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Study of Resected and Postmortem Lung Specimens

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Many of the difficulties traditionally associated with the study of lung pathology may originate in improper preparation of lung specimens for gross and microscopic work. I strongly favor fixing lung specimens in their distended state before dissection. Within the chest cavity, the lung is in a variable degree of distention, but opening the thorax during necropsy or following surgical excision results in a collapsed mass of deeply congested and slippery lung tissue that is difficult to section and assess. Much information can be missed by examining unfixed lungs, and little is gained from fixing such a lung in formalin for inspection at a subsequent date. One of the few instances in which examination is easier is in a lung with pulmonary edema, in which alveoli are fully distended with fluid, and the lung is firm and easy to cut in the fresh state. Even intrabronchial formalin perfusion may not wash away the edema fluid, and there is frequently excellent histologic detail (Color Fig. 5-1).

In most cases, significant disease can be overlooked if the lungs are fixed in the collapsed state. For diffuse lung disease, fixation by intrabronchial formalin perfusion becomes not only desirable but indispensable. In emphysema, for example, the lesions are displayed at advantage (Fig. 5-1). The lesions of interstitial pneumonia and fibrosis are also best demonstrated in distended, fixed lungs. The collapse of alveolar structures in the unfixed specimen is frequently misinterpreted as interstitial lung disease, a pitfall eliminated by appropriate fixation.

I do not recommend the time-honored practice of dissecting the lung by opening the bronchi lengthwise with scissors and forceps. Larger bronchi are hard structures that are particularly difficult to cut and to hold open for inspection in the fixed lung. Dissection with scissors produces extensive crushing of airways and can be carried out only to a certain degree, leaving many airways unopened. I recommend that dissection of the fixed lung be conducted by opening segmented bronchi and more distal airways with the aid of a metal probe and a sharp knife. The metal probe can be advanced into bronchi and through the alveolar tissue, even across the pleura, for further stability. The knife should slide freely along the probe at an angle of about 45° in a fast and steady motion.

After the major airways in the mediastinal aspect of the lung have been opened in approximately parallel planes, the specimen is placed face down on the dissection table, and the remainder of the lung is further sectioned in two or more parallel slices in the sagittal plane. At this stage, some pathologists prefer to cut the lung parenchyma in a breadloaf pattern, but I discourage this practice except for small subpleural lesions that can be best demonstrated in this manner. Even after the lung has been divided in this manner, the pulmonary arteries can be slit open with fine scissors to look for pulmonary emboli. The amount of information that can be gained from this method of dissection can be truly remarkable (Fig. 5-2).

Regardless of the techniques of fixation and dissection, the lungs should be weighed and measured. Weigh the lung in the fresh (*i.e.*, unfixed) state, and measure it after fixation.

SURGICAL PATHOLOGY SPECIMENS

The study of lung tissue obtained from a living patient by surgical means (*e.g.*, pneumonectomy, lobectomy, segmentectomy, wedge resection) presents the pathologist with an extraordinary opportunity for diagnostic work and research on lung disease. In handling specimens, the following steps should be followed in a systematic manner:

1. Uncontaminated tissue is secured and frozen for subsequent virologic investigations and studies of molecular biology (*e.g.*, gene rearrangement, *in situ* hybridization, oncogene analysis; see Chaps. 4 and 55). Although these

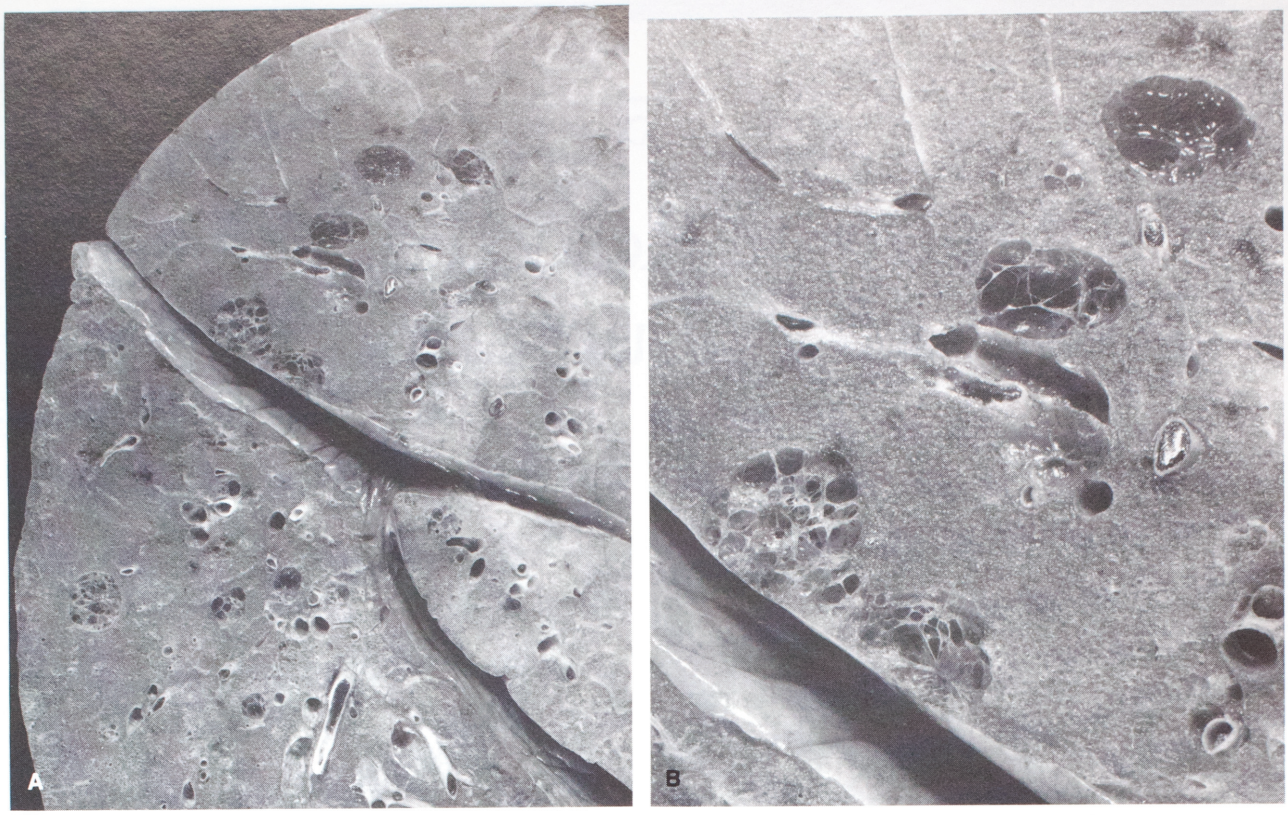

FIGURE 5-1. (**A**) In this peculiar example of centrilobular emphysema, the lesions involve all lobes of the lungs, but they are especially prevalent in the upper lobes. (**B**) A closer view reveals punch-out lesions of emphysema without anthracosis.

studies are routinely conducted in large academic institutions, practicing pathologists in small private institutions are not exempt from the responsibility of obtaining and preserving tissue samples until a decision is reached to proceed with additional investigations. Failure to secure such samples in lymphoid lesions, for example, is a disservice to the patient and may have medicolegal repercussions.

2. If the surgeon has not done so, obtain representative samples for bacteriologic and fungal cultures. Air-dried imprints of infectious lesions should always be obtained. Organisms are frequently seen better in these smears than in the histologic slides.
3. Small, 1-mm cubes of fresh tissue should be fixed in glutaraldehyde or Karnovsky fixative for eventual electron microscopic examination and kept in the refrigerator until needed. These studies are important for the diagnosis of tumors and viral infections. Cytologic smears are valuable for cell detail and for the recognition of viruses and other agents (see Chap. 3).
4. Samples of lung tissue may be obtained and fixed in special solutions for specific needs, such as B-5 fixative for lymphoid lesions, Michel fixative for immunofluorescence studies, and absolute alcohol for glycogen (see Chap. 4).
5. The main specimen should be kept in an adequate state for proper fixation and sampling for conventional histologic examination.

In practice, fixation of surgical specimens is accomplished by directly infusing formalin solutions from a large bottle or container with an attached rubber tube and appropriate cannula to fit into the bronchus. Direct endobronchial infusion with a plastic syringe is a viable alternative. A 50- or 100-mL plastic syringe is used to inflate the specimen to an appropriate volume. Areas of the specimen that cannot be inflated because of bronchial obstruction by tumor or other causes can be directly injected with formalin with a syringe and needle. Clamping of the bronchus is discouraged, because it is not needed and can damage the bronchial margin of resection for histologic analysis. The optimal duration of fixation varies with the specimen, but I recommend 2 to 3 days. Some specimens are ready for dissection after 1 day only, but others require several days, although slicing the specimen after the first day may accelerate the process of fixation.

AUTOPSY LUNG SPECIMENS

One step usually forgotten in the study of lungs at necropsy is the status of the parietal pleura. Important lesions (*e.g.*, pleural fibrosis, pleural plaques, tumors, granulomas, amyloidosis) can be overlooked if the parietal pleura is not examined (see Chap. 75).

The lungs should be extracted *en bloc* from the chest cavity and in continuity with a segment of trachea; a longer tracheal segment provides better handling of the specimen. The next step is to gently aspirate secretions that have accumulated within the trachea and large bronchi. Large tears on the pleura should be thoroughly sutured with silk thread before inflation. As in the case of surgically resected specimens, samples for special studies may be required from autopsied lungs, and the procedures are the same as previously described. To obtain bacteriologic samples, a patch of pleura must be burned with a hot metal knife to provide a sterile

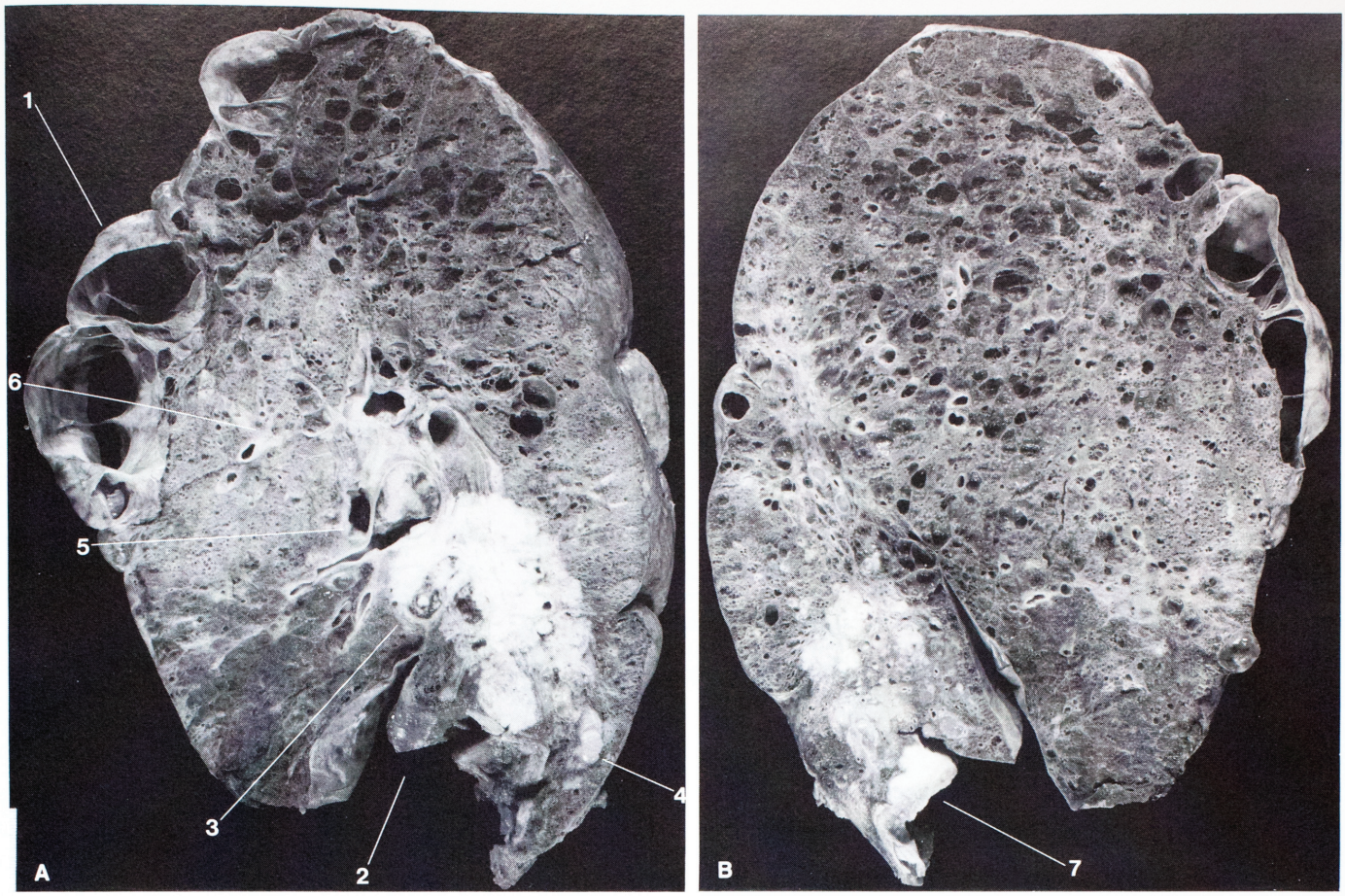

FIGURE 5-2. (**A**) An autopsy lung in a man with chronic obstructive lung disease and bronchogenic carcinoma reveals seven different lesions: 1, severe centrilobular emphysema with bullous formation in the anterior aspects of the lung; 2, chronic atelectasis and contraction of the lower right lobe; 3, bronchogenic carcinoma arising in the bronchus of the right lower lobe with infiltration of the lung parenchyma and involvement of intrapulmonary lymph nodes; 4, obstructive bronchiectasis with a mucus plug as a result of tumor obstruction; 5, pulmonary thromboembolus; 6, extensive confluent bronchopneumonia of the middle lung field. (**B**) The same specimen as seen from the lateral aspect shows pleural invasion by tumor (7).

point of entrance to obtain an uncontaminated sample of lung tissue.

Fixation

Fixation of entire sets of lungs can be carried out in a manner comparable to that already described for surgical specimens. In an active autopsy service, several sets of lungs can be fixed simultaneously using a device such as the one in Figure 5-3.[1] Longer fixation periods produce a better quality specimen (Fig. 5-4).

Dissection

Ideally, each lung should be dissected separately as described for surgical pathology specimens (*e.g.*, pneumonectomy specimens); this is what I call technique I (see Figs. 5-1, 5-2, and 5-4). It is easier than technique II and allows a thorough examination of the specimen. In technique II, both lungs are kept together with the mediastinum (Fig. 5-5). It requires resection of the pars membranacea of the trachea and extrapulmonary bronchi down to the hilus. With the aid of a metal probe appropriately placed in major bronchi in the frontal plane, the posterior one third of the lung is separated from the anterior two thirds. The remainder of each lung is divided in an anterior and a middle third, the latter containing the immediate branches of the main bronchi. The three main portions of each lobe can be further subdivided with the knife down to 2.5-cm-thick slices. Technique II has the advantage of demonstrating mediastinal disease in contiguity with lung disease or bilateral lung lesions. Both techniques are useful for confirming radiologic-pathologic correlation. If correlation with the computed tomography scan is needed, the lung is serially sectioned in a transverse plane at 2.5- to 5-cm intervals from the base of the lungs to the apices. Interesting lung specimens should be photographed whenever possible for record-keeping purposes, research, teaching, and publication. I recommend a black background for both color and black-and-white pictures. A ruler in centimeters should be affixed to the background, not the specimen.

SPECIAL FIXATION PROCEDURES

Formalin Vapor Fixation

As observed by Weibel and Vidone, liquid formalin fixation has a drawback.[2] The normal shrinkage of alveolar tissue induced by fixation takes place against an incompressible mass of fluid, pro-

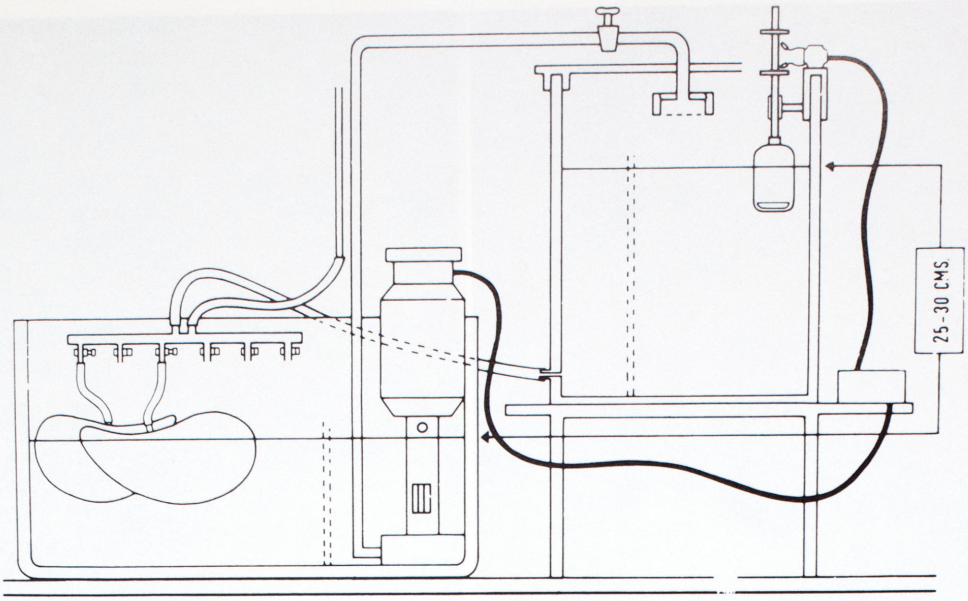

FIGURE 5-3. The Heard mechanical device is used to inflate lungs by continuous infusion of formalin. The lungs are contained in a receptacle (*left*). Formalin is moved by a pump to an upper container (*right*). The formalin pressure is controlled at 25 to 30 cm H_2O. (From Heard BE. Pathology of chronic bronchitis and emphysema. London: J & A Churchill, 1969:8.)

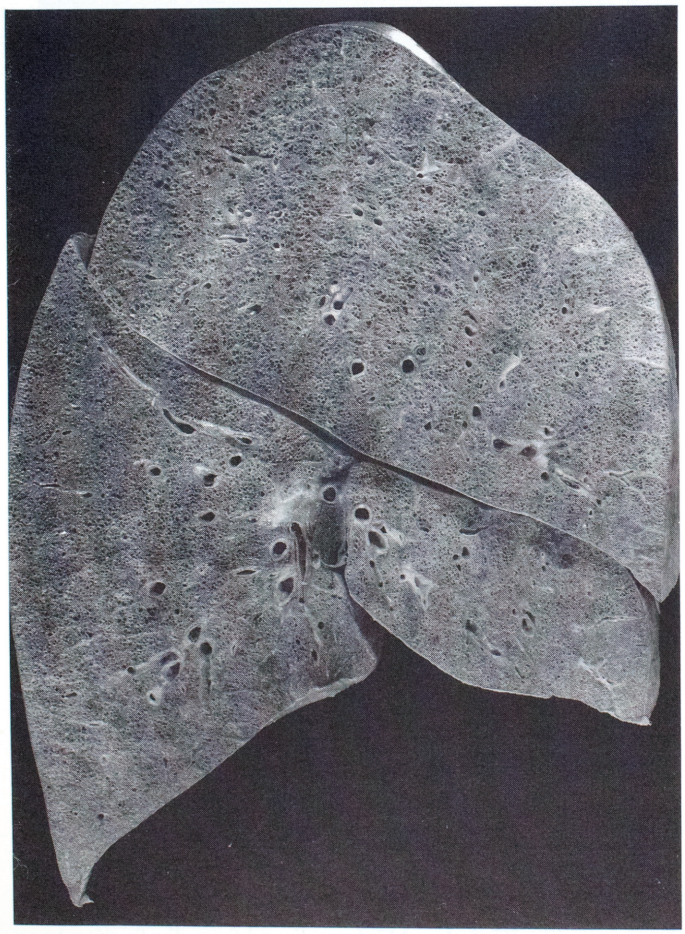

FIGURE 5-4. A unique case of panlobular emphysema in a specimen inflated by the Heard technique and dissected according to the type I method. Such cases are usually missed if not properly inflated.

ducing distortion of alveolar walls and flattening of hollow vascular structures. To overcome these limitations, the fixation of lungs by formalin fumes appears to be a solution, especially for specific research needs, such morphometry of the lung. I have found that intrabronchial formalin fixation is simple and produces well-preserved tissues for routine pathologic work. However, during the late 1950s and early 1960s, fixation of the lung by formalin fumes attracted considerable attention.

In 1959, Blumenthal and Boren described their technique of lung fixation by cold formaldehyde vapors.[3] Air was bubbled through a solution of concentrated formaldehyde at room temperature, producing formaldehyde vapor. After a fixation period of 3 to 5 days, the specimen was air-dried. Problems with this technique included poor cytologic detail, and the specimen became excessively brittle, particularly when stored.

In 1961, Cureton and Trapnell described a method of fixation using formaldehyde gas as a vapor above a concentrated solution of formalin within a carboy.[4] Air was intermittently bubbled through the formalin solution by means of a rubber hand syringe, and the airtight lung was continuously exposed to formaldehyde vapor for 2 to 3 days. A major drawback with this technique is that it requires an airtight lung; pleural tears must be meticulously searched for and ligated.

Pratt and Klugh devised another technique involving the use of formaldehyde fumes.[5] They constructed a respiratory box in which lung specimens were caused to absorb formaldehyde fumes intermittently for about 18 hours required for fixation. The lungs were dried in the inflated state by continuous application of pressure for 5 to 8 days. The researchers did not comment on the adequacy of tissue for histologic work.

In 1961, Weibel and Vidone described a method of delivering hot formalin fumes to a lung specimen contained within a plexiglass box from which air was extracted to create negative pressure and distention of the organ.[2] The method is useful for mor-

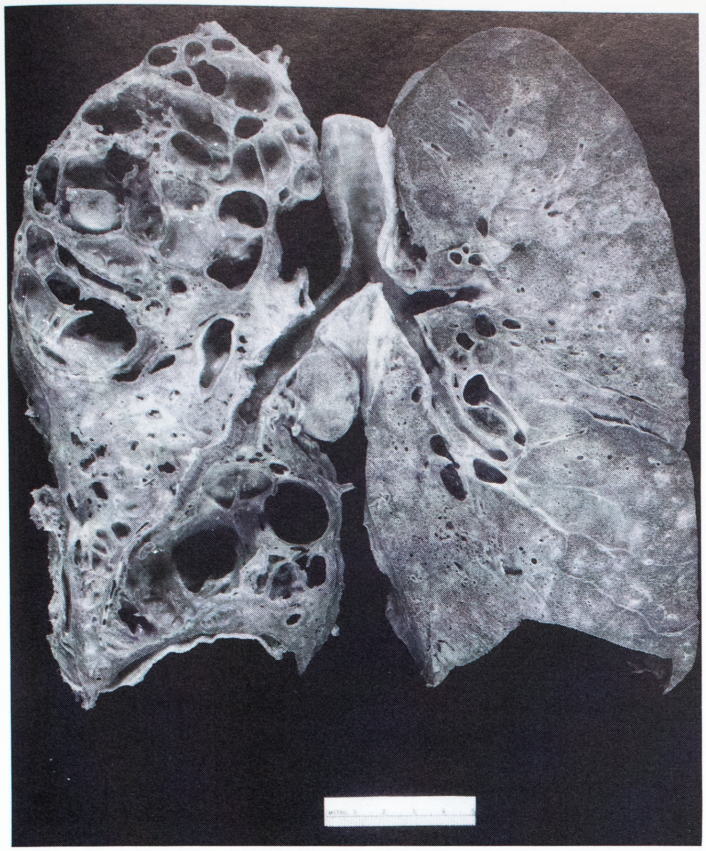

FIGURE 5-5. In an example of the type II method of dissection, which keeps the lungs together, the left lung is extensively involved by saccular bronchiectasis, and there is extensive confluent bronchopneumonia of the right lung. An enlarged hilar lymph node and an inspissated mucus ball are present in the left lung.

phometric work, such as counting alveolar surface area or volumetric proportion of different tissue compartments. However, serious drawbacks include the relatively complicated device and the need for correction factors because of shrinkage of the specimen, the difficulty in fixing specimens with pulmonary edema, and the need of later fixation of the lung with Zenker fluid for hardening. Besides being expensive, this method involves some hazards to the experimenter during the fixation procedure and in relation to handling large amounts of Zenker fluid; safe disposal of the latter is also a problem.

Glycol-Formalin Fixation Techniques

In 1962, Sills introduced a technique for fixation of the lung by instillation of the bronchus with a fluid fixative.[6] The fixative was prepared by mixing 95% ethyl alcohol with formalin in a ratio of 9:1. The resulting mixture was added to glycol in a 3:2 proportion. Fixation required 12 hours to 2 to 3 days. The fixed lung was then suspended in a bell jar to which a negative pressure was applied, inflating the specimen and drawing the liquid fixative from it. A well-dried specimen was obtained 2 to 3 days later. The lung could then be cut with a standard commercial meat-slicing apparatus.

With the Sills method, the glycol prevents undue hardening of the dried lung and prevents bacterial and fungal contamination as well. The alcohol and formalin in the fixative provide a safe way to handle specimens that may be infected with bacteria and tuberculosis. The resulting specimen has a spongy, dry texture without being brittle, and thin microscopic sections can be embedded, stained, and studied with a dissecting microscope.

Markarian and Dailey's method represents a modification of Sills' original method.[7] It has the advantage of simplicity of method and equipment. The end product is a specimen suitable for photography, gross demonstration, histopathologic examination, and radiographic-pathologic correlation. The lung is inflated through a bronchial cannula for 24 to 48 hours with a fixative containing polyethylene glycol 400 (50% of the solution), 95% ethyl alcohol (25%), 37% formaldehyde (10%), and water (15%). After fixation, the specimen is air-dried at a pressure of 25 to 30 mm Hg for 36 to 48 hours.

Markarian and Dailey's method can be used with injection of contrast material for arteriograms preceding the fixation procedure, and this is best done with the lung inflated with air or carbon dioxide.[7] For injections of vascular structures and the bronchial tree, they obtained excellent results using the technique of Schlesinger.[8]

PAPER-MOUNTED SECTIONS

The technique for the study of lung anatomy and pathology was originally devised by Gough and Wentworth in 1949, but it can be used in other organs as well.[9,10] The preparation can be stored like any other paper, and the samples are also convenient for sending through the mail. They last 20 years or longer, probably as long as the paper that supports them. The color of the preparation does not fade because the glycols used in the preparation process have high boiling points, and these preservatives are probably combined with hemoglobin and its derivatives.

With the Gough and Wentworth technique, slices of whole lungs, 400 μm thick, are mounted on paper or mounted in fluid in thin cases of plexiglass. The technique has been modified by Whimster for routine laboratory use.[11,12] The technique is ideal for the study of the correlation of structure and function, emphysema, and pneumoconiosis (Figs. 5-6 through 5-8), but it can be extended to practically any pulmonary pathologic process.

INJECTION METHODS

Two basic types of injection procedures are used to study vessels and other hollow anatomic structures of the lung and other organs. One is injection followed by corrosion of the tissue to leave a cast of the injected structure (see Fig. 1-6); the other is injection of soft substances that can be studied by standard histologic techniques.[13,14]

In its final form, a corrosion cast must be dry, reasonably rigid, and strong enough to be handled freely. The injection mass must be able to withstand drying without much shrinkage and the effects of strong acids and alkalis used in the corrosion process. An acetone solution of vinylete is suitable for such purpose as originally described by Liebow and colleagues.[15] The mass is colored with acetone-soluble dyes or lamp black. Shrinkage is minimized by continuous or intermittent injection of a material during a 2- to 3-day interval or by the addition of a diatomaceous earth filler. If lead bismuth or barium are added to the injection mass, roentgenographic studies can be carried out. Vinylete casts are most useful

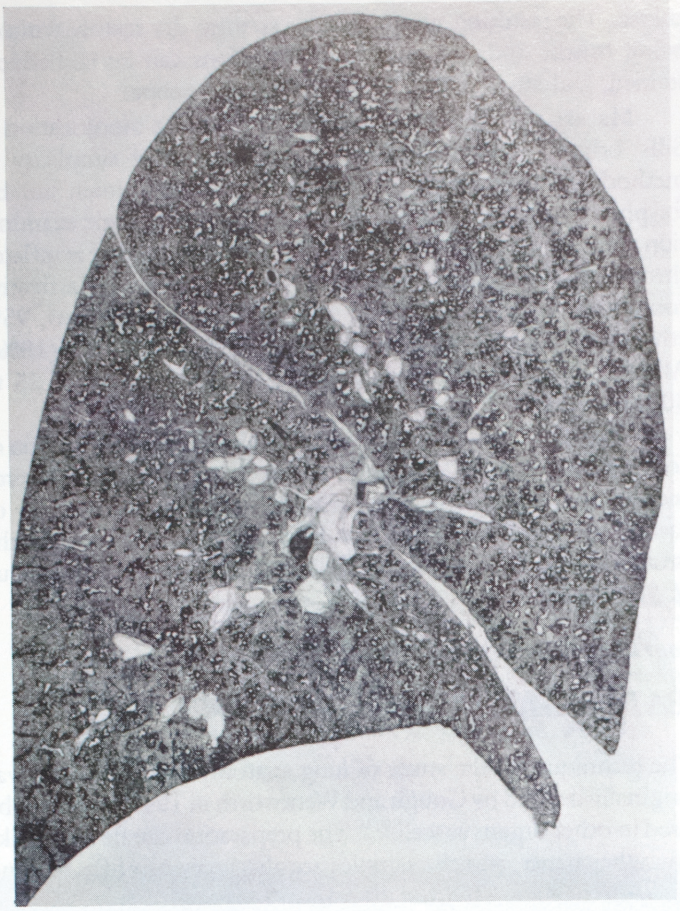

FIGURE 5-6. A study using the Gough and Wentworth technique for examining thin sections of the entire lung in an uncomplicated case of coal worker's pneumoconiosis. (Courtesy of the W.A.D. Anderson collection, Department of Pathology, University of Miami School of Medicine, Miami, FL.)

FIGURE 5-7. The Gough-Wentworth technique is used for a patient with complicated coal worker's pneumoconiosis. (Courtesy of the W.A.D. Anderson collection, Department of Pathology, University of Miami School of Medicine, Miami, FL.)

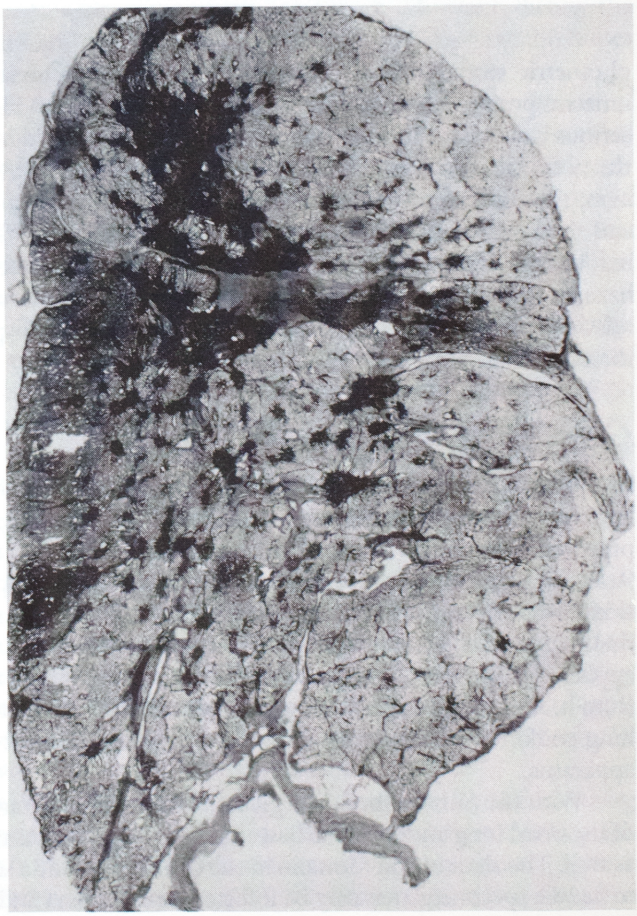

FIGURE 5-8. Silicosis with massive fibrosis and anthracosis is studied using the Gough-Wentworth technique. (Courtesy of the W.A.D. Anderson collection, Department of Pathology, University of Miami School of Medicine, Miami, FL.)

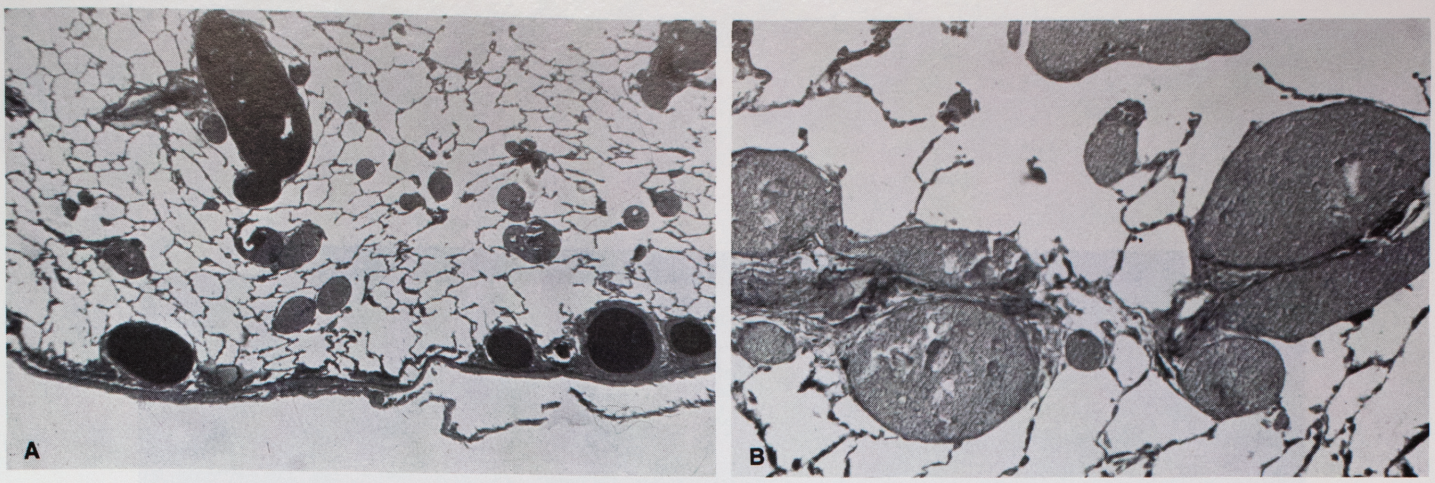

FIGURE 5-9. Vascular lesions in experimental pulmonary hypertension. (**A**) Injection of bronchial collaterals in a dog with severe pulmonary hypertension after systemic pulmonary arterial anastomosis. All vessels were injected from the aorta. (Elastic tissue stain; low magnification.) (**B**) A closer view of the same lung shows clusters of thin-walled bronchial collateral vessels around occluded pulmonary arteries. (Elastic tissue stain; intermediate magnification; from Saldana ME, Harley RA, Liebow AA, Carrington CB. Experimental extreme pulmonary hypertension and vascular disease in relation to polycythemia. Am J Pathol 1968;52:935.)

for studying vessels larger than 50 to 100 μm in diameter. Solidification of the injection mass is accomplished in few minutes to 1 hour by adding a precisely predetermined amount of formalin to the buffered gelatin solution immediately before injection.

An improved technique for corrosion casts of blood vessels was published by Batson in 1955,[16] and it recently has been applied by Redmond and colleagues in their study of the spleen[17] and by Nyström and associates in their description of the vascular anatomy of the hand.[18] This technique involves the use of Batson Corrosive Cast Compound No. 17 (Polysciences, Warrington, PA), or alternatively, Mercox CL-2R Resin (Vilene Company, Tokyo, Japan) has been used by Bugajski and colleagues in their study of the vascular supply of renal cell carcinomas.[19]

Hales and Carrington modified the Schlesinger method by substituting inert pigments of very small particle size for the barium salt.[14] The pigments are resistant to hydrogen peroxide bleaching, dehydration, and cleaning techniques and can be identified in histologic examinations (Color Fig. 5-2; Fig. 5-9). An alternative to this technique is the use of Microfil-MY 122 yellow silicon rubber (Canton Biomedical, Boulder, CO), as recently employed by Bugajski and colleagues in their study of renal cell carcinomas.[19] After the injection procedure, the tissues are cut in 1-mm-thick slices, cleared in ethanol-methylsalicylate, and examined under a stereomicroscope.

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