Inhaled Cellulosic and Plastic Fibers Found in Human Lung Tissue


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Abstract

We report the results of studies undertaken to determine whether inhaled plant (i.e., cellulosic; e.g., cotton) and plastic (e.g., polyester) fibers are present in human lungs and, if so, whether inhaled fibers are also present in human lung cancers. Specimens of lung cancer of different histological types and adjacent nonneoplastic lung tissue were obtained from patients undergoing a lung resection for removal of a tumor. With the protection of a laminar flow hood and safeguards to prevent contamination by extraneous fibers, fresh, nonfixed, and nonstained samples of lung tissue were compressed between two glass microscope slides. Specimens in these dual slide chambers were examined with a microscope configured to permit viewing with white light, fluorescent light, polarizing light, and phase-contrast illumination. Near-term fetal bovine lungs and nonlung human tumors were used as controls. In contrast to the observations of these control tissues, morphologically heterogeneous fibers were seen repetitively in freshly excised human lung tissue using polarized light. Inhaled fibers were present in 83% of nonneoplastic lung specimens (n = 67/81) and in 97% of malignant lung specimens (n = 32/33). Thus, of the 114 human lung specimens examined, fibers were observed in 99 (87%). Examination of histopathology slides of lung tissue with polarized light confirmed the presence of inhaled cellulosic and plastic fibers. Of 160 surgical histopathology lung tissue slides, 17 were selected for critical examination; of these, fibers were identified in 13 slides. The inhalation of mineral (e.g., asbestos) fibers has been described by many investigators; we believe, however, that this is the first report of inhaled nonmineral (e.g., plant and plastic) fibers. These are candidate agents contributing to the risk of lung cancer.

Introduction

In experiments in which we viewed resident macrophages in fresh human lung tissue with an epifluorescent microscope (1), we noted the presence of inhaled fluorescent fibers (2). This observation prompted us to examine further surgically excised human lung tissue that had not been fixed, sectioned, or stained for inhaled fibers of different types.

It is widely recognized that airborne fibers are ubiquitous. We hypothesize that some of these fibers may be inhaled. Furthermore, we theorize that some of these fibers may escape the mucociliary clearance mechanisms of the lung, particularly fibers inhaled by habitual smokers or individuals whose clearance mechanisms have been impaired (3, 4).

The rationale for pursuing this investigation is that most environmental fibers are cellulosic or plastic, and it is unlikely that either fiber type would be biodegraded in the lung. The inhaled fibers may induce a foreign body reaction, often mediated by macrophages. The inhaled fibers could remain sequestered within the lung for a prolonged time, possibly for life (2). Furthermore, these fibers often contain different dyes, mordants, plasticizers, and other chemicals, some of which may be toxic to lung tissue. These agents associated with the fiber, either bound or leached at various rates, may damage any one of several types of cells in the tissue microenvironment adjacent to the fiber. Inhaled cellulosic and plastic fibers may pose a health risk and, specifically, may be candidate confounders for acute and/or chronic inflammation as well as diverse nonmalignant and malignant lung diseases.

The inhalation of mineral (e.g., asbestos) fibers is widely recognized (5-16), and different protocols have been established to identify and enumerate asbestos and other mineral fibers in human lungs (12, 14-16). With the procedure used most frequently, human lung tissue is subjected to high-temperature ashing; then, the residue is examined for mineral fibers with a scanning electron microscope (12, 14-16). This technology has been used to examine the effects of fiber characteristics (e.g., length and width) on lung deposition, retention, and disease (10). In addition, the role of asbestos in the etiology of different human nonneoplastic (e.g., fibrosis) and malignant (e.g., mesothelioma and bronchogenic carcinoma) diseases have been documented in many epidemiological studies and animal experiments (reviewed in Refs. 5, 9-14, and 16).

By using fiber isolation and identification techniques similar to those that have proven successful for studying asbestos, the inhalation toxicology of manmade vitreous fibers (e.g., glasswool) and nonmineral organic fibers (e.g., slagwool, rockwool, and refractory ceramic fibers) have been studied in animals (17, 18) and ex vivo (19-22).

A manual and computer-assisted search of the literature, however, has failed to identify a publication describing the inhalation of plant and plastic fibers. We believe, therefore, that this is the first report of the inhalation of plant and plastic fibers.
Inhaled Fibers Found in Human Lung Tissue

Fig. 1. Inhaled fibers observed in nonneoplastic human lung samples from different patients. For each panel, the lung tissue sample was fresh, nonfixed, and nonstained and had been mounted in a dual-slide chamber as described in “Materials and Methods.” A, fresh human lung tissue (~0.05 g) compressed in a dual-slide chamber. A band of carbon exists in the lower right quadrant of the lung sample. Two dabs of silicone grease (arrows) appear as half-circles adjacent to the tissue (scale, 7 mm); B, two golden asbestos fibers (i.e., ferruginous bodies), one atop the other, in a fresh nonneoplastic lung sample from a patient with a squamous cell carcinoma of the lung and who was known to have had an occupational exposure to asbestos (white light, ×250). Inhaled fibers were also detected in a malignant lung tumor of the patient (see Fig. 2, O, polarized light and × white light); C, view with a fluorescent microscope of a nonneoplastic lung sample of a 52-year-old habitual smoker with a poorly differentiated adenocarcinoma of the lung. Numerous macrophages, some with ingested carbon, are prominent. Both the macrophages and lung parenchyma fluoresce (FITC filter, ×50); D, a wishbone-shaped inhaled fiber is inconspicuous in a lung sample that was viewed with a white-light microscope. The lung sample was from a former smoker and machine operator with a moderately differentiated adenocarcinoma (×50); E, same field as in D but as viewed with a fluorescent microscope. The bright red fluorescence exhibited by the wishbone-shaped plastic fiber enabled us to locate the fiber readily (TRITC filter, ×50); F, inhaled plastic fiber, displaying a bright yellow fluorescence, is present in fresh nonneoplastic lung tissue. Note also the apple-green lung parenchyma in the lower portion of the specimen (FITC filter, ×50); G, inhaled fiber entwining a blood capillary in a fresh nonneoplastic lung sample collected from a patient.
### Materials and Methods

#### Human Subjects

Included in our study were patients with primary lung cancers, patients with cancers that had metastasized to the lung, and deceased cancer patients from whom lung tissue and/or nonlung tissue had been collected at the time of autopsy (n = 114 specimens). Patient information was collected that included diagnosis, gender, age, occupation, and treatment status, including previous chemotherapy and/or radiation treatment. The anatomical site of the specimen resected from the lung was identified, and surgical pathology reports were obtained. In most cases, the smoking history of the patient was obtained by self-report. Of 92 patients whose smoking history was known, 22 (24%) were current smokers, 56 (61%) were former smokers, and 14 (15%) were individuals who had never smoked. The research protocol for the studies reported herein was approved by the Review Board of the Institute, and a signed informed consent was obtained from all patients or their representatives before enrollment.

#### Fresh Human Lung Tissue

Samples of the surgically excised cancers and adjacent nonneoplastic lung tissue were submitted for histopathology. Meanwhile, under the management of the Tissue Procurement Program at this Institute, a portion (>0.5 g) of the nonneoplastic lung tissue was collected (mean ± SD, 6.6 ± 6.9 g; n = 114 specimens) for our study of inhaled fibers. In cases in which the lung cancer was relatively large (≥0.4 g), a piece (mean ± SD, 2.9 ± 4.1 g; n = 26 specimens) of the tumor tissue was also collected for analysis of inhaled fibers. Each specimen was assigned a study number and placed in a new, wide-mouth, polypropylene specimen jar (Nalge Co., Rochester, NY) that had been flushed repetitively with fiber-free water. The specimen was then transported, usually within 1 h after excision, to the research laboratory. The excised lung tissue was handled in a manner to safeguard against contamination by extraneous (i.e., noninhaled) fibers.

#### Experiment and Tissue Controls

Different controls were used to define and monitor fiber contaminants. A partial listing included: (a) dual-slide chamber without tissue; (b) tissue from fetal bovine lungs; (c) surgically excised human nonlung tumors; (d) and surgically excised whole lung of a cadaveric organ donor.

Various controls were used in pilot studies to define the most suitable method for examining freshly collected human lung tissue. Besides its use in qualifying different parameters of the proposed dual-slide chamber method, the control tissues also served as the fiber-free tissue standard in examinations of human lung specimens.

A dual-slide chamber without tissue was also prepared. This control served as a means of monitoring fiber contaminants on the glass slides and the environment in which the chambers were assembled.

The fresh, near-term, fetal bovine lungs were obtained with certification from the United States Department of Agriculture and were donated by a licensed commercial meat packing plant. The prenat al bovine lungs served as lungs controls (i.e., no inhaled fibers). Therefore, any fibers detected were contaminants. The fetal bovine lung was cut into small specimens that were stored individually at −20°C. After thawing a specimen at room temperature, multiple samples of the control lung were collected and mounted for microscopic examination using the same procedure as that for samples of a fresh human lung specimen (see below).

Surgically excised human nonlung tumors were also used as control tissue. Tumors that proved most useful for routine purposes were very large (>40 g) leiomyosarcomas. The frozen tumor tissue was used as control tissue for repetitive examinations of different lung specimens.

Multiple specimens from a whole human lung were also used as a surgically excised tissue control. The lung was removed in surgery from a cadaver (nonsmoker) who was a heart transplant donor.

In all instances, samples collected for examination were obtained under the protection of a laminar flow hood. Furthermore, the sample was taken from the innermost part of the tissue specimen, thus excluding any fiber contaminants that may be on the surface of the specimen and that were from surgical sponges, drapes, clothes, and other sources.

The different control tissues were processed and examined using the dual-slide chamber as described below.

### Table 1  Inhaled fibers present in human nonneoplastic and malignant lung specimens

<table>
<thead>
<tr>
<th>Patient’s diagnosis</th>
<th>Lung specimens with inhaled fibers in:</th>
<th>Nonneoplastic tissue</th>
<th>Malignant tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squamous cell carcinoma</td>
<td>26/31†</td>
<td>9/9</td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>16/20</td>
<td>7/8</td>
<td></td>
</tr>
<tr>
<td>Other classifications</td>
<td>13/13</td>
<td>5/5</td>
<td></td>
</tr>
<tr>
<td>Metastatic lesions</td>
<td>6/9</td>
<td>4/4</td>
<td></td>
</tr>
<tr>
<td>Large cell carcinoma</td>
<td>4/6</td>
<td>3/3</td>
<td></td>
</tr>
<tr>
<td>Carcinoid tumors</td>
<td>2/2</td>
<td>1/1</td>
<td></td>
</tr>
<tr>
<td>Mesothelioma</td>
<td>ND</td>
<td>3/3</td>
<td></td>
</tr>
<tr>
<td>Subtotals</td>
<td>67/81 (83%)</td>
<td>32/33 (97%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>99/114 (87%)</td>
<td></td>
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</tbody>
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† Lung tissue was fresh, nonfixed, and nonstained.
1 Number of specimens with inhaled fibers/total number of specimens examined.
* ND, not done.
**Dual-Slide Chambers.** After the weight, gross appearance, and other features of the fresh human lung specimens were recorded, samples of each specimen were prepared for microscopic examination using the following method. A 10 × 20 cm stainless steel surgical instrument tray was washed, flushed thoroughly with filtered deionized water, and, without drying, placed in a laminar flow hood. A thoroughly washed pair of forceps and sharp-nose scissors, used for holding and cutting the lung specimen, were placed in the steel tray. Afterward, 15 ml of 95% ethanol were added to the tray that was then rotated to wet the instruments and the walls of the tray. The ethanol was ignited to burn or destroy any fibers that may have been present.

Most of the lung specimens were of sufficient size to permit us to select samples from the interior portion. This provided additional assurance that the observed fibers were inhaled fibers and not extraneous fibers that had contaminated the surface of the specimen. From each lung specimen, three samples were analyzed. Usually, however, sufficient tissue was available to permit us to study six or more samples.

A dual-slide chamber was prepared with 1 × 3-inch microscope slides washed and rinsed thoroughly with deionized water. The slides were maintained in covered glass staining dishes filled with deionized water to prevent exposure to airborne fibers and other contaminants. Working in the fiber-free environment of a laminar flow hood, a slide was retrieved from the dish, submerged and swirled in 95% ethanol, and ignited (i.e., flamed). After allowing the slide to cool, a fresh lung sample (~0.05 g) was placed on it. The slide was then added four to six dabs (~3 μl) of high-vacuum silicone grease (Dow Corning Corp., Midland, MI). The grease was used to bond together the two microscope slides that formed the chamber. The grease, expressed from a 5.0 ml plastic syringe, was distributed onto the slide and around the tissue such that when the tissue was compressed, the grease would not contact the lung tissue. For example, the lung samples were collected and mounted under the protection of a laminar flow hood with intake and exhaust HEPA filters (certified ≥0.3 μm particle exclusion). The efficiency of the flaming procedure for destroying fibers was demonstrated by examining with a stereo-zoom microscope and polarized light, before and after flaming, various items contaminated intentionally with a mixture of different fibers that included cellulotic and plastic fibers that differed with respect to color, diameter, length, surface morphology, and other features.

Detection of Inhaled Cellulosic and Plastic Fibers. A fiber, defined previously (9), was recognized as having a length: diameter ratio (i.e., an aspect ratio) of ≥3 and a length of ≥5 μm. A lung sample was viewed initially with white light to analyze tissue parenchyma, erythrocytes, leukocytes, carbon deposits, blood capillaries, other features of the fresh specimen, and to detect any artifacts or defects in the sample preparation. Subsequently, in the following sequence, the lung sample was examined with polarizing light, fluorescent light, and phase-contrast illumination to detect inhaled fibers. Microscope stage coordinates of a fiber in the fresh lung sample were recorded so that a particular fiber could be relocated quickly. For all specimens defined in Table 1, photographic documentation of inhaled fibers was obtained, usually with Kodak Ektachrome Elite film (ISO 400). Cellulosic and plastic fibers were recognized by their morphology and birefringence (23, 24). The ease with which the two fiber types can be distinguished is illustrated (see “Results”). A detailed schema used routinely by forensic pathologists and members in the fiber manufacturing industry has been presented elsewhere (23, 24).

**Microscopic Examination of Lung Tissue.** The mounted lung samples were examined with a microscope having vertical and transmitted light optics. The fluorescence system included filters that were optimal for viewing FITC and TRITC. FITC and TRITC are green- and red-appearing fluorochromes, respectively, that are commonly used as imaging markers for diverse applications, including the identification of leukocyte surface membrane antigens and receptors. Furthermore, the microscope had phase-contrast optics (Reichert-Jung, Cambridge Instruments, Inc. Buffalo, NY). The microscope was equipped also to permit viewing with polarized light. The polarized light configuration included an analyzer turret and a polarizer with a full-wavelength (530 nm) plate (Reichert-Jung). This microscope enabled us to view fresh lung samples, histopathology slides, and controls consisting of various types of fibers and particles with a ×10 or ×20 objective and to examine a selected microscopic field with white light (i.e., bright field), polarizing light, fluorescent light, and phase-contrast illumination. The dual-slide chamber could be inverted, which permitted viewing of the compressed tissue from either side. Furthermore, substitution of the upper slide in the dual-slide chamber with a thin (no. 2) microscope coverglass (22 × 50 mm) reduced the working distance. Thus, a lung sample in the coverglass and microscope slide chamber could be viewed with a ×40 objective that provided higher resolution of lung parenchyma and inhaled fibers. The ×40 objective, however, was not required for detecting inhaled fibers.

**Detection of Inhaled Cellulosic and Plastic Fibers.** A fiber, defined previously (9), was recognized as having a length: diameter ratio (i.e., an aspect ratio) of ≥3 and a length of ≥5 μm. A lung sample was viewed initially with white light to analyze tissue parenchyma, erythrocytes, leukocytes, carbon deposits, blood capillaries, other features of the fresh specimen, and to detect any artifacts or defects in the sample preparation. Subsequently, in the following sequence, the lung sample was examined with polarizing light, fluorescent light, and phase-contrast illumination to detect inhaled fibers. Microscope stage coordinates of a fiber in the fresh lung sample were recorded so that a particular fiber could be relocated quickly. For all specimens defined in Table 1, photographic documentation of inhaled fibers was obtained, usually with Kodak Ektachrome Elite film (ISO 400). Cellulosic and plastic fibers were recognized by their morphology and birefringence (23, 24). The ease with which the two fiber types can be distinguished is illustrated (see “Results”). A detailed schema used routinely by forensic pathologists and members in the fiber manufacturing industry has been presented elsewhere (23, 24).

**Safeguards against Extraneous Fibers.** Different precautions were used to prevent contaminating the specimen with foreign fibers. For example, the lung samples were collected and mounted under the protection of a laminar flow hood with intake and exhaust HEPA filters (certified ≥0.3 μm particle exclusion). The efficiency of the flaming procedure for destroying fibers was demonstrated by examining with a stereo-zoom microscope and polarized light, before and after flaming, various items contaminated intentionally with a mixture of different fibers that included cellulotic and plastic fibers that differed with respect to color, diameter, length, surface morphology, and other features.

The most common sources of contamination were glass items, including microscope slides, coverglasses, and glass specimen vessels. Glass has a high negative surface charge and attracts readily certain types of fibers. It proved advantageous, therefore, not to allow glass items to dry outside the fiber-free environment of the laminar flow hood. In addition, we substituted glass specimen vessels with polypropylene jars.

When working at the laminar flow hood, disposable non-woven plastic apparel was worn instead of long-sleeve cotton lab coats. To insure that the crystal-like structures observed in some lung samples could not be attributed to talc or starch from the glove, particle-free gloves were used, and gloved hands were washed and rinsed with filtered water.

The first step in an examination was the inspection of the lung tissue with a ×10 objective of the area surrounding the tissue (e.g., background). The presence of fibers in the background, indicative of contamination, would disqualify the sample; very few preparations, however, were rejected. As an

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1 The abbreviation used is: TRITC, tetramethylrhodamine 5-isothiocyanate.
additional safeguard, selected lung specimens were contaminated intentionally with a mixture that contained fibers from different sources, including a clothes dryer lint trap, carpet vacuum cleaner, floor dust, and glass surface of computer monitors. To this mixture we added color-coded reference fibers (e.g., cotton, paper, polyester, nylon, and other fibers). Fresh human lung specimens that had been exposed intentionally to short fibers of diverse origins proved useful in training us to detect different types of fibers and to distinguish inhaled fibers from contaminants. To cite but a single example, extrinsic fibers were: (a) on the surface and not within the lung tissue; (b) not discolored; (c) without surface carbon and tobacco tar; and (d) without adherent host inflammatory cells (e.g., macrophages).

**Inhaled Fibers in Histopathology Slides.** Having examined fresh lung specimens in dual-slide chambers, additional observations were performed of the same human lung specimen that had been prepared for clinical histopathology. The microscope slides of paraffin-embedded lung tissue that had been stained with H&E were prepared in a conventional manner by certified histopathology technicians of the Department of Pathology. We examined the lung tissue sections with white light and polarizing light for inhaled fibers.

**Results**

**Inhaled Fibers Discovered in Nonneoplastic Human Lung Tissue.** We conducted a study in which 81 surgically excised, fresh, nonneoplastic lung specimens from patients with lung cancer were examined for inhaled fibers. Of the 81 specimens, 67 (83%) contained one or more fibers (Table 1). Of the 31 nonneoplastic lung specimens from patients with a squamous cell carcinoma, 26 (84%) contained inhaled fibers. Fibers were identified in most of the other lung cancer patients studied, including those with an adenocarcinoma (n = 16 of 20; 80%), other classifications (n = 13 of 13; 100%), metastatic lesions (n = 6 of 9; 67%), large cell carcinoma (4 of 6; 67%), and carcinoid tumors (2 of 2; 100%; Table 1).

**Cancers in the Human Lung Contain Inhaled Fibers.** Having discovered fibers in nonneoplastic lung tissue, we sought to learn whether fibers were also present in freshly excised malignant human lung tissue. In this study, 32 of 33 (97%) of the malignant tumors contained fibers (Table 1). Fibers were detected in all squamous cell carcinomas (n = 9 of 9; 100%), adenocarcinomas (n = 7 of 8; 88%), other classifications (n = 5 of 5; 100%), metastatic lesions (n = 4 of 4; 100%), large cell carcinoma (n = 3 of 3; 100%), and a carcinoid tumor (n = 1 of 1; 100%; Table 1).

**Absence of Fibers in Fetal Bovine Lung Tissue.** Fibers were not present in prenatal bovine lung tissue processed and examined using the same procedures as those used in analyzing human lung tissue. For example, in one experiment, four samples from a fetal bovine lung specimen and four samples from a human nonneoplastic lung specimen were mounted in dual-slide chambers by each of four examiners. The 32 chambers of each bovine and human specimen were mounted in dual-slide chambers. No fibers were observed in the fetal bovine lung samples (n = 20 samples) by any of the five readers. In three human samples, some fibers were present as clusters (>10, >25, and >60 fibers/cluster). Fibers in these bundles could not be counted accurately. Three substitute human samples were prepared and analyzed. The number of fibers (mean ± SD) in the human nonneoplastic lung specimens was 3.9 ± 3.5 (n = 60 samples; median, 3; range, 0–16).

**Morphology of Inhaled Fibers in the Lungs of Cancer Patients.** Photographic documentation of inhaled fibers was obtained for 106 human lung specimens (nonneoplastic specimens, n = 76; malignant specimens, n = 30). Figs. 1 and 2 illustrate the diversity of the fibers observed using the dual-slide chamber method.

**Fresh Lung Tissue Examined by White Light and Phase Contrast Microscopy.** A low-power view of a compressed lung sample in the dual-slide chamber is shown in Fig. 1A. The tissue is distributed uniformly, and its color typifies most of the lung specimens. In this lung sample, as for most other samples collected from smokers, tobacco tar and/or carbon-like masses were visible with the naked eye. With white light microscopy, ferruginous bodies (6, 13, 14), consisting of asbestos fibers coated with bead-like structures, were prominent in some lung samples (Fig. 1B). The beads that give the ferruginous bodies their characteristic golden-brown color are from macrophages and are thought to be an iron-mucopolysaccharide-protein (e.g., ferritin or hemosiderin) complex (6, 14). Rarely were fibers other than asbestos fibers with ferruginous bodies observed with a white-light microscope.

When compared with white-light microscopy, phase-contrast microscopy offered no advantage for viewing inhaled fibers.
Inhaled Fibers Found in Human Lung Tissue

Fig. 2. Views of fibers in fresh human lung cancer specimens. In each panel, the tissue was fresh, nonfixed, and nonstained and had been mounted in a dual-slide chamber. A. inhaled fibers are not apparent in this view with a white light microscope. The sample was from a patient with a bronchoalveolar carcinoma (×100); B, view of the same field as that of A but with a polarizing microscope configured with a one-fourth wavelength retardation plate. An inhaled fiber deep within the lung cancer tissue is evident (×100); C, photograph of the same field as that of A and B but as observed with a full-wavelength retardation plate (×100); D, inhaled fiber observed with a microscope configured to permit viewing of the tissue with a mixture of white and polarized light. This illumination scheme enabled use to view simultaneously the inhaled fiber and macrophages. The macrophages are present as tobacco-brown phagocytes adjacent to the fiber (upper left). Note that the fiber becomes indistinct (upper left) as it penetrates deeper into the tissue; in this panel, neither end of the long inhaled fiber is visible (see E). The tissue was from a 40 pack-year smoker with a moderately differentiated adenocarcinoma of the lung (×100); E, this view shows the proximal end of the fiber displayed in D. Together, D and E illustrate a portion of the total length of the inhaled fiber. Macrophages with ingested tobacco tar are also visible (×100); F, a fiber present in tissue obtained from a homemaker and smoker (1.5 packs/day) is shown penetrating deep within the tissue (polarizing light, ×100); G, two fibers inside a squamous-cell carcinoma of the lung from a nurse who had smoked for 40 pack-years (polarized light, ×50); H, carbon-coated fiber in lung cancer tissue of a habitual cigarette smoker (polarized light; ×200); I, another view, with similar lighting conditions, of the same cancer sample as that in the previous panel. This photograph reveals another inhaled fiber, similar to that in H. This fiber, however, is twisted, lying beneath a mantle of carbon (×200); J, two inhaled cellulosic fibers are shown in a multifocal bronchiol-alveolar carcinoma of the lung (white and polarizing light; ×100); K, present are two fibers within a malignant mesothelioma collected at autopsy from a 50 pack-year smoker and pipe fitter with known asbestos exposure. One is a broad, green, cellulosic fiber. Perpendicular and to the right of this prominent fiber is a thinner fiber that lies deep...
Inhaled Fibers Detected with a Fluorescent Microscope. We first observed inhaled fluorescent fibers while viewing fresh lung tissue to define a method for isolating macrophages (Refs. 1 and 2; Fig. 1C). The ease with which fluorescent fibers could be detected is illustrated by comparing the image of a fiber viewed with white (Fig. 1D) and fluorescent (Fig. 1E) light. Fig. 1D is a view of a fresh, nonneoplastic human lung sample that contains a wishbone-shaped fiber. With this lighting, the inhaled plastic fiber is detectable but not prominent. However, when viewed with a fluorescent microscope, this same inhaled fiber is glaring (Fig. 1E). Another inhaled fluorescent fiber is shown in Fig. 1F. The fiber has a bulb-shaped terminus, a feature characteristic of certain plastic fibers (e.g., polyester) that have been processed for anti-piling (e.g., reduction of fiber balls on polyester sweaters). Inhaled fluorescent fibers were visualized with a fluorescent microscope configured with a TRITC (Fig. 1E) or FITC filter (Fig. 1F). In most instances, the inhaled fluorescent fibers were visible with both FITC and TRITC filters.

In lung specimens obtained from smokers, the macrophages (1, 2, 25) and lung parenchyma (1, 2) fluoresce. These resident phagocytic leukocytes, with ingested tar, and lung tissue could be viewed with either FITC (Fig. 1C) or TRITC filters. As viewed with a FITC filter configuration, the parenchyma was often a brilliant yellow-green (Fig. 1C). With a TRITC filter configuration, the macrophages and parenchyma glowed a bright, uniform red (not shown). Both the macrophages and parenchyma resisted photobleaching. Human lung tissue that had been stored in a refrigerator retained their high level of fluorescence for at least 1 week.

Fibers Detected in Nonneoplastic Human Lung Tissue with a Polarizing Microscope. Viewing compressed nonneoplastic human lung tissue with a polarizing microscope proved to be the most effective means of finding inhaled fibers (Fig. 1, G–N). The use of polarized light for observing inhaled fibers was demonstrated in the following scheme. A compressed lung sample in a dual-slide chamber was inspected with polarizing light until a fiber was detected. Then, without changing the microscopic field, the polarizing filters were removed from the light path so that the same field was viewed with white light (e.g., Fig. 1H versus 1F and 1J versus 1K). With polarized light, the fiber was pronounced; with white light, the same fiber was often difficult to detect.

Particularly important was the finding that inhaled fibers were discernible deep within the lung sample that had been compressed between two microscope slides (Fig. 1, G, H, J, and L). Moreover, some inhaled fibers had adherent macrophages (Fig. 1H). Other inhaled fibers were blanketed with erythrocytes (Fig. 1L). We could distinguish readily inhaled fibers from birefringent collagen and elastin tissue (Fig. 1O).

Variable numbers of inhaled fibers were observed in lung samples collected from different patients and in samples from the same lung specimen. A few lung samples contained no fibers. In contrast, other lung samples contained many fibers. Moreover, a single microscopic field may reveal one fiber (Fig. 1, G, H, and J), two fibers (Fig. 1, J–M), or an agglomerate of many fibers (Fig. 1N).

Inhaled fibers were heterogeneous with respect to length, width, surface morphology, birefringence, color, and other features. We have observed fibers that were >250 μm in length (Fig. 1G). Other inhaled fibers were very wide (~50 μm; Fig. 1J). Some fibers were distressed (e.g., frayed and discolored). Most all fibers were thought to be biorefractive (i.e., refractory to digestion or dissolution) and biopersistent (e.g., escaped lung clearance and residing in the lung indefinitely).

Some fibers had unique morphological and/or optical features that greatly facilitated their identification. As an example, cotton fibers are known to have a twisted, ribbon-like form (Refs. 23 and 24; Fig. 1N). Other inhaled cellulosic fibers had features that were consistent with paper fibers. Wood fibers were recognized easily (Fig. 1M) but were seen infrequently. Plastic fibers were also seen, many of which (e.g., nylon and polyester) were highly birefringent.

In addition to inhaled fibers, we observed inhaled crystal-like structures. The inhaled particles were very diverse with respect to size, shape, birefringence, and other attributes. With polarizing light, the particles were conspicuous (e.g., Fig. 1K). In contrast, with white light, the particles were not readily discernible (e.g., Fig. 1J). In some lung specimens, the distribution of the inhaled particles within the lung tissue was highly localized. In other specimens, the particles were distributed randomly in the tissue (Fig. 1K).

Fibers Seen in Malignant Lung Tissue with Polarizing Light. Presented in Fig. 2, A–L, are views of inhaled fibers found in different lung cancers. These photographs also illustrate the utility of polarized light for detecting fibers in freshly collected lung cancers (Fig. 2, A–C). Fibers observed within the lung tumors were inhaled and were not an artifact, as exemplified by observations in which the fibers were: (a) deep within the tissue (Fig. 2, B–G, I, J, and M); (b) coated with carbon (Fig. 2H); (c) surrounded by tar-laden macrophages (Fig. 2, D and E); and (d) situated beneath a field of carbon particles (Fig. 2I). As noted for inhaled fibers in nonneoplastic lung tissue, fibers present in tumor tissue were morphologically heterogeneous (Fig. 2, B–M). In contrast, rarely were fiber contaminants observed in human nonlung tumors (e.g., leiomyosarcoma).

The absence of fibers in the nonlung tumors is particularly noteworthy because these specimens were surgically excised and handled in the same environment and with operating room practices similar to those used for procuring lung tumors.

Inhaled Fibers Discovered in Histological Sections. We have demonstrated also the presence of inhaled fibers in paraffin-embedded sections of human lung tissue that had been prepared using common histology methods (Fig. 2, N and O). We have observed that inhaled fibers may be overlooked when viewing lung tissue sections with a white-light microscope. In contrast, inhaled fibers and fiber contaminants were viewed readily with a polarizing microscope.

within the malignant lung tissue. Also notable are the inhaled birefringent, amorphous structures at the top (polarized light, ×500; L, several fibrils, which appear to be components of a large fiber (polarized light: ×100); M, a view of a fiber within a squamous cell carcinoma of the lung (left lower lobe) of a 37-year-old welder who is a 40 pack-year smoker. The birefringence and surface morphology of the fiber suggest that it is plastic. Other birefringent matter is present to the left of the fiber (×50); N, paraffin-embedded thin (thickness, ~5 μm) section of a nonneoplastic sample from the right lower lobe of a 71-year-old pipe fitter with a 50 pack-year smoking history (white light, H&E stained; ×200); O, view of the same field as in N but at viewed with polarizing light. Shown is an inhaled fiber that has been cut longitudinally with a microtome knife. The fiber is with adherent macrophages, some of which are multinecrotic cells within the interstitial space. This area is associated with marked chronic and granulomatous inflammation and with foreign body type giant cells. The background parenchyma exhibits prominent emphysematous change, type II pneumocyte hyperplasia, anthracosis, interstitial fibrosis, and pulmonary arteriole muscular hypertrophy. Portions of this same fiber were observed in sections that had been cut immediately before and after the paraffin-embedded section that is shown (×200).
The study reported herein was conducted to learn whether inhaled fibers in surgical histopathology sections of human lung were observed in 13 of 17 sections (inhaled fibers screened, 17 slides were selected for critical examination using human lung specimens for inhaled fibers. Of the 160 slides conducted in which we screened 160 surgical pathology slides of for inhaled fibers. To test this hypothesis, a study was conducted in which we screened 160 slides. 17 slides were selected for critical examination using a multiparameter criteria that we have proposed for insuring the identification of inhaled fibers in paraffin block sections. Inhaled fibers were observed in 13 of 17 sections (inhaled fibers per slide: range, 1-3; \( n = 8 \) patients). Accordingly, the presence of fibers in surgical histopathology sections of human lung specimens supports further our thesis that airborne fibers are inhaled.

Discussion
The study reported herein was conducted to learn whether cellulosic and plastic fibers are inhaled and, if so, whether these fibers are present in both nonneoplastic and malignant lung tissues of patients with different histological types of lung cancer. To this end, pilot studies were conducted in which we developed different methods for viewing fresh, nonfixed, and nonstained human lung tissue from different pulmonary sites. Pilot experiments were then conducted with these procedures for viewing lung tissue for inhaled fibers. All fibers, except glass fibers, are birefringent (23, 24). In contrast, lung tissue is nonanisotropic. We, therefore, focused our attention on viewing fresh human lung tissue samples with a polarizing microscope. Noteworthy is that polarized microscopy has been described for identifying inhaled silica in paraffin-embedded sections of human lungs (26).

In one study, the examination of 114 human lung specimens that included 81 nonneoplastic and 33 malignant tissues with a dual-slide chamber method enabled us to document the discovery of inhaled cellulosic and/or plastic fibers in 99 (87%) of the cases. The inhaled fibers were heterogeneous (e.g., type, color, morphology, length, diameter, optical properties, and other characteristics). Most fibers exhibited little or no deterioration, and this observation supported our premise that the inhaled cellulosic and plastic fibers were bioresistant and bio-persistent.

In the pilot investigations, we tested various types and configurations of both glass and plastic viewing chambers. Microscope slides proved to be the easiest, cheapest, and cleanest means of constructing a chamber for viewing lung and other tissues. Of the various glues and bonding agents tested, silicone grease proved to be an effective and nontoxic substance for joining two glass slides. The combination of silicone grease and tape secured the tissue in a compressed position. Chambers containing compressed lung tissue suitable for viewing inhaled fibers could be stored at 4°C for as long as 2 days.

Different human and animal tissues were analyzed to identify a suitable control for evaluating human lung specimens for inhaled fibers. Tissues studied were: (a) human disease-free lung specimens and nonlungs specimens (e.g., liver) obtained from autopsies; (b) lung and nonlung tissue from livestock (e.g., pig); (c) lung tissue from small laboratory animals (e.g., mouse); (d) near-term fetal bovine lungs; and (e) surgically excised human tumors.

Fetal bovine lungs were selected for routine use because they afforded the following advantages: (a) bovine lungs were a more representative control than nonlung human tissue; (b) when compared with lungs of mice, rats, and other animals used in fiber inhalation studies, the size of the lung from a near-term bovine fetus (~24 kg) more closely represented that of an adult human lung; (c) prenatal lung tissue offered the assurance that no inhaled fibers were present; and (d) multiple samples from a single fetal bovine lung could be frozen so as to provide a readily available supply of control tissue.

Of different human tumors that been screened to define a suitable control for surgically excised lung tissue, leiomyosarcomas proved most advantageous due to their relatively large size and absence of in vivo exposure to airborne fibers and particles.

The most salient features by which our experimental approach differs from that used by others are the: (a) selection of fresh human lung tissue for examination; (b) compression, rather than cutting, of the lung tissue; (c) detection of fibers in lung samples that had not been ashed or digested chemically; (d) utilization of a polarizing microscope for viewing fresh tissue; and (e) incorporation of fiber identification schema and protocols used routinely by forensic pathologists.

A partial listing of the advantages afforded by the dual-slide chamber method included the following: (a) the lung sample did not require fixing, staining, or sectioning; avoiding these conventional but cumbersome and time-consuming histopathology procedures reduced the risk of sample contamination; (b) samples from a fresh lung specimen could be prepared for fiber analysis quickly (<15 min); (c) procedures used were technically simple and avoided cumbersome multiple-step methods that would subject the tissue to fiber contaminants; (d) the size of the lung sample required was small so that many chambers could be prepared, even if only a small amount of lung cancer or nonneoplastic tissue was available; (e) samples of lung specimens from different regions of the lung could be flattened to ~0.15 mm, allowing the samples to be viewed with either transmitted light or epi-illumination; (f) the dual-slide chamber could be inverted, allowing inhaled fibers in the compressed lung sample to be observed from two perspectives; (g) the chambers could be stored for at least 2 days. After allowing the refrigerated chamber to warm to room temperature, an inhaled fiber could be reexamined; in most instances, lung tissue in the stored chambers exhibited little or no deterioration or drying; and (h) studies reported herein were performed with fresh lung tissue, yet, lung tissue stored frozen or formalin-fixed also proved satisfactory for analysis of inhaled fibers.

In applying this technique to screen many lung specimens, we have identified the following limitations: (a) although the prescribed method was effective for detecting fibers, it is unsuitable for fiber enumeration; (b) the general morphology of the fiber in situ was sufficient to permit classification of the inhaled fibers as cellulosic or plastic but was usually inadequate for fiber subclassification within these two broad classifications (e.g., cellulosic: paper versus cotton; plastic: polyester versus nylon); (c) although the gross anatomical site of the resected lung specimen was known, the location of the fiber with respect to the alveolar and terminal bronchiole was unknown. Note-worthy, however, the respiratory tract from the larynx to the

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4 Crystalline silica is listed as a "Known Human Carcinogen" by the United States National Toxicology Program, 9th Report on Carcinogens (Attachment 3), January 1998.
terminal bronchiolar contains collagen (27, 28), and we observed this collagen with a polarizing microscope in diverse lung specimens including: (a) fresh lung samples, mounted using the prescribed dual-slide chamber method; (b) cryosections; and (c) paraffin-embedded sections. As illustrated in Figs. 1 and 2, we observed fibers in areas in which no collagen was discernible, suggesting that the inhaled fiber was at or near a terminal bronchiolar.

A manual and computer-assisted review of the scientific literature has failed to identify a publication describing inhaled cellulosic or plastic fibers. The major focus of pulmonary pathologists and inhalation toxicologists has been on asbestos due to its widely recognized association with different lung diseases (5–16).

Our observations challenge contemporary convictions that cellulosic and plastic fibers are not present in the human lung because: (a) the fiber types are too big; and (b) if inhaled, the fibers would be removed by different methods, including: (i) mechanical (e.g., coughing); (ii) physiological (e.g., mucociliary escalator); (iii) immunological (e.g., macrophage phagocytosis); and (iv) chemical (e.g., dissolution) mechanisms. We challenge this doctrine. We hold the opinion, supported by observations reported herein that: (a) many human air ducts are sufficiently large to allow penetration of various types of airborne nonmineral fibers; and (b) some of these inhaled fibers escape clearance mechanisms and remain in the lung as inhaled foreign bodies that resisted degradation and/or dissolution and that may induce acute and/or chronic inflammation.

The appearance of inhaled fibers may escape detection in surgical pathology slides. Failure to recognize inhaled fibers may be due to: (a) thin-sectioning (≤5 μm) of the lung tissue; thus, inhaled fibers would usually be cut transversely and would not be recognized; (b) contamination of pathology tissue with fibers during processing in an unprotected environment of a histology laboratory; (c) some plastic fibers would be dissolved by xylene used for paraffin clearing; (d) inhaled fibers are not conspicuous when the specimen is viewed with a conventional microscope; (e) histopathology slides of the lung are not routinely examined with a polarizing microscope; and (f) when observed by the pathologist the fibers are often assumed to be airborne contaminants.

A number of studies have been conducted of the structural organization of the tracheobronchial tree in humans (27, 28). Within the lung the airways divide, usually dichotomously, that give rise to five groups: trachea, main bronchi, terminal bronchiole, respiratory bronchiole, and alveolar duct. Each of these groups gives rise to an increased number of branches. One schema of numbering these branched areas is by generation, beginning with the trachea (generation 0) and terminating with the alveolar duct (generation 23). The number, diameter, and length of each generation has been defined (e.g., generation 17; 131,072 branches, 540 μm diameter, 1410 μm long; Refs. 27 and 28). The diameter of this respiratory bronchiole could readily accept any of the fibers shown in Figs. 1 and 2. For example, the fiber illustrated in Fig. 1F measures 155 μm in length, and this length is approximately one-fourth the diameter of the respiratory bronchiole. This and other fibers observed are relatively small, and some are pliable (Figs. 1K and 2J); thus, they could penetrate readily into the lung.

The mechanisms underlying asbestos-induced malignant transformation are being elucidated. The results of extensive in vivo and ex vivo studies suggest that the following are implicated: (a) fiber dimensions, surface properties, and durability; (b) fiber burden; (c) assorted chemicals adsorbed to the surface of the asbestoses, including carcinogens, tumor promoters, and toxins; (d) lung site and tissue microenvironment; (e) ionizing radiation; (f) radon gas; and (g) host-defense mechanisms, including lymphocytes and phagocytic leukocytes [i.e., macrophages and their cytokines (i.e., tumor necrosis factor-α) and reactive oxygen species (e.g., superoxide anion, O$_2^-$_]; Refs. 5 and 29–31]. The effect of these factors is also diverse, as evidenced by the multitude of aberrations that precede malignant transformation, including the alteration of chromosomes (e.g., deletions; Ref. 32) and the activation of proto-oncogenes (e.g., c-fos; Ref. 33).

Although the physical and chemical properties of cellulosic and plastic fibers and asbestos differ greatly, all three fiber types resist biodegradation. Accordingly, it would be reasonable to postulate that inhaled biopersistent cellulosic and plastic fibers, particularly those that contain mordants, dyes, and various chemicals, may contribute to different pulmonary diseases, including lung cancer. For example, the biological mechanism for the epidemiologically proven syncarcinogenicity of cigarette smoke and asbestos has been attributed to asbestos fibers that carry polycyclic aromatic hydrocarbons into the cells and influence their metabolism (i.e., polycyclic aromatic hydrocarbon carrier hypothesis; Ref. 34).

The identification of inhaled cellulosic and plastic fibers is claimed. We, however, make no affirmation that the observed inhaled fibers are etiological agents or confounders in lung pathogenesis. Correlation (e.g., lung disease versus asbestos, smoking, DNA adducts, and oncogenes) is, nevertheless, the hallmark of initial reports that have furnished the impetus for undertaking additional studies that, over a period of years, have provided insight and understanding into the complex and diverse mechanisms underlying malignant transformation.

Cellulosic and plastic fibers have also been observed in human lung tissue using a method in which the lung tissue is chemically digested, and inhaled fibers present in the digestion residue were collected onto a micropore membrane (35). In ongoing studies, we are focusing our efforts on expanding this technology to assay the distribution and deposition of various fiber types and crystals (36) in different anatomical sites of the lung.

More people die worldwide of lung cancer than any other cancer, and the incidence of lung cancer among both smokers and nonsmoker is increasing (3, 4). The high mortality of lung cancer that is not associated with smoking has fostered renewed interest in the risk assessment of occupational and environmental settings. Moreover, the morbidity and mortality worldwide of idiopathic nonmalignant lung diseases is increasing.

In this appraisal, we believe that it will be useful to investigate airborne cellulosic and plastic fibers because these fibers are ubiquitous, numerous, biopersistent, and often contain mordants, dyes, and toxic chemicals; thus, different types of nonmineral fibers could be assessed as candidate agents and confounders that may contribute to the pathogenesis of different pulmonary diseases, including lung cancer.

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References
Inhaled Fibers Found in Human Lung Tissue


Inhaled cellulosic and plastic fibers found in human lung tissue.
