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Postmortem inflation and fixation of human lungs

A technique for pathological and radiological correlations

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Wright, B. M., Slavin, G., Kreel, L., Callan, K., and Sandin, Brenda (1974). *Thorax*, **29**, 189–194. **Postmortem inflation and fixation of human lungs.** A method of fixing lungs by inflating them with heated formalin vapour is described. This method facilitates postmortem correlations between radiographic and histological appearances.

Lungs removed from the body at necropsy are collapsed and contain very little air. This gives a quite unnatural appearance to both gross and histological specimens and makes it impossible to compare postmortem and antemortem chest radiographs, because the pattern of lung shadows is made up of the contrast between the air-containing and the liquid-containing or solid parts of the lung.

Infusion of liquid into the lungs (Gough and Wentworth, 1949; Heard, 1966) serves to expand them but obliterates radiological contrast. Although fixation is good it produces histological artefacts, such as alveolar macrophages apparently free in the lumen of the alveoli and unattached to the alveolar walls (Duguid and Lambert, 1964).

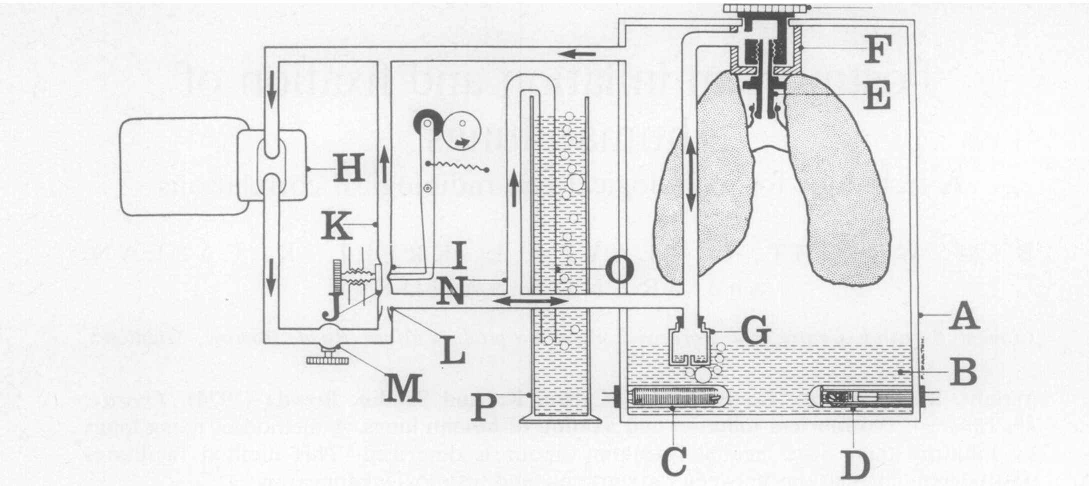
The logical procedure, therefore, is to inflate the lungs with gas and fix them while so inflated, and a number of attempts have been made to do this (Silverton, 1964). With the exception of simple drying of the lungs while inflated, which produces gross histological distortion, nearly all the methods described have been based on fixation with formaldehyde, either by blowing in the vapour from boiling formalin (Weibel and Vidone, 1961; Duguid and Lambert, 1964) or by inflating the lungs with air bubbled through cold formalin (Cureton and Trapnell, 1961). None of these procedures seems to have proved satisfactory for routine use, because of a lack of appreciation of the fundamental physical processes involved. These are as follows:

1. Normal uninjured lungs will not pass any appreciable volume of gas at physiological pressures. Lungs which have been injured during removal from the thorax will pass gas through any perforations but this will only expose a very limited part of the lung to fixation by the gas.
2. If steam and formaldehyde gas are blown into a lung held distended by air pressure, as in the method of Weibel and Vidone (1961), the steam will condense in the lungs so that water in which formaldehyde is dissolved will accumulate in the air spaces. Although some fixation by formaldehyde gas will occur, the method is primarily one of wet fixation. A substantial proportion, however, especially of the upper part of the lung, may remain relatively free of liquid because of drainage to the most dependent parts.
3. If the lung is fixed only from the inside, especially at room temperature, the outer parts may begin to decompose before formalin reaches them by diffusion from the airways.

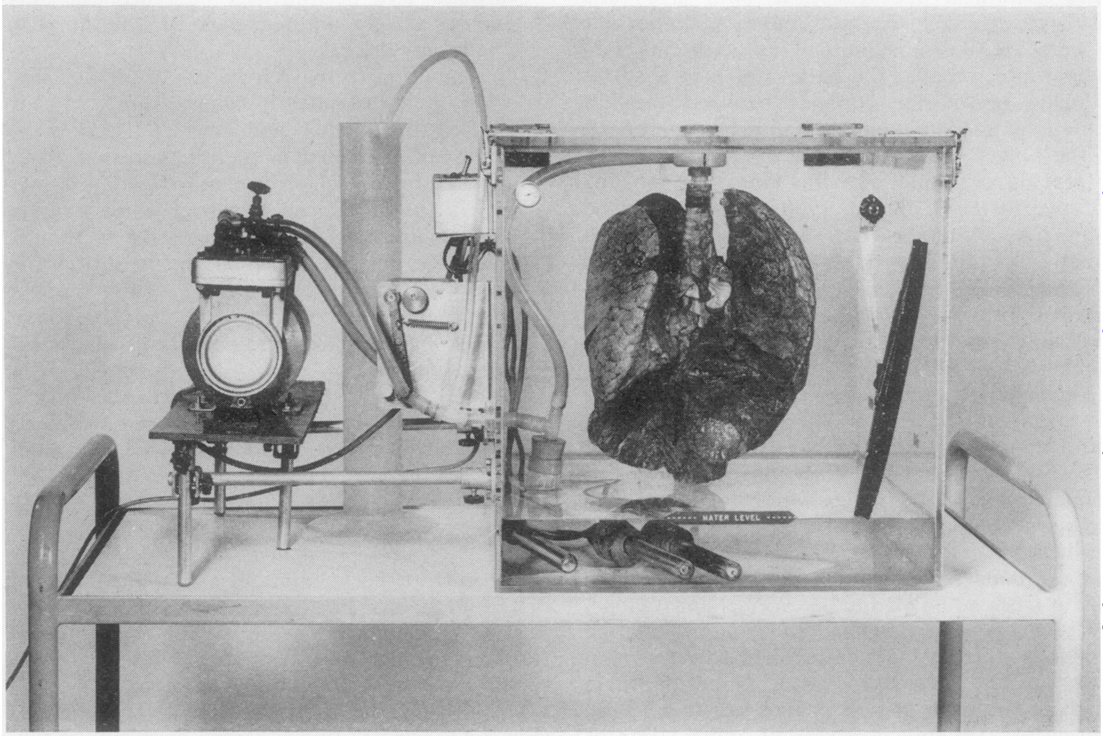
APPARATUS

After much experimentation, a simplified version of the method of Pratt and Klugh (1961) of causing the lung to breathe the vapour above heated formalin solution was adopted as follows:

The lungs are suspended in a Perspex box (A) over a 40% aqueous formaldehyde solution (B) (Fig. 1A). The solution is heated by means of two submerged 150 watt heaters (C), controlled by a thermostat (D), so that the temperature of the atmosphere above the solution is held at about 45°C. The lungs are removed from the thorax



(A)



(B)

FIG. 1. **(A)** *Diagram of inflation apparatus. For details see text.* **(B)** *Apparatus in use. Note that Perspex box together with pump fit easily onto a standard hospital trolley.*

taking special care to avoid pleural tears. Small perforations do not interfere with the method but larger tears must be oversewn. Mucus is removed by aspiration from the trachea. The trachea or bronchus is tied over a cannula (E), which is screwed into a boss (F) in the lid of the box and connected by a wide-bore tube inside the box to a water trap (G) and thence to an external circulating air pump (H). Air is drawn from the box and passed in and out of the lungs by the pump and by means of a cam-operated pinch-cock (I) with an adjustable platen (J), driven by a motor (not shown) which opens and closes a bypass (K) 15 times a minute. When the bypass is open, the flow through it is increased by means of an injector (L), which produces a slight negative pressure in the lungs. The rate of circulation can be controlled by a valve (M). The pump used is of the diaphragm type which can circulate the wet corrosive atmosphere without being damaged.

A T-piece (N) in the pipe leading to the lungs, but outside the box, is connected to a tube (O) which dips into a tall narrow vessel (a plastic measuring cylinder) (P) to act as a pressure gauge and safety vent. A windscreen wiper (seen in Fig. 2B) inside the front of the box makes it possible to see the lungs in spite of the condensation on its walls.

MODE OF OPERATION

The heated formalin solution gives off water vapour and formaldehyde which condense on the walls and lid of the box and on the lungs and then run back into the solution. The condensation of water vapour heats up the lungs and the box until the atmosphere is held at a temperature of 45°C while the liquid in the bottom is at about 50°C. This ensures that the atmosphere is always saturated with water vapour and prevents drying of the lungs. At the same time, except for the absorption of formaldehyde by the lungs, the composition of the formalin solution remains unchanged, and it may be used repeatedly. The elevated temperature increases the rate of fixation but is not high enough to damage the tissue.

The atmosphere drawn from the box cools down as it passes through the external circuit so that formalin solution accumulates in the piping. This is carried round the circuit and is eventually caught in the trap (G) in the box. This trap has a fine-bore tube projecting up from the bottom so that there is always an accumulation of liquid in the trap, but when it reaches the level of the top of the tube the liquid drains through it. A small amount of air also escapes from the trap during each inflation. As the trap is immersed in the liquid in the bottom of the box, it is held at a higher temperature than the lungs, so that the air leaving it is saturated at a rather higher temperature than in the lungs. As the air rises up the tube to the lung it cools to the ambient temperature, but any condensate on its walls runs back into the trap. As a result the atmosphere entering the lungs is saturated with water vapour at lung temperature so that the lungs are neither dehydrated nor water-logged.

The degree of inflation of the lungs can be controlled by the depth of immersion of the dip tube, and the amount of ventilation of the lungs by varying the pump output by means of the valve (M) on the outlet pipe. The inflation/deflation ratio can be varied by moving the platen (J) of the pinchcock. The pumping rate is adjusted so that, when satisfactory ventilation is being obtained, a certain amount of air escapes from the dip pipe at the end of each inflation. Provided this is substantially more than the amount escaping from the trap (G) there is a net loss of air from the system and the pressure in the box is always slightly below atmospheric. It is not therefore necessary for it to be hermetically sealed or to keep the apparatus in a fume cupboard, but it is desirable to keep it in a ventilated room. The air escaping from the dip tube (O) contains formaldehyde, but this is absorbed by the water until the latter becomes saturated, when it must be changed.

When the apparatus is up to temperature the atmosphere in the box is extremely irritating, so that the box should not be opened unless absolutely necessary. This should not normally be required until fixation is complete and the box has been allowed to cool again but if it is, a gas mask should preferably be worn. If one is not available, the breath should be held and the eyes and mouth kept tightly shut. The cannula is held in position in the top of the box by a screw (M), which is operated from outside, so that the lungs need not be rotated. It is, therefore, possible to remove the lungs from the box quickly and easily.

As the lungs harden, the amplitude of ventilation falls and more air escapes from the dip tube. The pumping rate should, therefore, be adjusted from time to time; it may also be necessary to adjust the pressure and the inflation/deflation ratio to ensure that the lungs finally fix at a satisfactory level of inflation.

The apparatus can be run overnight if required, but care should be taken to renew the dip tube water as late as possible and to ensure adequate room ventilation in case the water becomes saturated during the night. The time required for primary fixation varies but is usually of the order of five to six hours.

After fixation the lungs remain in an inflated position and retain their expanded contours (Fig. 2) so that radiographs may be taken at this stage.

RADIOGRAPHY

All radiographs are taken using Kodak Industrex C envelope packed industrial film and a 120 mA mobile *x*-ray unit with 0·8 mm focus.

ERECT ANTEROPOSTERIOR VIEW OF THE LUNGS The lungs and trachea are suspended just above the table top and are steadied against a thin Perspex sheet by a foam pad. A further pad is placed between the lungs to simulate the position of the heart in relation to each lung. A film is placed vertically against the Perspex behind the lungs and the mobile *x*-ray unit is positioned to ensure that the horizontal beam is centred midway between the lungs. Exposure factors are 50 kV, 50 mA, 3 seconds at 100 cm F.F.D.

LATERAL HORIZONTAL VIEW The lungs are separated and radiographed individually in the horizontal lateral position using a vertical beam. Exposure factors are 45 kV, 50 mA, 2 seconds at 100 cm F.F.D.

VIEW OF CORONAL SLICES Each lung is cut into 10 mm slices, which are placed in sequence on a film and marked for identification. Exposure factors are 45 kV, 50 mA, 2 seconds at 100 cm F.F.D.

A plastic sheet protects the film envelopes from the formalin-impregnated specimens.

PATHOLOGY

The apparatus has been in regular use in the mortuary of this hospital for one year and has proved satisfactory for the demonstration of macroscopic pathology. The sliced lungs are convenient to examine by naked eye and hand-lens. Macroscopic lesions are clearly discernible.

Comparison of antemortem and postmortem radiographs allows localization of specific lesions. Blocks are selected from these areas for histological examination. All blocks are further fixed for 72 hours in 10% formalin before histological processing in the usual way.

Fixation by this method is good but it is essential that the slicing and selection of blocks be done promptly. In solid areas of lung, e.g., infarction or consolidation, fixation by the formalin vapour may be incomplete and delays in block selection and secondary liquid fixation may lead to deterioration in histological appearances.

However, if the slices are promptly fixed further in 10% formaldehyde solution histological preparations by this technique are excellent. There is good preservation of bronchial mucosa and alveolar structures. Pulmonary interstitial tissue is clearly seen and lymphatics are well demonstrated. Dust-filled macrophages are seen adherent to alveolar walls and not lying loose in alveolar spaces. Fine radiographic detail may be obtained by taking films of lungs fixed in this way. Line shadows, whether due to bronchi, vessels, fissures or septal plates, are clearly seen, and soft tissue densities of the order of 1–2 mm can be distinguished on radiographs of sliced lungs. This technique is proving particularly valuable in the study of pulmonary oedema as an exact correlation is possible between the very fine shadows producing a punctate appearance and the histological demonstration of interstitial and intra-alveolar oedema. It is also possible to locate single small lesions within the lung fields by radiography, which might otherwise have been overlooked at necropsy.

ILLUSTRATIVE CASE

W.R., a 69-year-old man, was admitted in congestive cardiac failure associated with aortic stenosis. There was evidence of bronchopneumonia. Palpation of the abdomen revealed a large hard mass in the liver. Slight jaundice was present. At necropsy a massive primary hepatoma was found.

A chest radiograph confirmed the presence of pneumonia as well as a fine line shadow at the periphery of the left base. From previous work this was considered to be a pulmonary scar from an old infarct (Fig. 2A).

Detailed lung studies were undertaken to identify the fine line shadow seen at the left base on the chest radiograph. The radiograph of the whole insufflated lung confirmed the presence of the line shadow (Fig. 2B) which was shown much more clearly on the radiograph of the appropriate lung slice (Fig. 2C). A block of this slice was then taken (Fig. 2D) and three further thin slices

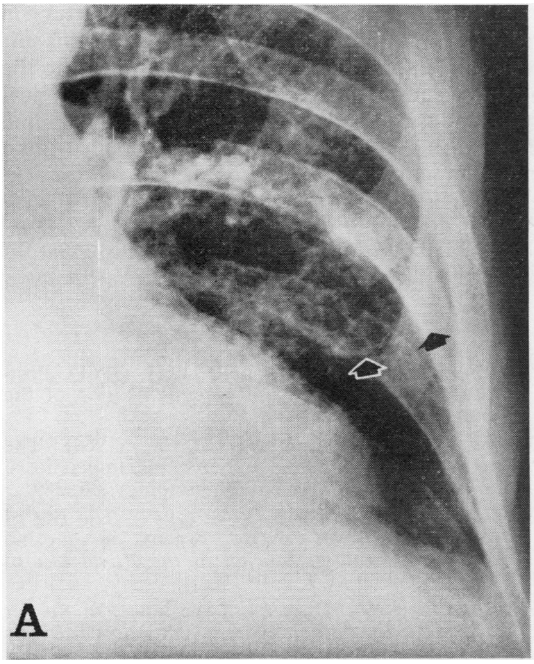


FIG. 2. (A) *Chest radiograph of left basal area to show fine line shadow (arrows) taken to be pulmonary scar due to old pulmonary infarct.*

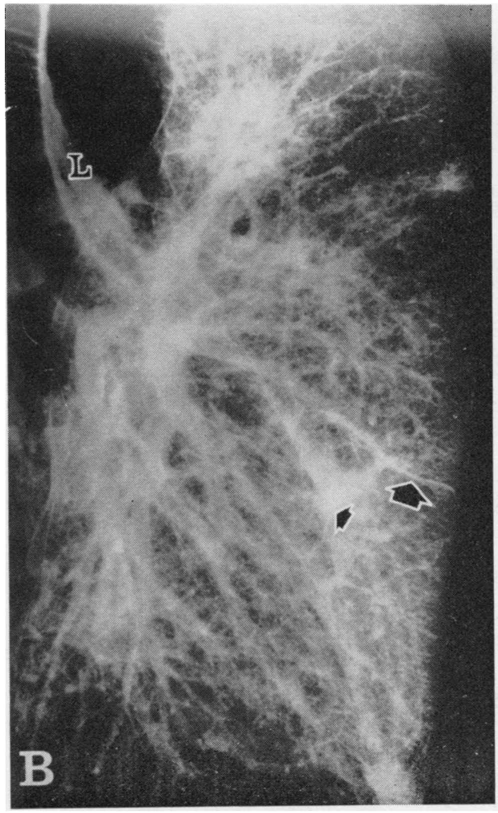


FIG. 2. (B) *Radiograph of left lung after insufflation showing appearance of line shadows.*

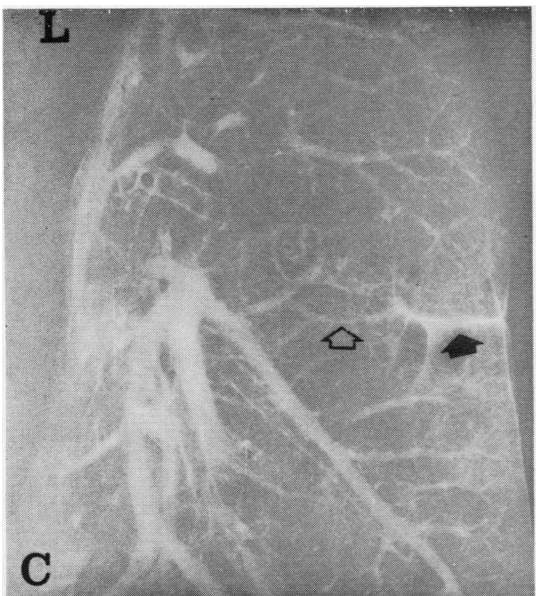


FIG. 2. (C) *Line shadows shown on appropriate lung slice.*

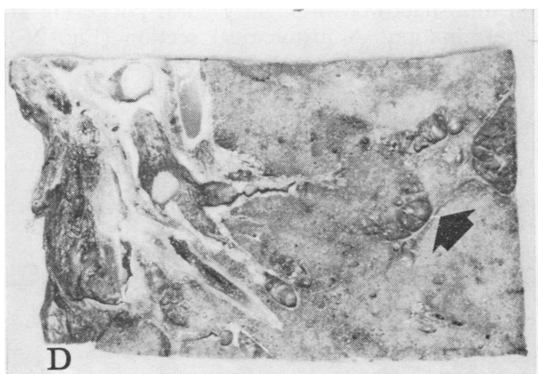


FIG. 2. (D) *Macroscopic appearance of pulmonary scar and surrounding emphysematous bullae.*

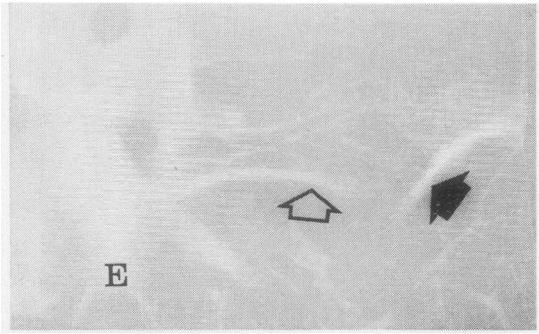


FIG. 2. (E) *Radiograph of thin lung slice showing pulmonary artery branch ending in curved line shadow.*

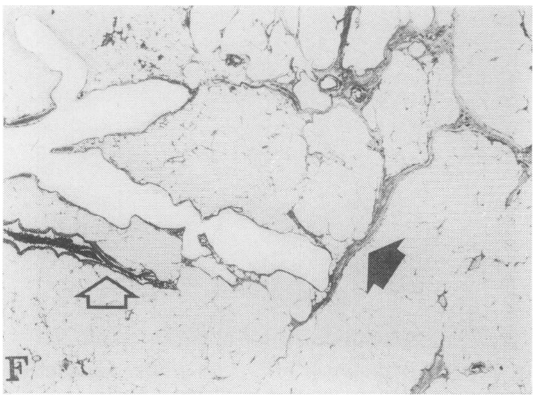


FIG. 2. (F) *Histological section showing pulmonary artery (open arrow head) and pulmonary scar (black arrow head).*

were made and radiographed (Fig. 2E). This clearly showed a pulmonary artery running into a thin line shadow with slight pleural puckering at its termination. A histological section (Fig. 2F) confirmed the presence of a pulmonary artery in relation to a fibrous pulmonary scar with surrounding emphysematous bullae abutting onto the pleural surface. This radiological–histological correlation thus demonstrated that a fine line shadow on a chest radiograph corresponded to an old pulmonary scar due to infarction.

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