A Simple Perfusion Apparatus for Lung Fixation[1] (37327)

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Optimally, microscopical studies of lungs are performed on tissue which is expanded and fixed in as near a normal state as possible (1). The techniques of perfusing whole organs with a life-supporting medium are extremely sophisticated (2), whereas the perfusion of a tissue-fixing medium can be accomplished with little difficulty (3). However, in the latter procedure, close attention to perfusion pressures and to the route of fluid instillation is critical if one is to achieve optimal results (4).

We have devised a simple perfusion apparatus for the instillation of fluids into the lungs of small animals at physiologically normal pressures (Fig. 1). This technique requires no sophisticated pumps or gauges and allows intratracheal or intra-arterial perfusion either simultaneously or successively. The components of the system are a liter Erlenmyer flask which is the fluid reservoir, a mercury manometer, a Propipette pipet filler (Will Scientific), Butterfly-needle (Abbott Laboratories) cannuli, various lengths of glass and rubber tubing (5/32 in. bore diam), and ring stands or another type of supportive framework. These components comprise a unit which can be assembled to fit one's own experimental design. In our laboratory, we use two units in parallel for simultaneous and sequential perfusion (Fig. 1). The central animal board of our apparatus is raised to approximately the same height as the fluid level so that gravity is not a factor in the final perfusion pressure.

The operation of the system first requires filling the flask with 200 ml of fluid and then calibrating the mercury manometer. The latter is done at ambient air pressure by simply measuring up from the mercury level with a millimeter ruler (2 mm divisions for each actual millimeter of pressure) and marking the tube before applying the pressure. Forcing air into the flask with the pipet filler creates a pressure both on the fluid and on the mercury (the tubing from the fluid remains clamped until flow is needed). The

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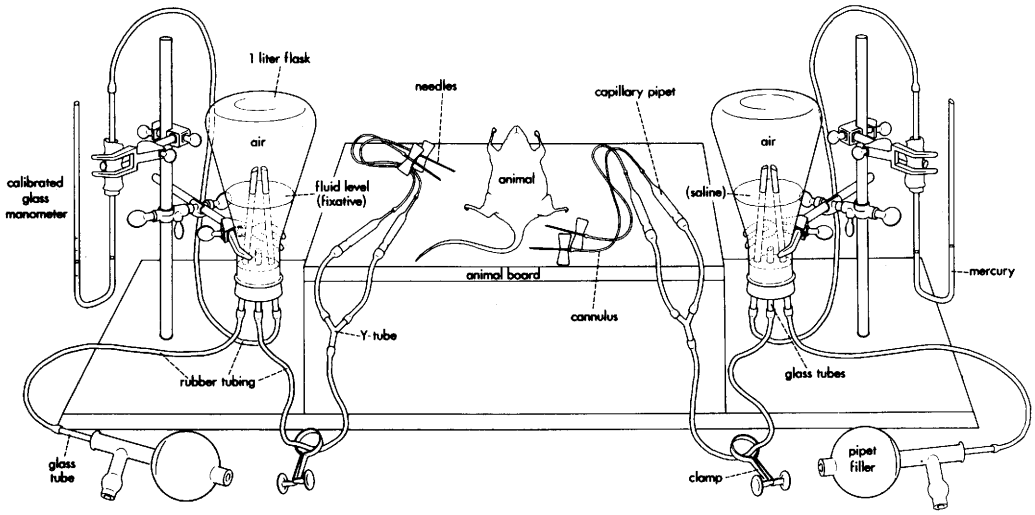
FIG. 1. Perfusion apparatus: This is a simple system which can supply fluids at pressures approximating physiologically normal levels. The height of the mercury in the manometer is adjusted by forcing air into the liter flask through pressure from the pipet filler. The mercury may be pushed up to any predescribed line on the manometer. The height of the mercury then measures the pressure upon the fluid which in turn is delivered to the individual cannuli when the fluid line is unclamped. This diagram indicates two cannuli from each flask, although the number is optional.

FIG. 2. Photomicrograph of a section through mouse lung which has been fixed with the perfusion apparatus diagrammed in Fig. 1. The patent capillary network is due to perfusion with saline prior to fixation with 4% glutaraldehyde. Numerous alveolar spaces (A) are expanded and well preserved (methyl blue; ×320).

FIG. 3. Electron micrograph of a portion of an interalveolar septum. Patent capillaries (C) are seen; one contains a polymorphonuclear leukocyte (PM). The attenuated endothelial (En) lining of the capillaries is preserved, as is the epithelial (Ep) lining of the alveolar (A) spaces. A type II pneumocyte, or granular epithelial cell (GEp) with numerous myelin inclusions is seen between two capillaries (×6500).

mercury level is then adjusted with the pipet filler to any predescribed line on the manometer, and when the fluid line is unclamped, air pressure drives the fluid through the cannuli.

In ultrastructural studies of murine lung disease, we have found that intravascular perfusion of a 4% aqueous solution of glutaraldehyde provides ideal fixation. First, the thoracic cavity of a Nembutal-anesthetized animal is opened to expose the heart and lungs. The needle cannula then is inserted into the right ventricle of the beating heart and phosphate-buffered saline is instilled



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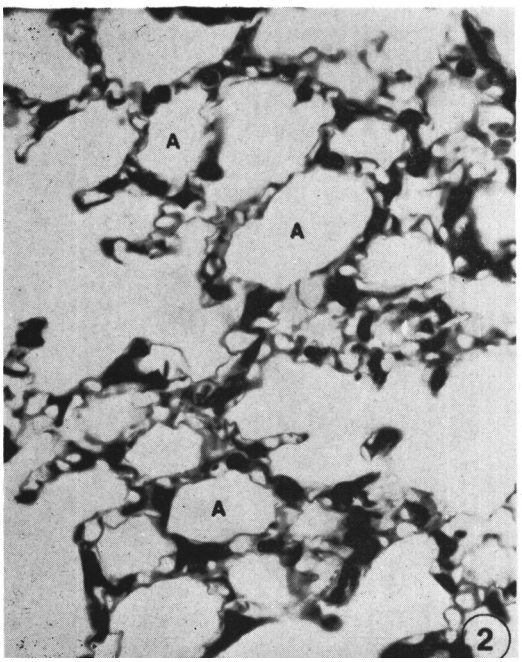

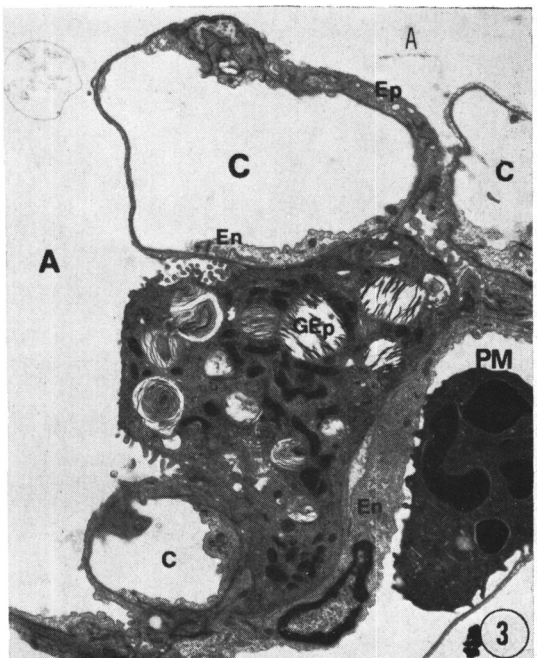

from one unit of the apparatus. The mercury manometer is preset at 15–20 mm of Hg, a pressure suggested as physiologically normal by several investigators (5, 6). A 30 sec perfusion of saline is sufficient to clear most blood cells from the pulmonary vasculature. The cannula from the saline-filled flask is then immediately replaced by the cannula from the fixative-filled flask. The heart generally ceases to beat 10–15 sec after the perfusion of fixative begins, although fluid continues to flow through the patent pulmonary artery into the lungs.

A photomicrograph of a mouse lung prepared in the manner described above is shown in Fig. 2. The absence of blood in the capillary network is a result of preliminary saline perfusion. Expansion and preservation of the alveolar architecture is a result of glutaraldehyde perfusion under controlled pressure. A high resolution electron micrograph (Fig. 3) demonstrates the excellent fixation provided by this perfusion technique.

Should one wish not to flush the vascular network, the cannula can be inserted directly into the trachea of the intact lung. This permits expansion of the lungs, but does cause occlusion and some compression of the capillary bed.

The diagram in Fig. 1 indicates the use of Y-tubes. This provides the option of a simultaneous intravascular and intratracheal perfusion of an experimental animal. In addition, we have used the Y-tube for the expansion and fixation of human lung biopsies wherein the cannuli are inserted into the natural orifices of exposed veins, arteries, and bronchi on the cut surface of a biopsy. Substantial portions of the tissue are expanded and good fixation under controlled pressure is provided.

Summary. An apparatus is described for the fixation–perfusion of the lungs of small animals. The advantages of this system are the simplicity of its components, its ease of operation, and the reproducibility of physiologically normal pressures within a closed system. A variety of solutions may be used, and excellent tissue preservation is a feature of this procedure.

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