

## UC Irvine

### UC Irvine Previously Published Works

**Title**

Inflated, dried whole lung specimens

**Permalink**

<https://escholarship.org/uc/item/1sw4k678>

**Journal**

The Anatomical Record, 231(2)

**ISSN**

0003-276X

**Authors**

Oldham, Michael J

Phalen, Robert F

**Publication Date**

1991-10-01

**DOI**

10.1002/ar.1092310216

**Copyright Information**

This work is made available under the terms of a Creative Commons Attribution License, available at <https://creativecommons.org/licenses/by/4.0/>

Peer reviewed

## Inflated, Dried Whole Lung Specimens

MICHAEL J. OLDHAM AND ROBERT F. PHALEN

*Community and Environmental Medicine, University of California, Irvine,  
California 92717*

**ABSTRACT** A method is described for preparing fully-inflated whole lung specimens that are suitable for instruction or research purposes. Undamaged lungs are removed from the body and then tracheally cannulated and lavaged with tap water more than 250 times. The treatment also includes rinsing blood from vessels with water. A final filling of the lung with alcohol is optional. The multiply rinsed lung is drained and inflated to 30 cm of H<sub>2</sub>O pressure with dehumidified air and held at that pressure until the tissue is completely dry. The resulting specimens are light in color and appear to be permanent if stored properly.

Inflated, dried lung specimens have a variety of applications, including their use as museum objects or teaching aids, and use in scientific investigations of inhaled air contaminants. Unlike most other organs, which retain their natural appearance after removal from the body, the lungs collapse to a non-physiologic size and shape as soon as the thoracic cavity is breached. Collapsed lungs are flaccid and dark in color in comparison with their appearance in their fully-inflated state. Because of the difficulties involved in removing the lungs from the thorax without tearing or cutting them, and the equipment needed for their proper inflation, it is fair to assume that few people have actually seen this organ in anything resembling its normal inflated appearance. Furthermore, in the deflated state, it is difficult to obtain samples of lung tissue that have known, describable locations with respect to their positions within the thoracic cavity. These and other limitations associated with collapsed lungs can largely be overcome by inflating and air-drying the entire organ. If the blood is completely removed, the lungs acquire a pale yellow to white color, against which accumulations of insoluble particles and other features can easily be seen. In addition, by removing most of the proteinaceous material before inflation-drying, a long-lasting, lifelike specimen can be prepared. With proper storage in a sealed, dry environment, deterioration can apparently be prevented.

Lungs have been dried and inflated by various investigators in order to stabilize the flexible bronchial airways so that they can be filled with metal, wax, plastic, or rubber in order to make anatomically realistic replica casts for teaching or anatomical study. Peterson (1935) described a method for making replica casts of lung airways. His procedure involved the removal of fresh, unperforated lungs, flushing the blood vessels with water, expanding the lungs with air through a glass cannula, and allowing the organ to dry in the expanded state for 36 hours. The dried lungs were then filled with molten (65°C) Wood's metal, cooled quickly (for 15 minutes), and then immersed in sodium hydroxide (about 2 molar) for 24 hours to retrieve the cast. Rahn and Ross (1957) reported the use of a simple technique for preparing "permanently preserved" inflated lung specimens. They connected the

tracheas of freshly excised lungs to a continuous air flow and dried them for a "few hours" (rat) to "several days" (cow). The dried lungs were used for measuring the dry weight of lung lobes or for making plastic casts of the airways for dead space volume determinations. Wolfe (1962) described a modification of the Rahn and Ross technique in which a 10% formalin solution was infused into the lung vasculature in order to sterilize the lung prior to air-drying. Wolfe used silicone rubber to make flexible casts of the airways. Recently, air-dried lungs have been used to evaluate *in situ* observations of airways obtained by computed tomography (Todo and Herman, 1986), to study the effect of pulmonary emphysema on the pattern of deposition of inhaled particles (Sweeney et al., 1987), and for producing flexible airway models for teaching purposes (Wang and Kraman, 1988).

In each of the above applications several criteria must be met for the technique to be successful. Most importantly, the lungs must be obtained undamaged so that a full inflation can be produced. The inflating air pressure for drying the specimen must be sufficient to prevent collapse, but it must not be so great as to rupture the organ. Also, the air should be very low in humidity and its flow maintained without interruption until the tissue is firm and dry. If each of these criteria is met, anatomically realistic-appearing specimens will be obtained. Although the above techniques produce impressive specimens, if blood or other tissue remain, the specimen will eventually darken and may develop an unpleasant odor—presumably due to bacterial degradation. Our method was developed in order to prevent the darkening and spoilage of the dried organ. In order to achieve these new objectives, it was necessary to essentially completely remove the blood, lipids, interstitial fluid, cellular cytoplasm, and external fatty tissue prior to air-drying. The additional steps proved to be more tedious than difficult.

Received December 11, 1990; accepted March 20, 1991.

## METHODS

### *Materials*

Ideally the lungs should be recently-acquired and fresh, although cold storage for a day or two or even fresh freezing followed by gentle thawing can substitute, if necessary. In any case, the lungs should have an intact trachea, and the organ should be free of major tears, cuts, or punctures. A deep laboratory sink with a source of cool, clean tap water and good lighting are useful. A shallow tray to hold the lung is needed so that the surface of the organ can be kept wet during the repeated water lavage. A cannula that can be inserted into the trachea and securely tied, along with a funnel and tubing that can be used to pass water into and out of the lung, are needed. Commercially available liquid soap for opening closed airways, and alcohol or bleach for killing microorganisms, are useful, but not always required. A modest set of surgical tools including a syringe, scissors, forceps, and clamps (e.g., hemostats) for closing leaks in the lungs should be available. For air-drying, a support stand with a clamp to support the lungs, a source of compressed dry air, and a 30 cm column of water (used as a pressure regulator) are needed. The preferred source of compressed dry air is an oilless compressor and refrigerated dryer. A Cole-Palmer oilless compressor (Model 07057-10) and Wilkerson refrigerated dryer model (A01-AH-POO) were used in this procedure. Compressed air from other sources (small air pumps, wall outlets, or compressed air tanks) is acceptable if oil free, less than 15% humidity, and filtered through a particulate filter (Motor Guard Corp. Model D-13 or equivalent). Satisfactory setups for rinsing and for drying the lungs are shown in Figure 1.

### *Procedures*

#### Tissue preparation

The external surfaces of the lungs and trachea should be trimmed free of fat and other adherent tissue, and the major blood vessels leading into and out of the lungs should be cut away. Care must be taken to avoid puncturing the parietal pleura, especially when the carinal lymph nodes are dissected away.

#### Degassing as an option

For large specimens (human lungs, for example) it is useful to prevent the trapping of large quantities of air within the lungs. Trapped air prevents the water from fully penetrating to all portions of the tissue and local regions of the specimen may not be fully rinsed. Our method of degassing is to inflate the fresh lungs with CO<sub>2</sub> gas from a tank and then allowing them to deflate. About 10 full inflation-deflation cycles are sufficient to replace most of the air with CO<sub>2</sub> if the pressure is regulated to about 30 cm of water. Because CO<sub>2</sub> is very soluble in water, it will completely dissolve as the lungs are subsequently lavaged, and water filling will be uniform.

#### Rinsing

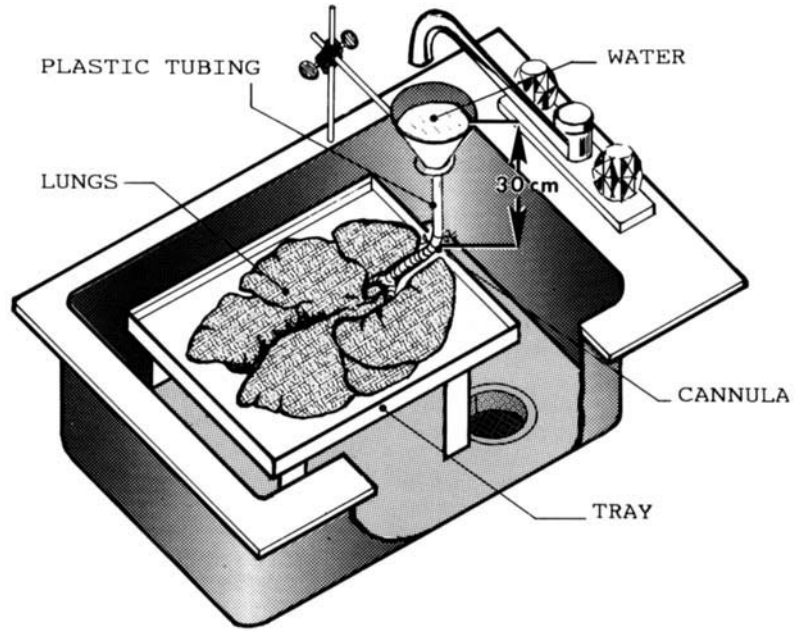
A tubular connector, or cannula, is inserted into the top of the trachea down about 2–3 cartilage rings, and is tied in tightly with strong string. It is important that this connector not slip out, especially when the wet

lungs are later hung for drying. A smooth-walled cannula is likely to slip, so a connector with ribs is recommended. A 30 cm long tube (rubber or flexible plastic) with a funnel on its end is attached to the cannula, and clean, cool tap water is poured into the funnel until the lungs are filled to a hydrostatic pressure of 30 cm. The funnel is then lowered below the level of the lungs and the water is allowed to flow out. After about 10 cycles of filling with water and emptying, the lungs will begin to lose their pink to red color (Fig. 2). At this time it is useful to flush the blood vessels of the lung 2–3 times by using a catheter-tip syringe. Also, at this time a small amount of liquid soap (10–20 ml for a human lung) can be poured into the trachea to aid in opening collapsed airways (repeated up to 5 times if lung is not fully inflated). Each lavage cycle will require about 10–30 seconds (for lungs from rats to humans), allowing approximately equal filling and emptying times. When at least 250 fill-empty cycles have been completed and the lungs have lost their pink color, three options are available prior to drying. First, if the presence of pathogens is suspected, one can fill the lungs with a dilute solution (10:1 with water) of laundry bleach. Bleach addition should be followed immediately by ten additional cycles of lavaging with water to prevent severe damage to the lungs. Bleach dissolves collagen and elastin in the lung and can destroy the specimen if too much is used or if it is not lavaged out immediately. Second, if no pathogens are suspected the lungs can be immediately connected to a source of dry air. The third option is to use absolute alcohol (isopropyl or ethyl) to fill the lungs for the last lavage cycle. Alcohol helps in the subsequent removal of water from the tissue and aids in killing any pathogens that may be present.

#### Drying

At this point the liquid in the tray holding the lungs is emptied and the lungs are connected to a dry air supply. The air flow is started and increased until the water column begins to bubble, producing a constant pressure within the line. A large lung, such as that from a human, will require a dry air flow of about 20–40 liters/minute. When enough water has left the lung so that it can be lifted to the drying stand without damage, it is attached upright to the stand by the tracheal cannula. This is the most likely time for the cannula to slip out; a second string tie should be made to secure the tracheal cannula. Because the trachea may tear under the weight of a wet lung, a smooth round bar should be placed under the first bifurcation and attached to the drying stand to provide added support for the organ. After about 30 minutes, most of the water will have left the lungs and the positions of the lobes can be adjusted to approximate their proper *in situ* orientation. Objects such as beakers, wood blocks, or cellulose sponges can be used to apply gentle pressure to the lung surfaces to aid in positioning the lobes. Complete drying of the lung will require about 12 hours (rat) to 4 or more days (human), so it is wise to make sure that dry air at 30 cm hydrostatic pressure can be supplied without any interruption for this length of time. A loss of air pressure during drying can lead to collapse of the lungs and permanent damage to the specimen. As the lungs dry, the surface may change

A.



B.

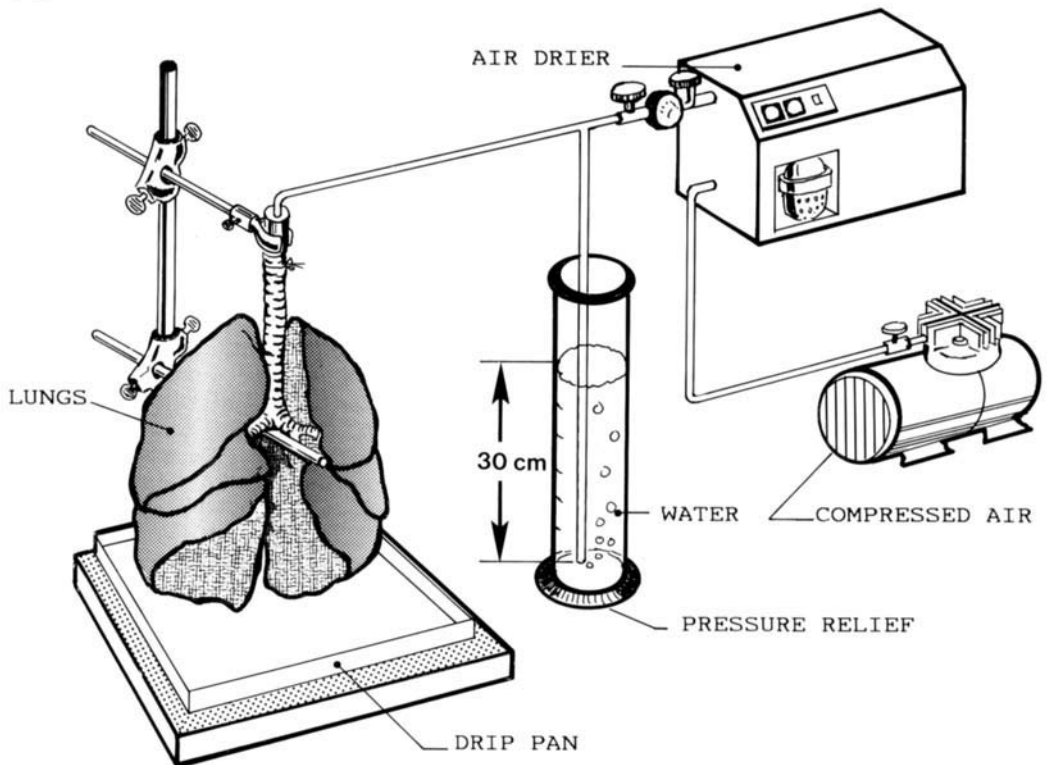


Fig. 1. A: Apparatus for repeated lavaging of the lungs with water. By limiting the pressure to about 30 cm of water column, rupture of the lungs is unlikely. B: Apparatus for drying the lungs. The water column in parallel with the lungs bubbles when 30 cm water pressure is reached, thereby regulating the inflation pressure to that value.

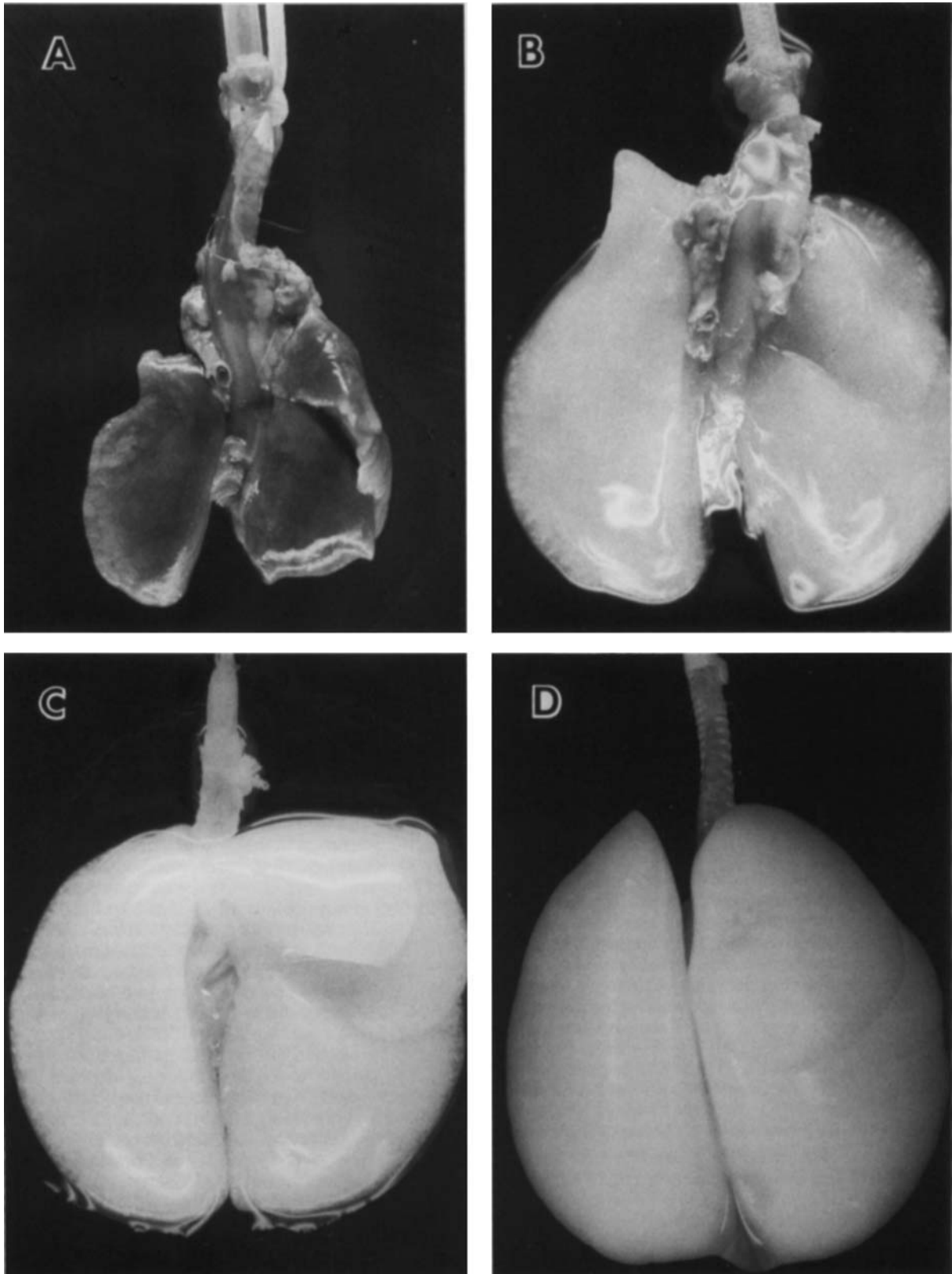


Fig. 2. **A:** Freshly excised rat lungs. **B:** The lungs after 10 fill and empty cycles with water. **C:** The lungs after 250 fill and empty cycles. **D:** The lungs after air inflation.



Fig. 3. Dried lung from an adult albino rabbit preserved for over 10 years.

from a white to a pale yellow, or they may retain a pure, white color.

#### Preservation

When the lungs are completely dry, they may be safely removed from the support stand and placed into a protective container containing a bag of desiccant. If desiccant is not used, the specimen should be kept out of damp or humid environments unless it is in a very well-sealed container. For long-term storage, a sealed container with enough color-indicating desiccant to cover the floor may be used. The desiccant should be changed as often as the color change dictates. Figure 3 shows a specimen (rabbit lung) that has been stored for over 10 years in this manner.

#### RESULTS

Figure 4 shows a human lung prepared by the described method. Dried lungs prepared by the above method will be white to light yellow in color and very light in weight. Adult human lungs with their full volume of blood weigh about 1200 grams, but a dried specimen prepared and dried as described will weigh only about 90–100 g. The trachea and major bronchi will be white to dark red-brown with the cartilage rings clearly visible. Air-dried lungs will not have the same overall shape that the lungs had in the thorax. In

the thorax, the thoracic walls, diaphragm, heart, and other tissues determine the shape of the lungs. When the lungs are removed and inflated their overall shape becomes more spherical and they will increase in width more than in length. Also, air-drying will produce some anisotropic shrinkage, which further distorts their shape. Yet, despite these artifacts, the dried lungs will appear to be anatomically lifelike if care is taken.

The lungs of all of the adult humans that we have water-rinsed and air-dried have numerous black deposits on their surfaces. These spots are present in smokers, ex-smokers, and non-smokers alike. The spots, which are carbonaceous deposits, are generally larger and more numerous on the upper surfaces of the upper lobes, and are generally smaller and less numerous on the lower surfaces on the lower lobes. Some human lungs, especially those from older individuals, have a deposit pattern of the lateral surfaces that give the organ a zebra-striped appearance. These stripes are apparently long term sequestration sites for accumulations inhaled insoluble particles.

#### DISCUSSION

Air-drying of the lungs is associated with significant shrinkage of the bronchi. This shrinkage can average more than 10% in airway diameters (Kilpper and Stidd, 1973). More importantly, one cannot expect this shrinkage to be uniform throughout the lungs. Therefore, if replica casts are made from air-dried lungs, such casts should not be used for detailed measurements of airway dimensions or for making dead-space volume determinations. For such purposes, casts made in situ will have more realistic dimensions (Yeh et al., 1975).

When working with mammalian tissues, safety considerations can be important. A variety of fungal, viral, bacterial and other infectious organisms may be present in the blood or lung tissue of deceased animals. Our human lungs were acquired through an organ donor bank and they had been tested for HIV (human immunodeficiency virus) and hepatitis antibodies. Only those lungs that were antibody negative were used for air-drying. However, examination gloves and laboratory coats are worn by those handling the tissue. Any lung that appears to be infected with fungi or bacteria should be handled with extreme caution, if at all. Precautions such as tight-fitting masks or respirators, double gloves with taped wrists, and the use of a biological hazard hood are recommended when working with suspicious specimens.

The permanence of dried lungs has not been evaluated, and it may never be completely defined. As mentioned previously, we have a specimen that was stored for over 10 years in a sealed jar without any apparent change in appearance. As another means of preservation we place a few grams of paradichlorobenzene ("moth balls" or toilet deodorizer are inexpensive sources) in the storage containers of particularly valuable dried lung specimens. This popular insecticide can act as a mucus membrane irritant, and it is capable of producing liver injury, so it should be handled with care.

It is apparent that repeated water lavage removes all but minute traces of the blood from the lungs. Furthermore, given the magnitude of the change in weight of

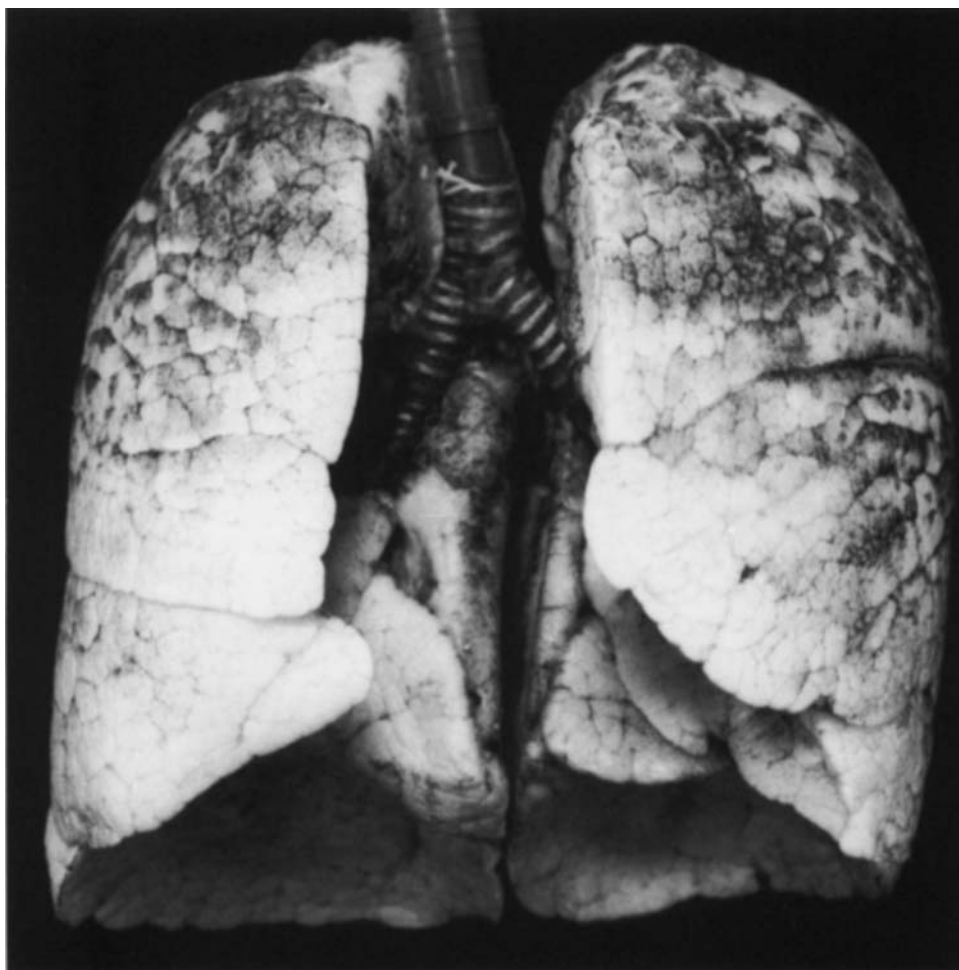


Fig. 4. Human lung that has been repeatedly lavaged (approximately 250 fill and empty cycles) with water and air-dried for 7 days at an inflation pressure of 30 cm of water. The subject was a 71 year old male ex-smoker of cigarettes.

the lungs, it is clear that additional tissue components are removed. Tap water is hypotonic with respect to physiological fluids, so it is presumed that widespread cell lysis during repeated rinses facilitates the removal of intracellular and extracellular fluids and non-bound proteins, lipids, and other substances. After the thorough washing procedure, the collagen and elastin that form the lung tissue matrix should remain. Snyder et al., 1975 have indicated that for men the lung weighs 1170 g with blood, 540 gm without blood, 260 gm without blood and water, and that 22% of this waterless weight is elastin and collagen. The elastin and collagen weight of the lungs is about 57 gm. Thus, our dried human lung specimens that average about 100 gm in weight probably consist primarily of collagen and elastin. The remaining content is unknown, but it would probably include inorganic air contaminants and other insoluble substances.

The procedure reported here for preparing lung specimens is a modest refinement of the method reported by Peterson in 1935. His technique involved rinsing blood from the vessels followed by inflation and drying. Our

refinement is to repeatedly rinse the airways with hypotonic water. The major drawback of our method is that the steps are time-consuming, requiring about 8 man hours of effort for the human lung. However, the repeatedly-rinsed lung has a striking appearance and longevity that can justify the additional effort.

#### ACKNOWLEDGMENTS

Supported by the NHLBI (grant RO1-HL39682) and the Charles C. Stocking Trust. We thank Dr. Robert Kilpper, Richard Mannix, Tuan Nguyen, and Marie Sorensen for advise, technical editing, artwork, and word processing. We also thank the University of California, Irvine, Organ and Tissue Bank, and especially Jesus Hernandez and Anna Nicole E'nama for providing the human lung specimens. Dr. Phalen is partially supported by the University of California, Irvine Southern Occupational Health Center.

#### LITERATURE CITED

Kilpper, R.W., and P.J. Stidd 1973 A wet-lung technique for obtaining silastic rubber casts of the respiratory airways. *Anat. Rec.*, 176: 279-288.

- Peterson, A.K. 1935 On the preparation of Wood's metal casts of the lungs. *Anat. Rec.*, 61:261-272.
- Rahn, H., and B.B. Ross 1957 Bronchial tree casts, lobe weights and anatomical dead space measurements in the dog's lung. *J. Appl. Physiol.*, 10:154-157.
- Snyder, W.S., M.J. Cook, L.R. Karhausen, E.S. Nasset, G.P. Howells, and I.H. Tipton 1975 *Report of the Task Group on Reference Man*, published for the International Commission on Radiological Protection, Pergamon Press, NY.
- Sweeney, T.D., J.D. Brian, S.A. Leavitt, and J.J. Godleski 1987 Emphysema alters the deposition pattern of inhaled particles in hamsters. *Am. J. Pathol.*, 128:19-28.
- Todo, G., and P.G. Herman 1986 High-resolution computed tomography of the pig lung. *Invest. Radiol.*, 21:689-696.
- Wang, P.M., and S.S. Kraman 1988 Construction of a flexible airway model for teaching. *Anat. Rec.*, 221:780-781.
- Wolfe, K.B. 1962 A method for preparing mammalian lungs for anatomical study. *Lab. Digest*, 25 June: 9-12.
- Yeh, H-C, A.J. Hulbert, R.F. Phalen, D.J. Velasquez, and T.D. Harris 1975 A stereoradiographic technique and its application to the evaluation of lung casts. *Invest. Radiol.*, 10:351-357.