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Preparation of whole lung macrosections

Dwight Leroy Snyder
University of Nebraska Medical Center

THE PREPARATION OF WHOLE LUNG MACROSECTIONS

Dwight LeRoy Snyder

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INTRODUCTION

In recent years there has been a marked increase in interest concerning the so called "chronic pulmonary diseases" which so commonly afflict mankind. Included in this general category are such entities as pulmonary emphysema, chronic bronchitis, bronchial asthma, and pulmonary fibrosis. In addition, it has become more and more apparent that further study of these conditions is required in order to categorize them into a standard classification and to gain more knowledge concerning their etiology, morphology, and patho-anatomy. It is generally agreed among investigators that inflation-fixation of both normal and diseased whole lungs has proved to be essential to these studies. An overall three dimensional study of lungs in their physiologic state of inflation makes possible an examination of the small delicate structures of lung morphology. Also the airway and vascular arborizations of the entire organ and the relationships of larger anatomical units such as alveoli and capillary beds can be investigated.

These simple and inexpensive techniques of processing lung tissue will undoubtedly be used more extensively in the future. As is pointed out by Kleinerman, (14) we now possess more knowledge of the microscopic components of lungs than we do of the equally important and readily visible gross relationships of anatomy. We now measure lung diffusing capacity, ventilation and perfusion

ratios, lung compliance, and bronchial resistance to airflow in addition to other highly refined pulmonary functions. To more completely understand the anatomic relationships governing these functions it is necessary to observe the ventilatory unit as a whole. The relationships of alveoli, lobules and lobes to one another with their associated passageways for blood and gases, including the network of elastic and reticular material which support and bind them together, must be studied in healthy as well as in diseased lungs.

The purpose of this paper is to describe a technique used by the author for the inflation, fixation, sectioning and lamination of sections of whole lung specimens. The relative merits of this method and those of several other techniques of whole lung fixation will be discussed. Finally, the results of these various techniques will be evaluated with emphasis on their application to research, education, and the study of chronic pulmonary disease in man.

METHODS

A total of eighteen lungs have been prepared in our project using the formalin fume technique first described by Blumenthal and Boren in 1959. (2) This method of preparing fixed, dried whole lungs is very simple and requires a minimum of equipment. The sectioning and subsequent lamination of specimens which are used in this study is a rapid and quite unique method which was described by Cote' in 1962. (5)

1. Fixation and Drying. - The material required includes a source of continuous compressed air with a regulator, a large side arm flask, small plastic cannulas, and an assortment of tubing, clamps and stands. The work should be done under a fume hood to prevent the escape of formalin fumes into the room.

The lungs obtained for our studies have all been removed during routine autopsy. The necessity of removing the lungs with the visceral pleura intact has presented no special problems except when extensive adhesions or pleural disease is encountered. It is then usually necessary to dissect out the parietal pleura along with the lung and visceral pleura. In addition, it is frequently necessary to remove portions of the diaphragm with the lung. This excess tissue can be easily dissected away later in the process, either before or after inflation. Small lacerations are sometimes unavoidable. These can either be sutured closed with some degree of success or they can be sealed off by using standard paper clips. If larger tears occur, the lung is best discarded in view of the difficulty encountered rendering them air tight. In severing the bronchi, care must be exercised to allow a sufficient length of bronchus into which the cannula can be easily inserted and firmly secured. The cannula can be held in place by purse string sutures or by winding string tightly around the ends. The cannulated lungs are then suspended from a ring stand by a wire through the hilar

vessels and placed into the fume hood. The cannula is connected to the side arm flask which is filled with 10 percent formalin solution. I used a large ten liter flask in order to avoid repeated fillings with solution. The side arm flask is then connected to the source of compressed air in such a manner to allow bubbling of air through the liquid. The airflow is adjusted with the pressure regulator to a point which will allow inflation of the lungs sufficient to barely separate the fissures. This end point is considered to be very close to physiologic lung expansion by Cote'. (5) I have needed a flow between two and eight liters per minute, depending on the amount of leakage in the system. After initial adjustment the flow of air seldom requires changing throughout the entire course of fixation or drying.

The time required for fixing and subsequent drying of the lungs varies with the condition of the specimen. Lungs with free airways require much less time than do those with bronchial obstruction. In the usual cases, formalin is bubbled for three days, after which it is replaced by 95 percent alcohol. This is bubbled for three more days. Absolute alcohol is then used for two days and finally compressed air alone for approximately five additional days. The lung should then be thoroughly fixed, dried and ready for sectioning.

This is a somewhat lengthy process for preparing tissue for study. It is, however, one which takes very little time or

monitoring on the part of the operator. It should also be pointed out that more than one lung can be prepared at the same time. I have had as many as four lungs being processed on a single air outlet. The airflow required to maintain the desired level of inflation in all specimens was only ten liters per minute. In such cases of multiple fixation, the flow of air to each specimen is controlled by individual screw clamps on the tubing just proximal to each lung cannula.

Another practice which I have found useful is that of freezing excised lungs for later processing. Excess blood and mucous are drained or gently expressed from the lung and the lungs are inserted into plastic bags to minimize drying. They are then placed in a freezer at minus 15 degrees centigrade. Later thawing at room temperature and fixation by the same techniques as for fresh specimens results in the same quality of finished product.

The dried lungs at the end of processing are very light in weight, light brown in color, and quite firm with a consistency not unlike that of dried bread. They are somewhat fragile but may be handled or stored without worry of distortion or deterioration.

2. Sectioning and Mounting of Tissue. - A commercial meat slicer with a circular cutting blade is used in this project for sectioning the lungs. The Hobart model 1612 was chosen because of its stable and easily operated carriage, its readily adjustable

size of cut and its built in sharpening device. I have found that a very sharp cutting edge must be maintained for good results in sectioning.

The lung is first cut into three or four thick sagittal sections using a long serrated bread knife. These parts are then easily handled on the carriage of the slicer which is adjusted to a cut of 400 to 800 microns. Thicker sections are possible if stereoscopic viewing or special preparations warrant it, but for lamination purposes a thickness of approximately 500 microns is optimum. The operation of the machine is quite simple; a whole lung can be easily sectioned in 30 to 40 minutes.

For purposes of preparing permanent mounts of these specimens, a process of lamination using a standard flat office copying machine previously suggested by Cote' (5) is used in this project. The machine is a Premier model 19 Thermofax which has been reworked to use standard 110 volts. Two methods of mounting for lamination have been used. The first method consists of mounting the tissue between a rigid sheet of card weight paper and a sheet of transparent laminant. This gives a good background for gross study of tissue structure and is quite durable. The second method consists of mounting the tissue section between two sheets of lamininant to produce a more pliable product which lends itself to study by stereoscopy and which can be included as sheets in

catalogues, reports, or pamphlets. The card paper is available as single coated back-up stock and the transparent sheets are regular laminating stock, both of which are available at most office copying retail outlets. The lamination process is accomplished by simply lining up the sheets and inserting them into the machine. If so desired, a typewritten insertion may be laminated with the specimen for descriptive or labelling purposes.

3. Results. - Tissue which we have prepared by this technique has shown no signs of deterioration in almost two years and there is no reason to believe that it will not withstand may years without showing change. It has been noted, however, that frequent handling of the specimens will eventually cause some flaking off of tissue.

In addition to the lamination of fume fixed tissue just described, several staining techniques have been tried. The stained tissue was embedded in parafin, sectioned on a microtome and mounted on standard glass slides. Microscopic examination of these preparations revealed good retention of cellular detail which was, in most respects, comparable to standard histologic techniques. There was, however, some distortion of the tissue, which most likely was a result of the drying process.

DISCUSSION

The methods described here for the study of whole lungs are by no means the first, nor are they the only techniques devised for this purpose. In a resumé of previous work done in the investigation of lung structure, Miller (16) described the work of several early investigators and their contribution to our fund of knowledge concerning this subject. Included in this resumé are such names as Malpighi, who in the seventeenth century used whole lungs into which he injected mercury in proving that no direct communication existed between the vessels and bronchi. Willis (1622-1675) and Reisseissen (1778-1828) also used mercury injected into the bronchi of whole lungs. In addition, these men used air to inflate and dry lung specimens in the course of their investigations. Other early investigators who made important contributions throughout history are Addison (1793-1860), Rossignol (1815-1870), and Waters (1826-1912). The latter first described the "terminal bronchiole" and the "air sac" as we know them today. These workers all included air inflated and dried lung tissue in their studies. Mandl (1812-1881) injected whole lungs with gelatin, dried and inflated them with air, and later made sections for microscopic studies. These techniques have since been performed by many investigators with reported good results. An interesting method used by Rindfleisch (1836-1908), and Loeschcke (1882-) was

the corrosion technique. This consisted of filling the bronchi with Wood's metal and then, after hardening, the organic matter was corroded away chemically to leave a case of the air passages. Modifications of this method have been devised for the airways and blood vessels, both of which are very effective in demonstrating the arborization of these structures.

More recently there have been numerous investigators who all claim very satisfactory results from various techniques of inflation and fixation of lungs recovered from cadavers. Many of these workers use combinations of studies which include, in addition to inflation-fixation, postmortem pulmonary function studies, x-ray techniques on specially prepared lungs, and latex or dye injections of the bronchial and vascular trees.

Moolten (18) in 1935 used "illuminating gas" to fix the hemoglobin of lungs a permanent red after inflating them in an evacuated bell jar. The fixative is introduced through a hole in the jar which communicates with the bronchus. The inflation of whole lungs with vacuum instead of positive pressure is considered more physiologic by some investigators. This technique, although used quite extensively in the inflation-fixation process, has the disadvantage of being more complicated and requiring much more elaborate equipment.

Gough and Wentworth, (7) in 1949, described their well known "paper section technique" which has been one of the early

classics in the preparation of macrosections of distended, fixed lung tissue. Their original work was for the purpose of studying respiratory disease in coal workers, but success with the technique prompted its application to all forms of lung disease. Liquid formaldehyde from a reservoir four feet above the lung was used to fix and distend the tissue. The lung was floated in the fixative and covered with a cloth soaked in the same fluid. After several days the lungs were cut into blocks and submerged in the fixative for long periods of time to destroy proteolytic tissue and bacterial enzymes. These blocks were then embedded in gelatin and sectioned on a special microtome which could take blocks up to 10 x 8 3/4 inches. A unique method of storing and displaying the macrosections has been devised by these workers. Wet sections are placed on Perspex which is then flooded with a mixture of gelatin and glycerin and covered with filter paper. When the paper is thoroughly dry it is stripped off the Perspex along with the firmly attached macrosection. This leaves a specimen with a glossy surface which can be studied grossly or microscopically. This technic is obviously quite involved and lengthy but has been used extensively and with apparent good results.

In his extensive studies on pulmonary emphysema, Heard (11) has described a technique which is basically the same as that of Gough and Wentworth but with more precise measurement of lung

distention. In addition, he devised a method of determining the volume of the lung throughout the fixation period. A constant pressure of 25-30 cm. of formaldehyde is maintained throughout the entire two week period of fixation. Heard calculated that this is sufficient to produce a near physiologic distention. The lung volume is measured by a fluid displacement technique and is useful in determining tissue shrinkage and recoil during fixation. The prepared specimens are sliced 0.8 cm. thick and stored in 5 percent formalin fluid for further staining or for direct study. A method of staining which greatly enhances the opacity of the lung tissue was also described by Heard. Barium sulfate is precipitated on the tissue by bringing it into contact first with barium nitrate and then with sodium sulfate solutions. The resulting white coloration is very effective in showing structures which normally are not readily seen. This unique method of staining was also used by Fischer in 1964. (6)

The use of formalin fluid under continuous pressure to distend and fix whole lungs was used by Wyatt (27) in the preparation of 275 specimens which he described in 1959. A modification which he introduced was the injection of latex dye into the pulmonary vessels prior to inflation in order to better visualize normal and abnormal blood channels.

In 1964, Fischer (6) combined antemortem and postmortem findings in his "Multiple Technique Concept" of lung studies. In

addition to antemortem and postmortem pulmonary function studies and x-rays, he used the Gough technique of preparing "paper sections" as well as the injection of pulmonary vasculature with latex dyes.

Other workers who used the same Gough technique or modifications thereof are Sills, in 1962, (21) and Kleinerman, in 1964. (14) Sills first fixed the tissues by instilling formalin fluid into the bronchial tree while the lung was submerged in the same fluid. He then inflated it to the desired level in a bell jar vacuum. This two step procedure was further modified by the addition of iodine fumes to the air used in inflation and drying. The iodine was added to render the bronchial tree more radiopaque for later x-ray studies.

Blumenthal and Boren, in 1959, (2) first described the technique of using formalin fumes rather than fluid for fixing lung tissue. Further improvement of this method was described by Cote',in 1963, (5) who devised a method of sectioning and mounting the prepared lungs which is both rapid and inexpensive. These methods of formalin fume fixation, sectioning with a circular bladed meat slicer and lamination of the sections with an office copying macine are all ones which are used in present study and have previously been described. It should be pointed out here that prior to the article of Cote', the use of a circular bladed meat cutter for this same purpose was described by Sills, in 1963. (21)

The formalin fume technique has been used by several others since its rather recent introduction in 1959. In 1960, Jones (13) used this method to prepare several normal as well as diseased lungs. He found that over distention, with rupture of alveolar membranes, is a problem if too much pressure is used. He mentioned also that several disease processes render the lung unfit for this method of fixation. Among others, he had trouble with lungs which had tumors, obstructive diseases, and fibrotic changes.

An interesting modification of the fume fixation was described by Hentel and Longfield in 1960. (12) These workers passed air not through the formalin but over it and produced fumes by the venturi effect. After a minimum of seven days fixation the lungs were sectioned by using a scalloped meat cutting blade in a regular band saw. The sections were then stained under vacuum using standard histologic techniques.

In 1961, Pratt and Klugh (20) did many postmortem studies of pulmonary function by using an enclosed box and a vacuum pump. Included in their studies were expiratory flow rate, residual volume and static inflation and deflation. Fixation of the lung was then accomplished by use of formalin fumes through a "breathing mechanism". A ported cylinder and piston arrangement and a one way check valve were used to alternately inflate and deflate the lung during fixation. After 12-18 hours the fixed lung, still collap-

sibile, was dried by a constant air pressure until the weight ceased to decrease. Five to eight days were required to reach this end point. The lungs were then sectioned using a knife blade on a band saw and studied by stereoscopic microscope. Various staining techniques were utilized.

In 1961, a new and rapid method for inflation-fixation of lungs was described by Weibel and Vidone. (25) Formalin steam is utilized for the fixation process and an evacuated chamber provides the inflation. After only two hours of such fixation the lung is removed from the apparatus and soaked in Zenker's solution overnight. Such rapid processing is possible because of the high temperature attained. The tissue is then sliced in 2 cm. sections and re-soaked in Zenker's solution for 12 hours. After this treatment the thick sections are somewhat hardened but still quite damp and pliable. The slices may be embedded or cut into thinner slices for various staining techniques. A tracing made of the lung at the beginning of fixation and later after completion are compared in order to compute the amount of shrinkage. The technique apparently produces good cellular detail and is quite simple once the rather elaborate apparatus is assembled. In 1964, this same technique was used by Greenberg (8) who claimed excellent results.

Tobin, in 1952, (23) studied pulmonary diseases and structure by injecting pulmonary vessels, bronchi and bronchial vessels with

latex dye. He then inflated and dried the tissue with compressed air alone which was passed through calcium chloride chips. He later used two percent agar in warm ten percent formalin in addition to the dye for the injection since this helped to fix the tissue. Minimal distortion of airways was noted and relative proportions were retained in lung structure. A certain amount of distortion was encountered in the cells because of drying without first fixing the tissue. The investigator points out that drying of this tissue should be no more detrimental to microscopy than the drying of a blood slide prior to staining it. The method of distending lungs with air alone has been used for many years. In 1862, Waters (24) and many others previously mentioned used this technique. Because of the danger of spreading infection, the method is considered unsafe in lungs which are diseased and, according to Sills, (21) should not be considered in the study of lung pathology.

EVALUATION OF METHODS

The selection of a single technique for the preparation of gross macrosections which is superior to the others would be difficult to make. Each author has pointed out the advantages of the process which he has described, and it would appear that each one is the ideal.

It was pointed out by Heard, in 1960, (10) that a certain amount of standardization of technique would be beneficial to the

study of pulmonary disease. If methods of study were the same, the results of various studies could be more closely compared with each other. Interest in gross preparations of whole lungs is gaining in popularity and as more work is done in this field, the best and simplest technique will undoubtedly evolve as the one most often used.

The first variation in procedure to be evaluated is the method of inflating the lung. One has the choice of either placing the lung in an evacuated chamber or using positive pressure to the airway. The vacuum method is purported by some to be more physiologic. There are, however, several disadvantages. It is more complicated to set up and operate, more elaborate equipment is required, and only one lung can be processed at a time. There have been no difficulties reported by those workers using the positive pressure method. In my own experience, this much simpler method has been very satisfactory in all respects.

Several factors must be considered in choosing the best method of fixation. Each of the three methods reviewed, formalin steam, vapor and fluid, are apparently quite satisfactory but each has its good and bad features. Weibel and Vidone (25) believe the following conditions should be satisfied: (a) lung distention should be controllable, (b) tissue shrinkage should either be negligible or should be accurately measurable, (c) tissue detail

should be well preserved, and (d) the method should not be excessively slow. These conditions are satisfied in the formalin steam method, however, it is not without disadvantages. Greenberg (8) reports shrinkage of 18 percent utilizing formalin steam. This amount of change, even though measurable, is bound to produce distortion of the tissue involved. Blumenthal and Boren (2) reported only 3 to 5 percent shrinkage with the fume technique and Heard, (10) utilizing fluid fixative, found almost no change in the volume of his specimens. Other drawbacks to the steam system are its requirement for a rather elaborate apparatus and the inherent dangers of using steam in an enclosed system. In addition, there is some discoloration of the tissue as well as distortion and shrinkage.

The formalin fume technique is simple, requires very little equipment and may be used to fix several lungs at one time. The method results in specimens which are permanently fixed and easily mounted. Good cellular detail is retained according to Cote' (5) and by Blumenthal and Boren. (2) Disadvantages, according to Boren, (3) are that inflammatory changes in the bronchi and bronchioles are not well demonstrated, alveolar walls are slightly thinner than in wet preparations, and the finished specimens are dry. This latter complaints may be desirable in certain cases. It makes the tissue more amenable for sectioning and lamination as described by Cote' (5) and it does not alter the predominant

lung pathology in most cases. Other drawbacks to the fume method according to Heard (9) are the increased difficulty encountered with pleural leaks and the fact that the size of alveolar spaces may not be comparable to that found in life. The latter objection is based on the lack of an accurate means of measuring volume variations during fixation in the fume technique.

The fluid method of fixation does not have as many drawbacks as the other two. It is simple, inexpensive, and makes possible very good cellular detail with little distortion of tissue. The finished product is a wet preparation which can be used in standard slide preparations or for macrosections. Sills (21) cut out cylinders of the fixed tissue with a cork cutter for conventional histologic preparations and then inserted a cork into the space for completion of the macrosection technique. According to Kleinerman (14) the technique is suitable for microscopic viewing of very small abnormalities in capillary distribution, alterations in reticulum and elastic networks, and changes in alveolar walls, as well as for showing three dimensions gross structural changes of the whole lung.

Wet sections may not be as well adapted to the lamination process of mounting as are the fume fixed dry specimens. Cote' (5) reports some clouding of the laminant after a period of time with wet preparations. In my own limited experience with wet

tissue lamination, good preservation is still apparent after 18 months. There is no question that dry lung tissue, however, is much easier to handle and to store than is wet tissue.

A limitation which is present regardless of the technique used is the problem of removing whole lungs from the cadaver with the visceral pleura intact. I have had to give up many good specimens because of adhesions, tumors and other extensive pleural involvement which prevented the removal of a lung fit for fixation. In many cases, the more valuable specimens are the ones which can not be used.

Another problem is that of obstruction of the airways by fibrosis, tumor, edema or bronchitis. This prevents adequate fixation of the whole lung if fixation is via the air passages. Jones (13) feels the whole process of inflation-fixation of fibrotic and diseased lungs is valueless because such conditions tend to inhibit the diffusion of fumes and fixatives. I have found in the present study that this difficulty can most always be solved by increasing the time for fixation and drying to allow more thorough diffusion. The difficulties have been mainly with lungs which are edematous and ones which contain tumor. This problem, however, is not nearly so bothersome if the fluid fixation method is used. Heard (10) points out that the lungs are floating in fixative and hence are being fixed both from the outside and via

the bronchial passages. It is for this reason, and some others which have been mentioned, that many investigators favor the fluid method of fixation.

APPLICATIONS OF THE TECHNIQUES

The adaptability of good quality gross macrosections in microscopic, stereoscopic and gross study of chronic pulmonary diseases has been previously discussed. Boren (3) pointed out that only one-two thousanths of the lung substance is viewed by examination of a standard lung tissue slide, whereas, the whole lung is routinely visualized in the macrosectioned method. Boren also observed that with this process it is possible to dissect the conducting airways to explore them in their entirety. According to him, alterations in the finer lung structure are not nearly so easily detected or studied in collapsed lung as in tissue which has been inflated.

Many investigators have described the use of fixed whole lungs for x-ray examination of the bronchial tree after perfusion with radiopaque material. Included here are Leopold and Gough (17) who used lead particles, Petit (19) who used lead nitrate, and Wyatt (27) and Fischer (6) who both used barium sulfate. In addition to x-ray techniques, some investigators use injection of dyes or fresco paints into the vascular or bronchial tree prior to fixation for later direct visualization of these constituents.

Such workers include Wyatt, (27) Fischer, (6) and Tobin. (23) Cote' (5) has suggested several uses for macrosections in addition to study purposes. He includes a representative section of each lung in the pathology report of the autopsy case. Sections are also included in reports sent to the Armed Forces Institute of Pathology. In addition, the sections are useful in teaching laymen or students about emphysema or other lung diseases. I have had may requests for both a normal and emphysematous specimen to be used for comparison in anti-smoking campaigns.

Blumenthal and Boren (2) have used the technique as a screening test for determining whether or not various pulmonary lesions can be produced experimentally in animals. A rather unique application of the inflation-fixation process was described by Moolten,(18) in 1935, who used fixed whole lungs to teach bronchoscopy to students.

During a symposium on emphysema held in 1958, in London, England, (4) much enthusiasm was generated concerning this method of research. At this symposium it was noted that emphysema can be diagnosed and classified consistently only when inflation-fixation techniques have been completed on the lung in question. Intrabronchial infusion of fixatives was recommended as the simplest technique. Also mentioned was the examination of serial sections and the use of stereoscopic microscopy in the study of emphysema.

A symposium on emphysema and the "chronic bronchitis" syndrome (22) was held at Aspen, Colorado, in June, 1958. At this meeting a special committee on the preparation of human lungs for macroscopic and microscopic study made several recommendations worthy of mention. They suggested that all lungs being studied for pulmonary disease should be fixed *in toto* via the air passages before being sectioned for either macroscopic or microscopic examination. This allows the examiner to view the tissue more nearly in its physiologic state.

The committee reported that non-distended lung tissue has approximately 12 percent shrinkage of alveolar diameter during formalin fixation. In addition, the sectioning of this tissue, even with the sharpest of knives, collapses the alveoli by another 40 percent. It was the belief of the committee that many pathologists have never seen lung tissue in the structure maintained throughout life because they have never distended it. Further discussion led to the proposal that all specimens of lung tissue be prepared for examination in this manner. The method of preparation favored by this committee was liquid perfusion of the air passages with formalin while the lung floats in the same fluid. Both thick sections for stereoscopy and thin ones for embedding and conventional staining were recommended.

SUMMARY

A simple technique has been devised to fix and dry whole lungs in their expanded physiologic state. This method, utilizing formalin fumes under pressure, was first used by Blumenthal and Boren in 1959. (2) The method was further improved by Cote' (5) who devised a method of sectioning and laminating this fixed and distended lung tissue.

The lung is removed from cadaver material with its visceral pleura intact. This allows inflation to the desired level with minimal leakage. A cannula is inserted into the main stem bronchus of the lung or into the trachea if both lungs are obtained. Compressed air is bubbled through formalin contained in a side arm flask. The formalin fumes are then connected to the lung via a section of tubing from the side arm to the cannula. The level of distention is controlled by a regulator which controls the flow of compressed air.

After three days of fixation, the formalin is replaced by 95 percent alcohol and the process is continued for three more days. Absolute alcohol is then used for two days and finally compressed air alone for approximately five additional days. The lung should then be thoroughly fixed, dried, and ready for sectioning and lamination.

The sectioning is accomplished by using a commercial meat slicer of good quality adjusted to a cut of 400 to 800 microns. The whole lung can be sectioned quite easily in one hour. The thin sections are then laminated by using a commercially available flat office copying machine. Two methods of mounting for lamination are used. The first method consists of mounting the tissue between a rigid sheet of card weight paper and a sheet of transparent laminant. This gives a good background for gross study of tissue structure. The second method consists of mounting the tissue between two sheets of laminants to produce a more pliable product which lends itself to study by stereomicroscopy and can be included in such things as pathology reports, catalogues of lung pathology, and study pamphlets.

Several different techniques have been described in the literature for the preparation of lung macrosections. Formalin perfusion of the bronchial airways is used by most investigators and is in the form of either fumes, steam or liquid. The steam method takes less time than the others but the high temperatures lead to some tissue shrinkage and distortion. In addition, a rather elaborate apparatus is required to control the steam. The fume technique is simple and lends itself well to the lamination process described. However, it is quite slow and produces specimens which are dry. This is considered undesirable by many

investigators. The fluid method of fixation is also quite slow but it is simple, leads to more thorough fixation, and produces very little shrinkage or distortion of the tissue. This latter method is the one preferred by the majority of investigators.

There are limitations which are common to the preparation of macrosections no matter which method of distention and fixation is used. It is frequently difficult, because of various disease states, to obtain a whole lung with the visceral pleura intact. Unless tears in the pleura are small ones which can be repaired, the lung must be discarded. Disease states which cause partial or complete obstruction of lung air passages create another problem. Tissue beyond the obstruction is sometimes inadequately fixed and is then subject to later deterioration. This latter drawback is overcome by increasing the fixation time or by the use of external as well as intrabronchial fixation.

The relatively simple and inexpensive methods of preparing whole lung macrosections have made this form of study increasingly popular over the past few years. It is considered a very useful and necessary tool in the study of chronic pulmonary diseases. In addition, these sections are excellent for display and for educational purposes as well as for making pathology reports and medical conferences more meaningful.

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APPENDIX

The following three lung specimens are submitted as examples of the formalin fume technique of inflation-fixation. This tissue was fixed and laminated in the Summer of 1963.

The first specimen is normal lung tissue taken from a 62 year old, non-smoking female patient.

The second specimen was taken from a 72 year old male who died of myocardial infarction. It is an example of generalized pan-lobular emphysema. There was no recorded history of dyspnea or shortness of breath.

Specimen number three is an example of centri-lobular emphysema taken from a 67 year old male who died of pneumonia of the opposite lung secondary to bronchogenic carcinoma. He had a long history of dyspnea, shortness of breath and an increased A-P chest diameter.