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An Airway Perfusion Apparatus for Whole Lung Fixation^{1,2}

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Summary | An apparatus was designed and built for the constant pressure fixation of up to 48 small to medium sized animal lungs by tracheal perfusion. The system had a 40-liter fixative capacity, automatically maintained selectable fixative fluid levels in all reservoirs, and filtered and recirculated fixation fluid for periods up to several days.

Key Words | Perfusion apparatus — Tissue preservation — Lung

Distending the lungs by perfusion of fixative through the airways is considered by most investigators as the best method of preparing pulmonary parenchyma for microscopic observation when the translocation of overlying secretions or exudates is of little concern. This method assures that the configuration of the respiratory units of the lung will be preserved in a state similar to that *in vivo* and that fixation will be sufficiently rapid to prevent deterioration.

In order to perform high quality fixation by airway perfusion, several problems must be solved. The fixative pressure should be constant, and the initial fixative flow rate adequate to insure that an equilibrium inflation volume is reached before significant fixation occurs. In addition, the lung should be immersed in fixative in order to provide mechanical support and prevent drying of the outer surface. Finally, a means must be provided for discarding or recycling fixative that passes through the lungs.

General Description

The apparatus (Figure 1) was designed and built for the fixation by tracheal perfusion of lungs ranging in size from those of mice up to those of medium-sized dogs. The apparatus was constructed of 0.635-cm thick clear acrylic plastic and had a 40-liter fixative capacity which was distributed among three separate reservoirs. Circulation of the fluid between reservoirs was continuously maintained and controlled by two means. A magnetic drive, centrifugal pump transferred fluid from the lower to the upper reservoir. The pump was actuated by a diaphragmatic, pressure-sensitive switch mounted on the plastic frame above the upper reservoir. The tip end of a 60 ml disposable, plastic catheter tip syringe barrel was

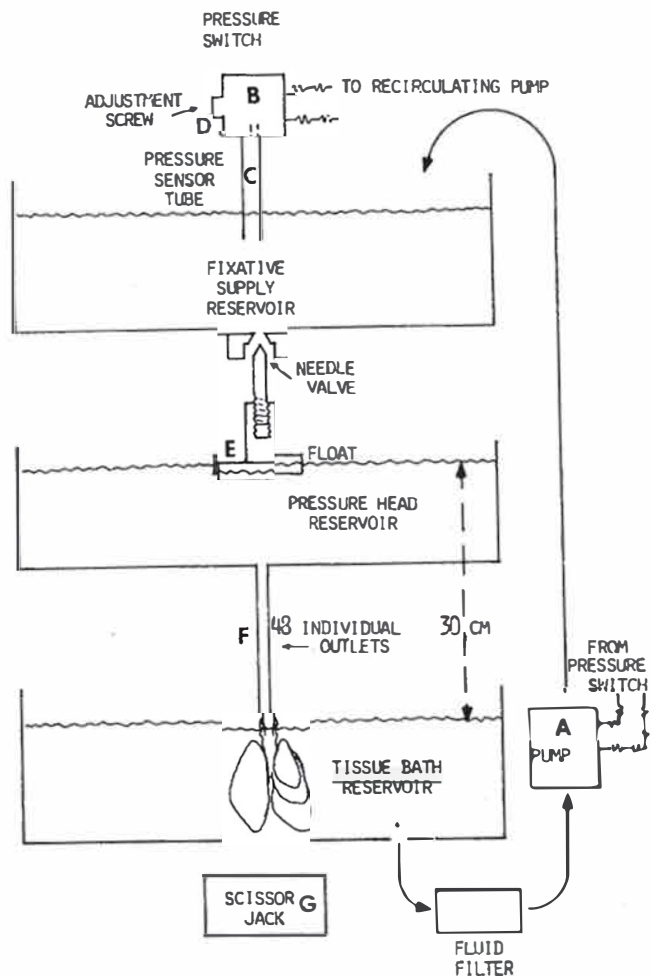


Figure 1
Diagram of perfusion apparatus for whole lung fixation: A=pump; B=switch; C=syringe; D=adjust screw; E=styrofoam platform; F=outlet tube; and G=scissor jack.

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attached to the high pressure port. The base of the barrel was partially submerged in the fluid of the upper reservoir. The pressure differential in the barrel created by changes in the reservoir fluid level actuated the switch, turning the pump on. An adjustment screw in the diaphragm assembly of the switch permitted adjustment of the pressure differential necessary for switch activation.

Because of the fluid surge created by the pumping system, the middle reservoir was used to maintain the hydrostatic fixative pressure level. This fluid level was controlled by a needle valve seated on an adjustable height plastic post, which was mounted on a floating styrofoam platform; a valve guard maintained the proper seating of the needle to its valve in the base of the upper reservoir. Therefore, a small drop in the fluid level of the middle reservoir lowered the float-mounted needle valve, and fluid entered from the upper reservoir raising the fluid level to the set point.

The bottom of the middle reservoir had 48 individual outlet tubes, each designed for connecting an individual lung to the reservoir. Each of these outlet units was composed of a tube adapter mounted flush in the base of the reservoir and secured by a neoprene "O" ring, a length of flexible tubing, a one-way stopcock, and a needle adapted cannula. The cannula was a 15-gauge syringe needle which was shortened and fitted with an appropriate length of polyethylene tubing suitable for insertion into the trachea. The entire outlet assembly was made of disposable, inexpensive, plastic items. The method of mounting these units to the reservoir base permitted easy replacement.

The lower reservoir was mounted on a laboratory scissor jack so that the level of that reservoir could be adjusted to assure that the desired pressure head was maintained and that tissues were totally submerged in the fixative throughout the period of perfusion. In the event that residual air in the lung caused it to float, a weight was attached to the lung.

A cylindrical filter chamber containing fine spun glass and micropore filter wafers was connected in the recirculation fluid line between the lower and upper reservoirs. Particulate contaminants were effectively removed from the fixative by this filter.

All reservoirs were equipped with drain spouts for easy cleanup. Two or more small lower reservoirs were used side by side in place of the larger single unit described, permitting different lungs to be perfused at different hydrostatic pressures simultaneously. A small reservoir also reduced the quantity of fixative needed when only a few lungs were being fixed. When two lower reservoirs were used, individual siphon tubes connected by a "Y" permitted use of the single recirculating pump.

The entire apparatus was placed inside of a fume canopy during usage to provide for adequate removal of noxious or toxic vapors associated with various fixative solutions.

Materials used in the construction of the apparatus were all resistant to attack by common fixative solutions. Individual pieces of plastic were welded together by painting with a suitable solvent and clamped until the joint was secure.

The list of materials needed for construction is as follows: (a) 0.635-cm clear acrylic plastic sheet; (b) solvent for fusing acrylic plastic pieces was reagent grade 1, 2 dichloroethane;³ (c) magnetic drive centrifugal pump;⁴ (d) disposable plastic male adapters,⁵ disposable plastic female adapters,⁶ disposable plastic one-way stopcocks;⁷ (e) low differential pressure switch,⁸ and (f) laboratory scissor jack.⁹

Discussion

The accuracy of morphometric analysis of fixed lung tissue depends in great part on the ability to minimize variations in lung inflation during fixation. The rate of infusion of fluid fixative also can be a determining factor in the resultant degree of inflation of the lung. Therefore, it was necessary that the flow rate through the individual outlets be limited by the resistance of the attached lung rather than the resistance created by either the length or diameter of the outlet tube or attached adapters. The initial rate of fixative flow must be great enough so that the rate of fixation does not cause hardening of under inflated tissue. This is critical to uniform inflation in those cases where a rapid fixative such as glutaraldehyde is used (1). The apparatus described was designed to permit precise control of both pressure and initial flow and still be useful for simultaneous fixation of different sized lungs.

The use of individual stopcocks for each outlet permitted selection of the number of outlets used at any one time and permitted limitation of perfusion flow rates in those cases where minimal disturbance of substances in airways is of greater importance than uniform inflation. The use of clear plastic for container construction and disposable plastic fittings facilitated easy cleanup, reduced chemical contamination over extended periods of use, and lent itself to easy adaptation to a variety of procedures.

We have used 10% neutral phosphate buffered formalin fixative fluid. The flow rate through the individual outlets without the resistance of an attached lung was in excess of 100 ml/minute. The device has been used, trouble free, for a period of over 1 year to fix both rat and dog lungs for histologic and morphometric analysis. Approximately 500 lungs have been fixed for periods varying from 1 to 5 days. In no case did the apparatus fail to perform optimally.

References

1. Hayatdavoudi JD, Crapo F, Miller J, et al. Factors determining degree of inflation in intratracheally fixed rat lungs. *J Appl Physiol Respir Environ Exercise Physiol* 1980; 48:389-93

³Mallinckrodt, St Louis, MO.

⁴1-catalog #7105-00. Cole-Palmer Instrument Co, Chicago, IL.

⁵48-No 59-150. Pharmaseal Corporation, Glendale, CA.

⁶48-No 59-157. Pharmaseal Corporation, Glendale, CA.

⁷48-No 71-1084. Pharmaseal Corporation, Glendale, CA.

⁸1-model 1823-S. Dwyer Instrument, Michigan City, IN.

⁹1-catalog 14-673-10. Fisher Scientific Co, Pittsburgh, PA.