Stereoscopic Study of the Inflated Lung*

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Stereomicroscopic study of the inflated pulmonary parenchyma offers a three-dimensional approach to the structure, vascular details and cellularity of lungs. The gross characteristics and the interrelationships of the parenchymal structures are more clearly observed. Preliminary observations on the unstained alveolar and pleural aspects of the lungs were documented by Joannides, whose observations did not differ greatly from the concept obtained by Miller* following reconstructional studies.

Inflation and drying of fresh lungs is nothing new, and has been used primarily to provide museum specimens for instructional purposes. In an effort to simulate the in vivo structure of the lung, other investigators have used Woods metal, colored gelatin, formaldehyde, Kaiserling solution and liquid latex. All of these methods have been employed to demonstrate primarily one facet of the pulmonary parenchyma, such as the vascular bed, the bronchiolar structure, or the alveolar membrane.

In our studies, we have combined the efforts of many previous investigators, using stereomicroscopic studies of serial sections of inflated lungs, both unstained and specifically stained sections. At first, the three-dimensional view of pulmonary parenchyma, either stained or unstained, seems

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FIGURE 1: Flared ends of medicine droppers in upper and lower lobe bronchi with purse string suture in place.

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to present a bizarre, sponge-like structure. With further study, one is presented with a concept of coordinated structures that opens up new vistas in the field of normal configuration and pathologic change. The material accepts almost all biological stains, and the method of plastic impregnation facilitates handling and shipment to other observers without distortion of the tissues or possibility of breakage. Stereomicroscopic serial section study may provide a more concise understanding of various disease processes that affect the lungs, for example, emphysema and bronchitis. Boren and Blumenthal have aptly demonstrated in their preliminary studies that these stained sections provide a greater understanding of the component portions of the pulmonary parenchyma.

Lungs or lobes, obtained from necropsy or surgery, may be prepared either in the fresh state or placed in the deep freeze for processing at a convenient time. The frozen lungs demonstrate satisfactory inflation of the parenchyma after five months in the deep freeze. It is noteworthy to state that frozen lungs should be thawed gradually in lukewarm water or else left at room temperature with a moistened towel over the pleura to prevent drying and cracking.

The preparation of material for fume inflation necessitates a preliminary careful observation for pleural tears. These can be controlled by small ligatures, using size 0 black silk. The pulmonary artery is tightly sutured and the veins left open to provide drainage of residual blood in the lungs. Boren has also sutured the veins in order to provide prominence of the vascular system. In our hands, this procedure of venous closure increases the brittle state of the tissue and causes excess fragmentation when the lungs are being cut.

The bronchial stump is cannulized, using the flared end of medicine droppers for the upper and lower lobes (Fig. 1). A purse string suture is passed through the secondary carina, anchoring each cannula separately in place. The lower lobe cannula should not be placed too deeply in order to avoid obstruction of the right middle lobe bronchus, and
similar care should be taken not to obstruct the lingular bronchus on the left. The use of two cannulae with the Y tube allows individual adjustment of flow to the upper and lower lobes. Y tube connections are placed to both cannulae and the lung is ready for inflation which should begin gradually until the specimen is completely expanded. We have been using the criterion of fissure approximation as an indication of an expanded lung within the thoracic space. When the fissures are approximated, the air flow is adjusted so that the lung maintains this state without further pressure variations (Fig. 2). The specimen is maintained on the manifold system for a minimum of seven days. A shorter period of time will invariably result in shrinkage and distortion after the lungs have been removed from the inflation apparatus.

The inflation manifold (Fig. 3) is constructed of copper tubing with valves and outlets into containers for formaldehyde, drying agent, and 95 per cent alcohol. The compressed air is passed over the solutions, utilizing the Venturi principle, in order to saturate the air volume with fume, rather than liquid material. The air desiccant is interspersed between the formaldehyde solution and the alcohol mixture in order to avoid transport of formaldehyde droplets into the lung. The formaldehyde and alcohol fumes serve to fix the lung in the inflated state as well as preserve some of the natural coloration of the tissue. A small quantity of glacial acetic acid added to the alcohol mixture will enhance nuclear staining techniques. It is probably best not to exceed pressures of 20 to 30 cm. of water passing through the manifold into the lung. Of utmost importance is a compressor with adequate output and reserve. A pressure reducing valve inserted between the compressor and the manifold will avoid excess surges of compressed air with possible rupture of pulmonary tissue. Such reducing valve also allows the use of increased flow of air through the manifold without increasing the pressure excessively.

The lungs are sectioned by using a scalloped meat cutting blade on a conventional band saw. For our purposes, we devised a carriage con-

![Image](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21345/ on 06/25/2017)
connected to a screw type band saw "gate" which permits sections as thin as one millimeter. In order to study interalveolar relationships, we sectioned most of our material in 2 mm. slices. Although the tissue can be manually held in the carriage, we cemented one end of the lung to the side of the band saw gate, in order to obtain consistent serial sections without endangering the fingers of the operator (Fig. 4).

The staining procedures follow standard histologic techniques plus the fact that all phases must be accomplished under vacuum. This provides for a thorough staining of these sections which are considerably thicker than the standard 5 mμ specimen. Constant observation is necessary to prevent overstaining or under differentiation. The method of plastic impregnation is best accomplished in a vacuum oven. At present, we believe that the easiest and safest to work with are the apoxy resins. For routine study, the stained sections may also be mounted in "Permunt."

The stereoscopic study of pulmonary parenchyma prepared and stained as described above provides the observer with a more comprehensive understanding of pulmonary anatomy, histology, and pathology (Fig. 5). Single plane observations made with the conventional microscope do not easily permit a continuous study of all adjacent areas, above and below the focal plane being studied. Extension of disease to adjacent areas can be observed more readily, especially in such instances as pulmonary emphysema. The variations in the degenerative processes involving pulmonary parenchyma can be studied in contiguous levels of tissue without resorting to the tedious study of single plane serial sections. With the application of histochemical staining techniques, it is possible to observe the normal and abnormal course of the alveolar capillary bed, pigment aggregates, depositions of fibrous tissue and other evidences of alveolar degenerative involvement. Bronchial and broncho-alveolar structures

FIGURE 4: Lung being sectioned with band saw.
FIGURE 5: Stereoscopic photomicrogram of pulmonary parenchyma.

with the major ramifications can be observed more clearly and perhaps provide a better understanding of pulmonary ventilation. The stereoscopic observations of pulmonary parenchyma in general provide a three-dimensional aspect which is more comprehensive than conventional microscopy and assists in better understanding of the normal and diseased structure.

SUMMARY

A method for inflation of pulmonary parenchyma is described which, combined with histochemical staining and stereoscopic study, may shed more light on our present concept of the anatomy, pathology, and physiology of the lungs.

RESUMEN

Se describe aqui un método para inflar el parénquima pulmonar, el que combinado con la coloración histoquímica y el estudio estereoscópico, puede proporcionar cierta luz sobre nuestro concepto actual de la anatomía, la patología y la fisiología pulmonar.

RESUMÉ

Les auteurs décrivent une méthode d'insufflation du parenchyme pulmonaire qui, associée à une coloration histochemique et une étude stéréoscopique, peut apporter davantage de lumière sur notre conception actuelle de l'anatomie, de la pathologie et de la physiologie des poumons.

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REFERENCES

5 Boren, H., and Blumenthal, B.: Personal Observation and Communication.