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Ferruginous Bodies in Human Lungs

Prevalence at Random Autopsies

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Ferruginous bodies with a transparent central core or no visible central filament were present in 97 of 100 autopsies; they were found in 55 of 56 men (98%) and in 42 of 44 women (95.5%). The youngest subject with ferruginous bodies was a 24-year-old woman. The bodies were generally more abundant in the lungs of the men. Thirty-two patients in this series had some form of malignant disease, but there was no apparent association between the malignancies and the relative abundance of ferruginous bodies. There were two cases of primary lung cancer in men, but no case of mesothelioma, nor of asbestosis.

The significance of these findings from an epidemiologic point of view must await identification of the central core of the ferruginous bodies, but in any event, pulmonary fibrosis or neoplasm is rarely associated with their presence in the lungs of these autopsied city dwellers not occupationally exposed to asbestos dust.

IN RECENT years studies have been conducted in a number of cities of the prevalence of ferruginous bodies in the lungs of city dwellers at autopsy.

In 1960 in Cape Town, South Africa, Thomson et al[1] reported finding that 30% of the men and 20% of the women at autopsy were found to have asbestos bodies present in their lungs.

The following year, Thomson and Graves[2] reported finding a similar prevalence in Miami, Fla. Men and women showed positive results in 31.6% and 20.4% respectively.

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In 1964 in Pittsburgh, Cauna, et al[3] reported finding 47% and 34%, respectively, in men and women autopsied.

The same year, Anjilvel and Thurlbeck[4] studied autopsies in Montreal, and found 57% in men and 34% in women.

Meanwhile, Meurman[5] was finding an overall prevalence of 70% in the urban element of a regional survey for asbestos bodies in Finland.

In 1966, Ghezzi, et al[6] reported finding 54% and 44%, respectively, in men and women autopsied in Milan, Italy.

As it appears that all the series studied so far have been of comparable age range and occupational backgrounds, the most likely explanation for this trend is that progressively more intensive searches are being conducted. The techniques employed in the foregoing studies, with the exception of the Finnish, have been basically the same: examination of unstained air-dried smears of juice expressed from cut portions of fresh postmortem lung or scraped from a cut lung surface. Some took their samples from the lung bases (Thomson et al[1,2]) and some from both upper and lower lobes (Cauna et al[3]). Anjilvel and Thurlbeck[4] sampled only left lungs. Meurman[5] examined 20 micron sections stained for iron by Perle's stain in his search for bodies.

In all the above studies, their authors have defined the bodies morphologically in similar terms, and have described them as "asbestos bodies" on the grounds that they considered only asbestos fibers to be responsible for the formation of such bodies and to be at their core. However, as Meurman[5] points out, bodies with a striking or even

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complete resemblance to asbestos bodies have been found to have at their core, not asbestos, but elongated or fibrous particles of a variety of other minerals including biotite-fusain, hornblende-rutile, diatomaceous earth, graphite, and carborundum. Moreover, it has recently been shown by Gross et al[7] that bodies morphologically indistinguishable from true asbestos bodies can be produced in hamsters by three different man-made, nonasbestos fibrous materials (namely, silicon carbide "whiskers", ceramic aluminum silicate, and fibrous-glass). The term "ferruginous body" has, therefore, been adopted by the authors, to describe all such bodies unless and until the central fiber has been positively identified as asbestos. This generic term encompasses both asbestos and pseudo-asbestos bodies as previously employed.

In this paper, the ferruginous body is defined as an elongated golden to reddish brown structure usually with clubbed ends; the shaft which often shows a segmented or beaded appearance is usually straight, but sometimes curvilinear with a tendency towards symmetry; usually it is from 3 to 5μ in diameter and 20 to 100μ in length. The coating contains iron demonstrable by Perle's stain (prussian blue reaction), and which is probably composed of ferritin or a ferritin-like protein material (Davis[8] and Collet, written communication, June 1966); it may cover the structure completely, masking the central fiber from direct view, or may be incomplete in the central portion of the shaft or in the interstices of the body, revealing an expanse of naked fiber. In this study, only ferruginous bodies with transparent or no visible central fibers were counted.

Materials and Methods

The 100 lungs comprising the material for this study were supplied to us, formalin-fixed, through the central autopsy room of the Presbyterian University Hospital, Pittsburgh, and came from autopsies performed on patients who died in five metropolitan Pittsburgh hospitals during the period from late 1966 through June 1967. The lungs were not, therefore, from consecutive autopsies and were unselected by us. There were 56 men, age range 37 to 87 years (mean age 65), and 44 women, age range 16 to 90 (mean age 59).

Thirteen of the men and six of the women were Negroes. Death had resulted from a wide variety of causes; 32 died from malignant conditions, including two cases of primary lung cancer (both men). There was no evidence of mesothelioma in any of the patients at autopsy. The lungs were examined superficially for calcified pleural plaques, and none were found; however, as some of the lungs were incomplete when received, there is a possibility that their presence may have been missed in some instances.

The lungs were sliced longitudinally on a commercial electric "bacon slicer," into slices approximately 3 mm in thickness and a total of about 300 gm (wet weight) was sliced in this way in each case. The slices were floated on the surface of about 2½ inches of domestic laundry bleach, undiluted (active constituent 5.25% sodium hypochlorite), contained in individual plastic pails of 9½ inches internal diameter at the base. Immediately as the lung tissue came into contact with the bleach, there was vigorous effervescence with the evolution of chlorine, which ceased after a few hours. Only the surface in contact appeared to be attacked. The pails were left undisturbed in a fume hood for 18 to 24 hours. At the end of this period the floating lung slices were seen to have shrunk considerably in area. The attacked undersurface was seen to be bleached yellowish white. At the bottom of the bucket, a dirty-looking grey to black sediment had formed. In some cases this was firmly adherent to the bottom and sides of the bucket as a greyish film. In others, the sediment appeared to be quite loose and readily resuspended in the spent bleach. In the former cases with an adherent film it was possible, after picking off and discarding the shriveled lung tissue, to decant all the spent bleach which was also discarded. The film was then dissolved off the inner surface of the pail by gentle agitation with about 200 ml of a 50% mixture of chloroform and 95% ethanol. The turbid, grey suspension so obtained was then decanted into a liter-separating funnel and shaken with an equal volume of clean water and allowed to stand. In the cases in which the sediment was nonadherent, about three fourths of the spent bleach only was carefully decanted, leaving most of the sediment in the remaining one fourth bleach. This was then mixed in the bucket with a similar volume of the chloroform-alcohol mixture and the whole swirled around the pail a few times and then decanted into a separating funnel.

On standing for ten minutes or so, the contents of the separating funnel were seen to separate into three layers. The lower, denser chlo-

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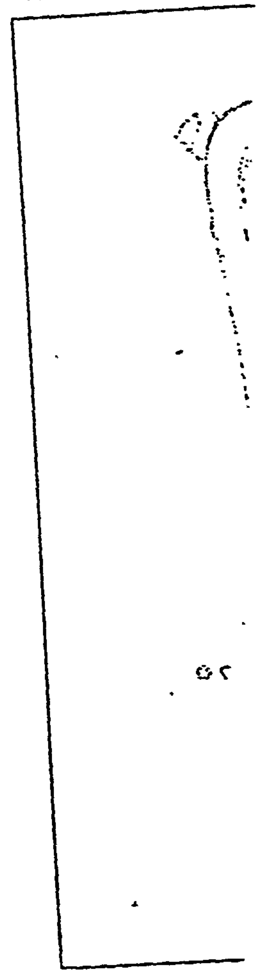

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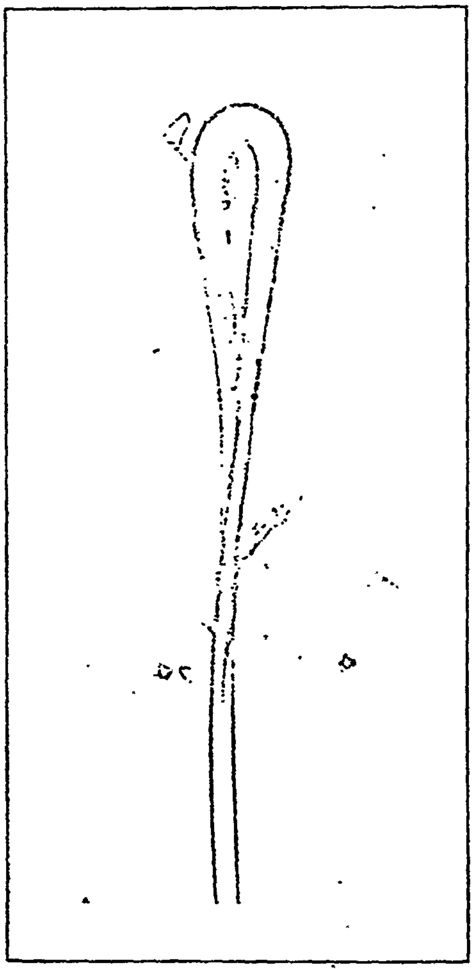
roform layer was more or less clear and amber colored and contained the denser, mainly inorganic, insoluble, mineral residue from the digested portion of the lung, plus any ferruginous bodies that might be present. The upper watery layer was turbid and grey, and contained sodium chloride, any undecomposed sodium hypochlorite and other soluble products of the digestion of lung tissue and blood, formalin, and a variable amount of organic debris in suspension. At the interface between the two main layers was invariably a dark grey to jet black layer, from 1 mm to 2 cm in depth, according to the amount of carbonaceous material present in the original lung, consisting mainly of carbon

Fig 1.—End of naked fiber seen in concentrate smear from human lung in this series. Appearance implies fusion during formation of fiber (unstained, × 1100).



together with a certain amount of lipidic material (there was also evidence that some lipid material was held in solution in the chloroform). The lower layer only was run off into an elongated glass tube with ground-glass stopper, mixed with a similar volume of fresh clean water and vigorously shaken. This time the emulsion formed was usually much more stable and was poured into 50-ml centrifuge tubes and spun for ten minutes at 2,000 rpm. Once more there was a three-layer separation with relatively little carbonaceous material at the chloroform-water interface, plus a dense deposit at the very apex of the tube, from 1 to 3 mm in depth and ranging in color from dirty white through greyish pink to a distinct reddish brown. Occasionally this deposit was almost black, indicating the persistence of carbonaceous material adherent to the denser matter. In such cases, a further separation was performed by resuspending the deposit in more chloroform-water emulsion and recentrifuging. Both chloroform and watery layers were finally decanted and discarded, and any carbonaceous-lipid ring adherent to the walls of the tube removed with cotton wool swabs before the tube was righted. The apical deposit was suspended in two or three drops of distilled water, plus two drops of dimethyl sulphoxide as a preservative and was then ready for microscopy as a smear.

Smears were prepared from a drop of the suspension of as near constant size as possible, using uniform pipettes held vertically, and allowing a free-falling drop to form and fall onto the slide. The smear was allowed to form by the natural spread of the drop only. It was found feasible to examine the smears initially in the wet state under low power (16 mm), under which conditions the majority of the ferruginous bodies were very readily seen and counted under transmitted light. Subsequently, as they became air dried, they were coverslipped with a mounting medium (Permount) and reexamined under high power for confirmation of very small bodies or otherwise doubtful structures.

Quantitative Estimation of Relative Abundance.—It is appreciated that employing a technique of this kind, with possibilities of sampling variation at almost every stage, only a very crude estimate as to the relative abundance of ferruginous bodies in the original lung tissue can be formed. However, the specimens did seem to form naturally into three categories. There were those in which examination of several smears (five was the maximum number practicable with the amount of material avail-

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able) was necessary to find a single ferruginous body. These were arbitrarily classified as "Abundance Category 1" and were taken to include those where no more than one body was found in any one smear.

Next, those specimens in which from two to five bodies were found in any one smear were placed in "Abundance Category 2" and lastly, those yielding over five bodies in any one smear were classified as "Abundance Category 3." The three negatives in this series yielded no bodies in five smears (approximately 2,000 low-power fields). The specimen in category 3 with the most bodies showed 32 in one smear. For comparison, a smear was prepared by the same technique, from the lung of a known clinical case of asbestosis. The resulting density of asbestos bodies was so high that they could not be counted with any accuracy, but would be of the order of many hundred in one smear.

Routine histological sections stained with hematoxylin and eosin were prepared from 20 of the lungs showing the most ferruginous bodies by the digestion technique.

Results

Ferruginous Bodies.—Ferruginous bodies were found in all but one of the lungs taken from men (98%), an 89-year-old white man.

Ferruginous bodies were found in all but two of the lungs taken from women (95.5%). The two negative results were found in the lungs of white females: one 16 years of age, and the other 42.

The Abundance Categories, mean ages in each category, and percentage in each category with malignant disease for both sexes are given in the Table.

The relative abundance of ferruginous bodies is significantly higher in men than in women ($X^2 = 8.973$, indicating that sex and abundance category are associated, at a 97.5% significance level). The age range for men was 37 to 87, and for women, 24 to 90. There seems to be a slight tendency for the relative abundance to increase with age, but the range of mean ages between the different abundance categories is small compared to the overall age ranges, and it was not possible to demonstrate statistical significance for this tendency.

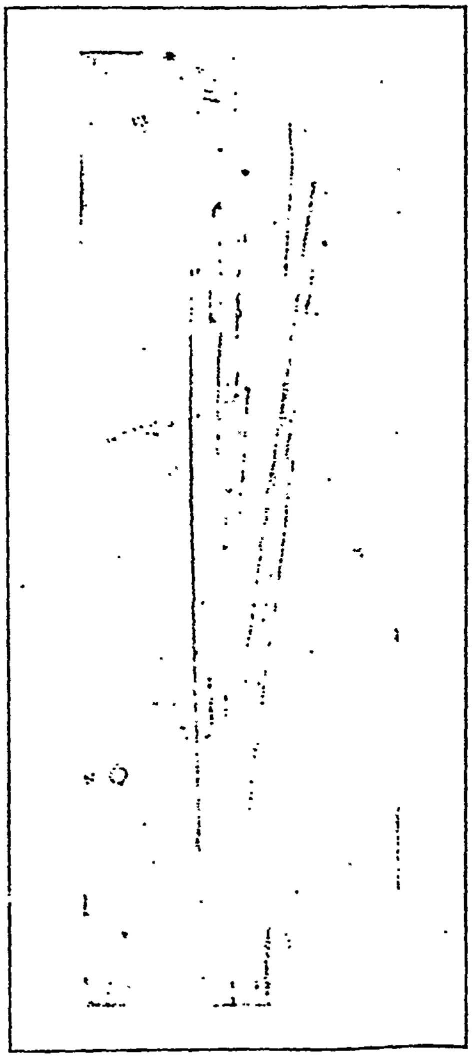
There does not appear to be any definite association between malignancy in general and relative abundance of ferruginous bodies in the lungs in this series.

There was definite histologic evidence of interstitial pulmonary fibrosis in only two of the lungs examined in category 3, but this was not of the type seen in asbestosis.

The two cases of primary carcinoma of the lung were both in category 2.

Naked Fibers.—The digest concentrates contain not only ferruginous bodies, but varying amounts of insoluble and relatively dense debris, denser, that is, than 1.5 gm/cc, the density of chloroform. Where carbon

Fig 2.—One end of naked fiber showing multiple longitudinal fission as seen in asbestos fibers (unstained, × 1100).



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persists in the concentrate from heavily pigmented lungs, it presumably does so by adhesion to denser mineral matter. Of particular interest, however, is the presence of what appear, in the light microscope at least, to be naked fibers of various types. Nearly all are translucent and rectilinear. Their range of dimensions is similar to that for the ferruginous bodies. Most appear to have fractured ends, and the fracture is usually conchoidal in appearance. Some, however, have fused ends or a "shepherd's crook" configuration (Fig 1). The latter we consider to be undoubtedly man-made, either "mineral wool" or fibrous glass, both of which materials are made from fused material which is spun and drawn out in a jet of steam or air. One naked fiber was found which shows the characteristic longitudinal fission associated with asbestos fibers (Fig 2). Whether such fibers were destined to become ferruginous bodies, but for the supervening death of the host, we cannot say.

Comment

Fibrous dust particles would appear to be part of the modern urban environment, at least of industrial cities. Cralley et al[9] comment upon their ubiquity in a recent paper. They give a long list of materials of animal, vegetable, and mineral origin, both natural and synthetic, which have the capability of forming fibers of respirable dimensions. As far as the naked fibers in our concentrates are concerned, organic materials may be ruled out as being unlikely to survive the prolonged action of sodium hypochlorite, and also being of lower density than chloroform. However, once such a fiber has become coated to form a ferruginous body, it may well survive into the concentrate.

Ferruginous Bodies.—In this series, only ferruginous bodies with transparent or invisible central filaments have been counted, as these are potentially, though not necessarily, asbestos bodies. However, in many smears additional ferruginous bodies were seen with opaque black central filaments (Fig 3).

A small number of ferruginous bodies were seen which exhibit longitudinal fission of the central filament and multiple extremities. Figure 4 shows such a body with one

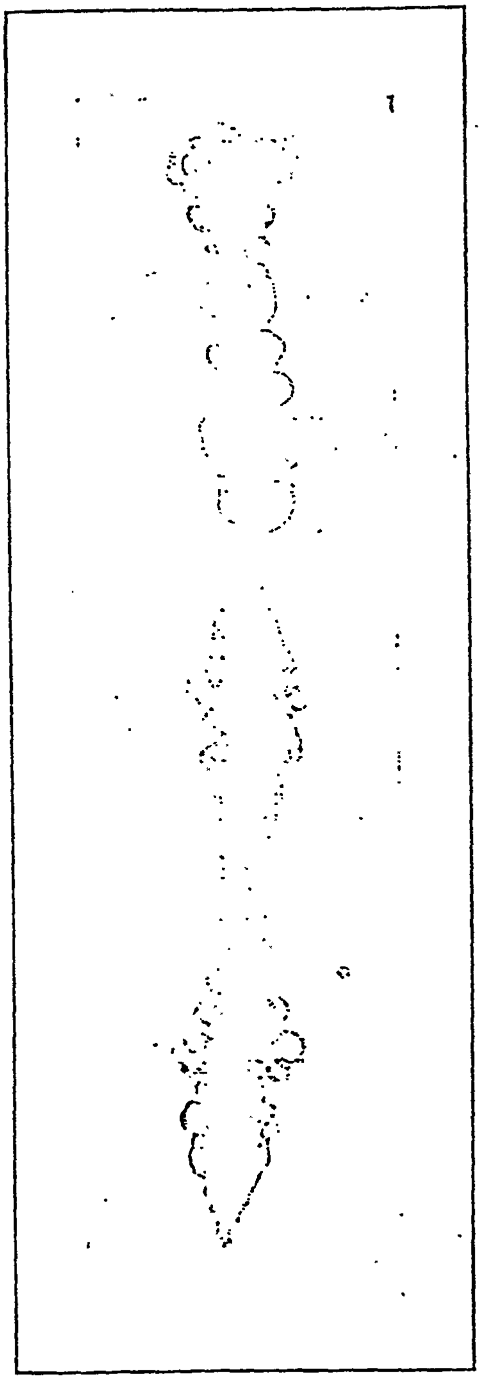

Fig 3.—Ferruginous body showing marked beading or segmentation in coating, and opaque central filament (unstained, × 1100).

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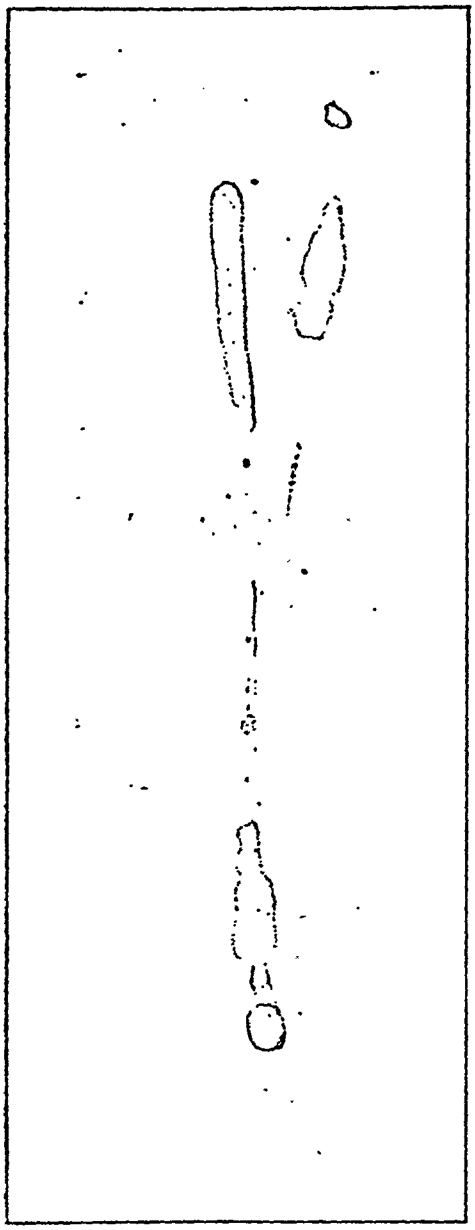

Fig 4.—Ferruginous body showing bifurcation of shaft and one double end. Appearance strongly suggestive of asbestos body (unstained, × 1100).

double end. Such bodies are almost certainly asbestos bodies, for we know of no other material which undergoes longitudinal fission on this microscopic scale.

*Distribution of Cases**

Abundance Category	No. of Cases	Distribution (%)	Mean Age	With Malignancy (%)
Men				
1	21	38	62	33
2	15	27	64	40
3	19	35	70	21
Total	55	100	65	33
Women				
1	29	69	57	31
2	8	19	68	38
3	5	12	64	20
Total	42	100	60	31

*Mean age, prevalence of malignancy by sex, and abundance of ferruginous bodies.

However, full and positive identification of both central filaments and naked fibers will have to await the further development of physical or physicochemical techniques. Positive identification of asbestos fibers by their distinctive electron-diffraction pattern is already underway in this laboratory.

Significance of Findings.—There is an urgent need for more data in order to form any estimate of the significance of ferruginous bodies and naked fibers in the human lung in terms of pathogenesis, actual or potential. The definite facts are few. It is now widely accepted that asbestos dust, if inhaled in sufficient quantity such as only occurs in those occupationally exposed in the absence of adequate dust control, may be carcinogenic to the human lung. In rats, the inhalation of chrysotile asbestos dust in high concentrations has been associated with a 35% prevalence of lung cancer.[10] There is also strong evidence that crocidolite, a particular variety of asbestos largely mined in South Africa and little used in the United States, may cause pleural mesothelioma—a rare tumor in man, after a time lag of many years.

Lastly, there is the established fact that primary carcinoma of the lung has increased in incidence in most industrialized countries over the past 50 years. However, little or nothing is known at present about the quantitative relationships involved. In the case of asbestos, what is the minimum pathogenic or carcinogenic dose? The only data on this question are afforded by the observation that the excess experience of lung cancer shown by certain asbestos industry workers in the 1930's and 1940's has been greatly reduced or altogether eliminated by the implementation of dust-control measures in the plant.[11] However, it is unlikely that such dust-control measures have reduced the level of exposure of the workers to respirable asbestos dust as low as that of the general population. Is the asbestos fiber rendered completely and permanently innocuous to the human body once it has become coated to form an asbestos body so that only the naked asbestos fibers are pathogenic? Which if any of the nonasbestos fibers, coated or uncoated, are pathogenic to the human lung and in what quantity? Can ability of nonasbestos fibers to provoke ferruginous body formation be related in any degree to pathogenicity? There is evidence from animal experiments that it cannot. Gross et al[7,12] have shown that fibrous glass, ceramic aluminum silicate fibers, and silicon carbide whiskers are relatively inert in animal lungs, beyond the fact of ferruginous body formation.

In conclusion it can be said that at this time there is no evidence that the presence of asbestos dust particles or dust particles of other fibrous materials, coated or uncoated with ferruginous material, in the lungs of individuals not occupationally exposed to these dusts in industrial urban communities, is making any contribution to pulmonary disease, inflammatory or neoplastic.

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Chairman Emmanuel Farber, MD, Department of Pathology, University of Pittsburgh School of Medicine, provided the lungs investigated in this study.

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