necessary to modify our previously published method for isolating ferruginous bodies from the lungs¹ in order to obtain quantitative yields. In this paper, we are reporting the details of these methods, as well as the results obtained on the lungs of a small number (seven) of urban dwellers (selected only in respect to sex), of a long-time employee of an asbestos products factory, and of two men who had been exposed for a working lifetime to dust in a factory producing fibrous glass. (From a factory which pioneered the production of fibrous glass, we have on hand for detailed study the lungs of 18 long-time employees who had died of natural causes. The above two workers are the first and second on which data have become available.)

Identification of both the naked fibers, as well as the cores of ferruginous bodies derived from lungs of these urban dwellers, will be studied and reported in a future paper.

Materials and Methods

Preparation of Dry Lung Tissue.—Formaldehyde-preserved lungs were washed with running tap water for 24 hours and then cut into thin slices by means of a meat slicer. The slices were extracted with three portions of acetone and three portions of dry ether for six to eight days, vacuum dried at 50°C for 48 hours, and then weighed.

Isolation of Dust From Lungs.—The defatted, dried lung tissue was digested by heating with formamide according to the method of Thomas and Stegemann.⁶ However, in order to remove more of the associated impurities and to render the dust more readily dispersible, it was subjected to more vigorous washing. In addition to the solvents used by Thomas and Stegemann, we used a methanol-chloroform mixture to wash the dust. This additional manipulation resulted in the removal of some unidentified brown colored impurities and made the dust more easily dispersible.

The procedure was carried out as follows: about 30 gm of dry tissue was placed into a 2,000-ml flask containing 1,000 ml of dry formamide (Fisher, reagent grade), and the mixture was kept under continuous stirring at $130 \pm 5^\circ\text{C}$ for 72 hours. The resulting liquid digest was centrifuged in 200-ml flasks at 2,000 g for one hour. The sediment, consisting of crude impure dust, was collected into a single flask and washed three to four times with 150-ml portions of warm formamide. The sediment was submitted to further digestion with 200 ml of formamide for 24 hours, and it was then washed with 150-ml portions of the following warm solvents: formamide; formamide-water (1:1); water; methanol; methanol-chloroform (3:2); chloroform-hexane (2:3); and *n*-hexane. The sediment was kept in contact with each of the solvents successively under continuous stirring at least for two hours and was repeatedly washed with several portions of each solvent until the washings were clear and colorless. It was finally dispersed in a small volume of hexane, vacuum dried at 50°C for 24 hours, and weighed.

Separation of Naked Fibers From Dust.—Ten milligrams of the isolated dust was suspended in 5 ml of 70% perchloric acid and heated in a sand bath at 160 to 180°C until the carbonaceous material disappeared (about 30