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Casting the Lungs *In-situ*

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ABSTRACT A method for producing flexible silicone rubber casts of the airways of the lungs *in-situ* is described. Casts are made to correspond to lung volumes occurring during normal breathing. The lung is prepared for casting by replacing the air within with CO_2 followed by filling with degassed physiological saline. The saline dissolves the CO_2 gas within the airways allowing for a bubble-free finished cast. Casting compound is then slowly injected through the trachea. The saline diffuses out of the lung and passes out of the thorax through several small slits in the thoracic wall. After the injection is completed, the cast lung is allowed to cure *in-situ* before it is removed and the tissue digested away. Finished casts have an overall shape corresponding closely to the shape of the thorax. Casts produced by this *in-situ* method appear to have more realistic geometrical relationships than those produced from excised lungs.

The accurate description of the anatomical details of respiratory airways is essential for producing satisfactory lung models for predicting particle deposition and localized dose patterns for inhaled materials within the respiratory tract in man and other animals. Morphometric data currently available are incomplete and therefore inadequate for these purposes. Equations for predicting regional particle deposition within the lungs are commonly based on the inertial, gravitational and diffusional forces acting on inhaled particles (Beeckmans, '65). The anatomical features considered to be of importance in particle deposition are airway segment shapes and sizes (usually diameters and lengths), the inclination of airways to the force of gravity, and the branching angles. Radii of curvature of airway segments and branch regions, airflow patterns, air mixing within the lungs and cyclic changes in structural relationships during breathing must also be considered in meaningful deposition calculations.

Flexible casts of the airways are useful for replicating anatomical detail. Two major criteria for evaluating such casts are fidelity to original airway anatomy and suitability of the cast for precise morphometry. This report presents a method for producing acceptable flexible casts of the respiratory system by the injection of a casting material *in-situ*. This method appears to overcome some short-comings inherent in making casts from excised lungs.

METHODS

After an examination of several casting materials, including paraffin, latex, low melting point metals, and several varieties of silicone molding compounds, two silicone rubber industrial molding compounds were chosen. These two materials, described in table 1, produce strong, flexible casts with good representation of anatomical detail of structures as small as 100 μ. They also cure at room temperature and have very small shrinkages during curing.

The procedure for making lung casts in the thorax is outlined in figure 1. Before the cast is made, several measurements of the functional residual capacity and the tidal volume are taken if possible. These measurements are used to determine the

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TABLE 1

Physical properties of silicone rubber industrial molding compounds

	Silastic E [1]	RTV-700 [2]
Uncured properties		
color	white	white/green/red
density	1.12 gm/cm^3	1.07 gm/cm^3
viscosity	1,200 poises	600 poises
Cured properties		
tensile strength	700 psi	600 psi
elongation at rupture	400%	400%
temp. stability (upper limit)	500° F	—
shrinkage (after 7 days)	0.1%	about 1.2%

[1] From Dow Corning Bulletin 61-008a, 1970, Midland, Michigan.
[2] General Electric Product data sheet, Silicone Products Department, Waterford, New York.

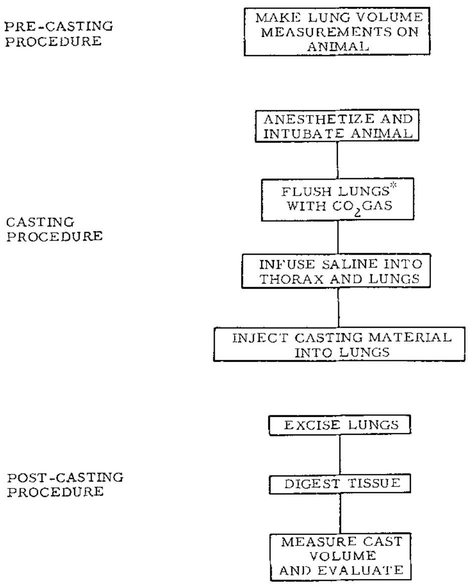


Fig. 1 Flow chart for preparing a cast of the lung *in-situ*. *At this time, the animal dies.

injection volume and to evaluate the final cast fidelity.

The experimental animal is anesthetized with sodium pentobarbital and the trachea is intubated. The animal is then placed on its back and the tracheal tube connected to a CO_2 reservior. Breathing this gas kills the anesthetized animal and replaces O_2 and N_2 in the lungs with CO_2. The CO_2 is considerably more water soluble than O_2 and N_2 and helps to produce a bubble-free cast. When casting the lungs of cadavers, as in the case of the human, the lungs are flushed with CO_2 using a respirator pump.

The inside of the thorax is then vented to the atmosphere by making six or more small ventral-lateral incisions between the ribs and through the thoracic wall allowing the lungs to collapse. The space between the lungs and parietal pleura is filled with physiological saline that has been previously degassed under vacuum. This provides support for the lungs during the remainder of the casting procedure. In the case of a rodent, the entire body may be suspended in degassed saline.

Degassed saline is next slowly introduced into the lungs through the tracheal catheter using the pressure of a 5- to 10-cm column of saline. This pressure inflates the lungs and enhances the diffusion of saline out of the lungs which are permeable to saline. Several (3 to 5) lung volumes of saline are infused, dissolving the CO_2 gas within. This saline infusion requires about 15 to 30 minutes. Fluid, diffusing from the lungs, flows out of the thoracic incisions during the infusion. These incisions, on each side of the thorax, at the apex, mid-region and near the diaphragm, are situated so that fluid will not be trapped within the thorax and interfere with full inflation of the lungs. The animal has now been prepared to receive the casting material.

The casting material is thoroughly mixed with its catalyst using an electric stirrer and is then degassed under vacuum. A syringe-type injection system (fig. 2) is used to slowly inject catalyzed silicone rubber into the lungs through the tracheal catheter. Syringes with volumes of 50 cm^3, 4 l and 10 l are used for injecting rodents,

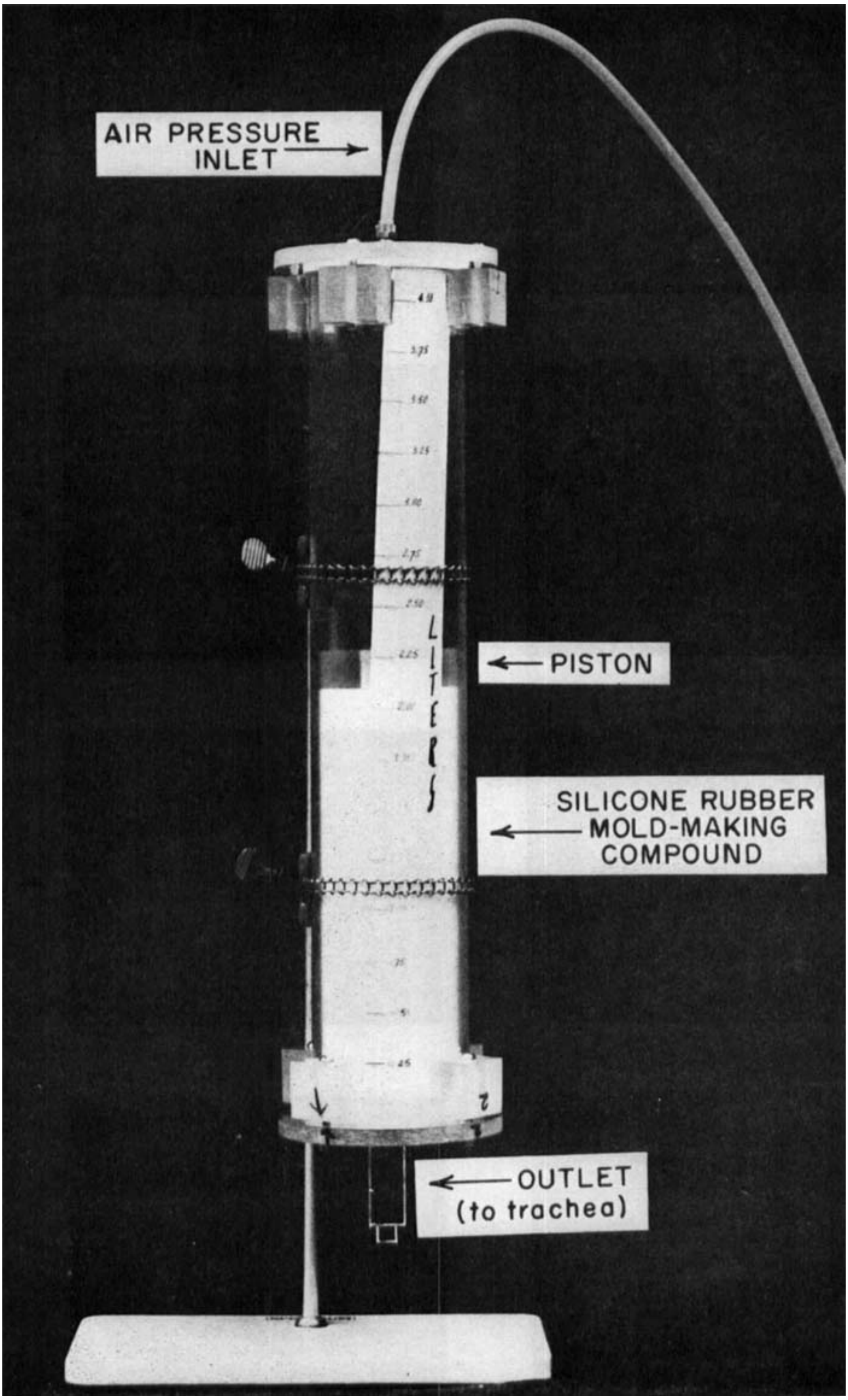
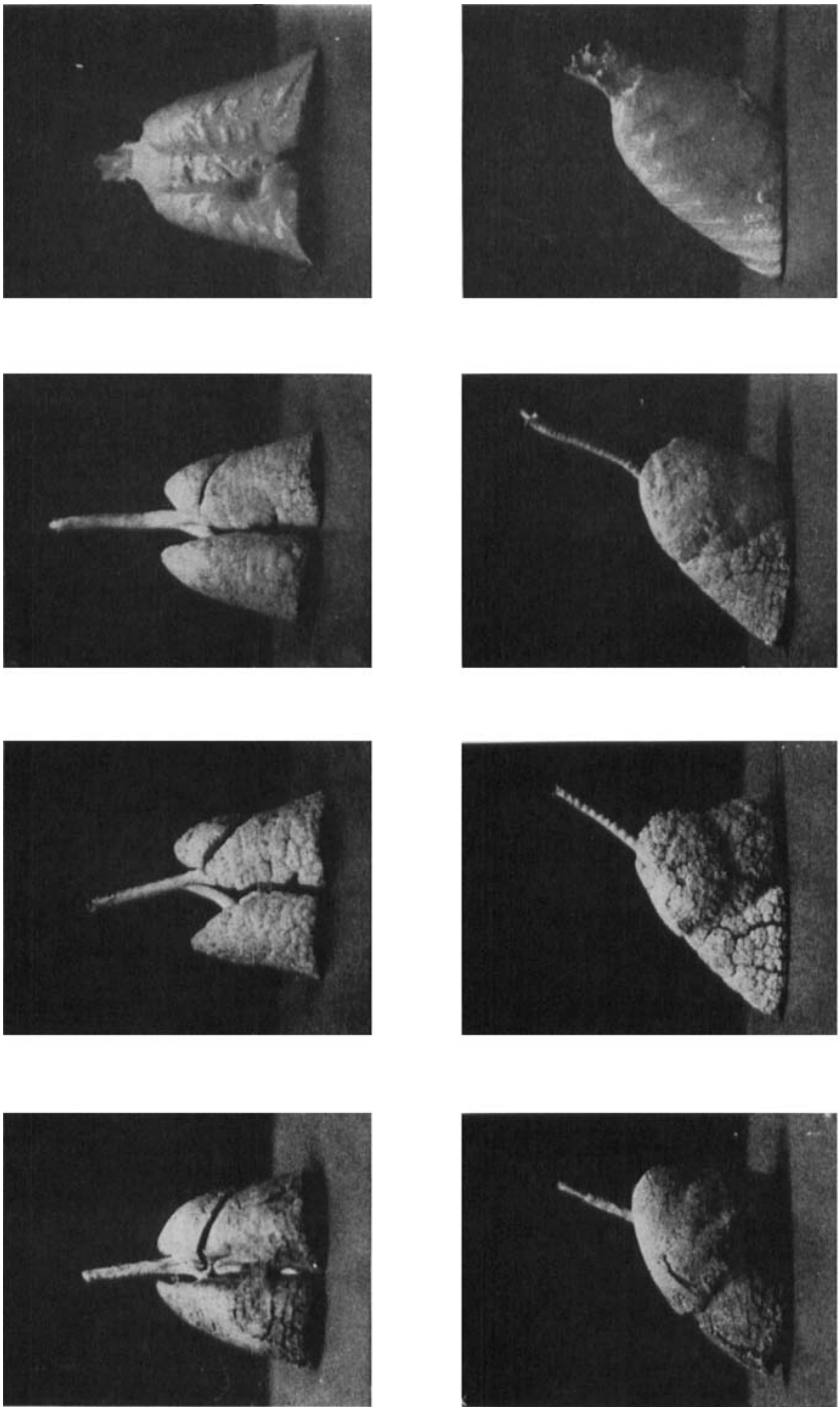


Fig. 2 Air-driven syringe (diameter, 4 inches) used for the metered dispensing of silicone rubber mold-making materials.



1 2 3 4

Fig. 3 A comparison of rat lung casts made using excised lungs (1 and 2) with a cast made using the *in-situ* technique (3). A cast of the thoracic cavity of a rat of the same size and sex is on the far right (4).

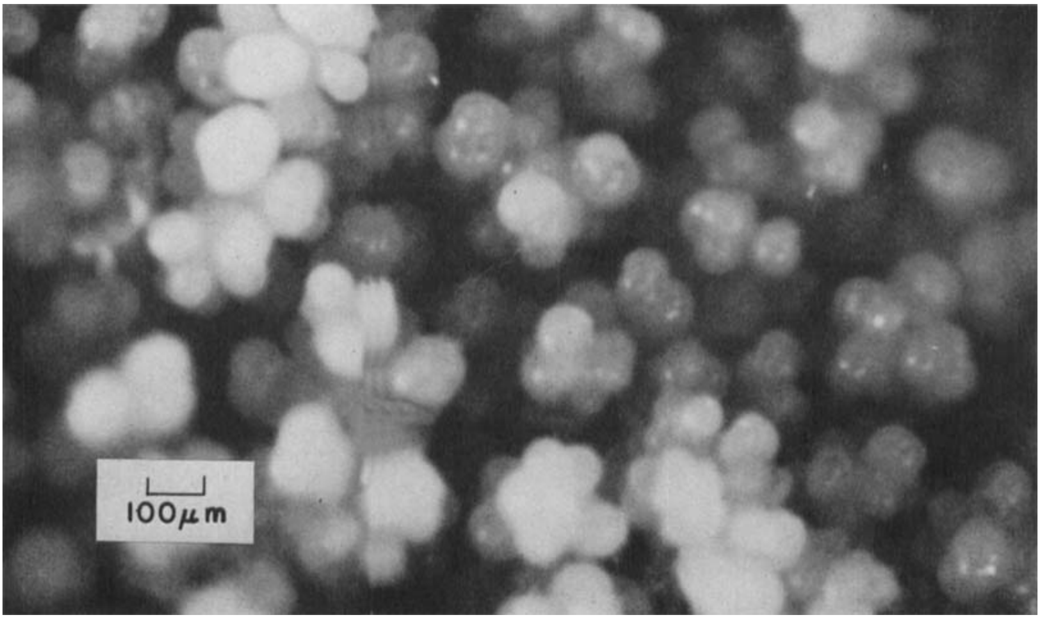


Fig. 4 Clusters of alveoli seen upon microscopic examination of an *in-situ* cast of a rat lung.

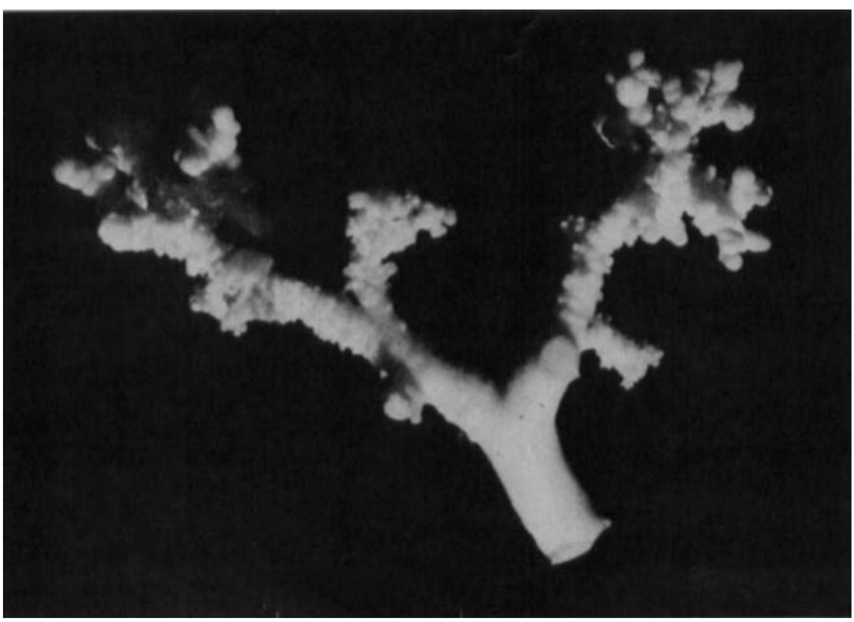


Fig. 5 Distal airway regions from an *in-situ* cast of a dog lung. The cast has been trimmed so that distal airway detail is visible. Due to the low filling volume the alveoli are not completely cast. Photo supplied by Dr. T. L. Chiffelle.

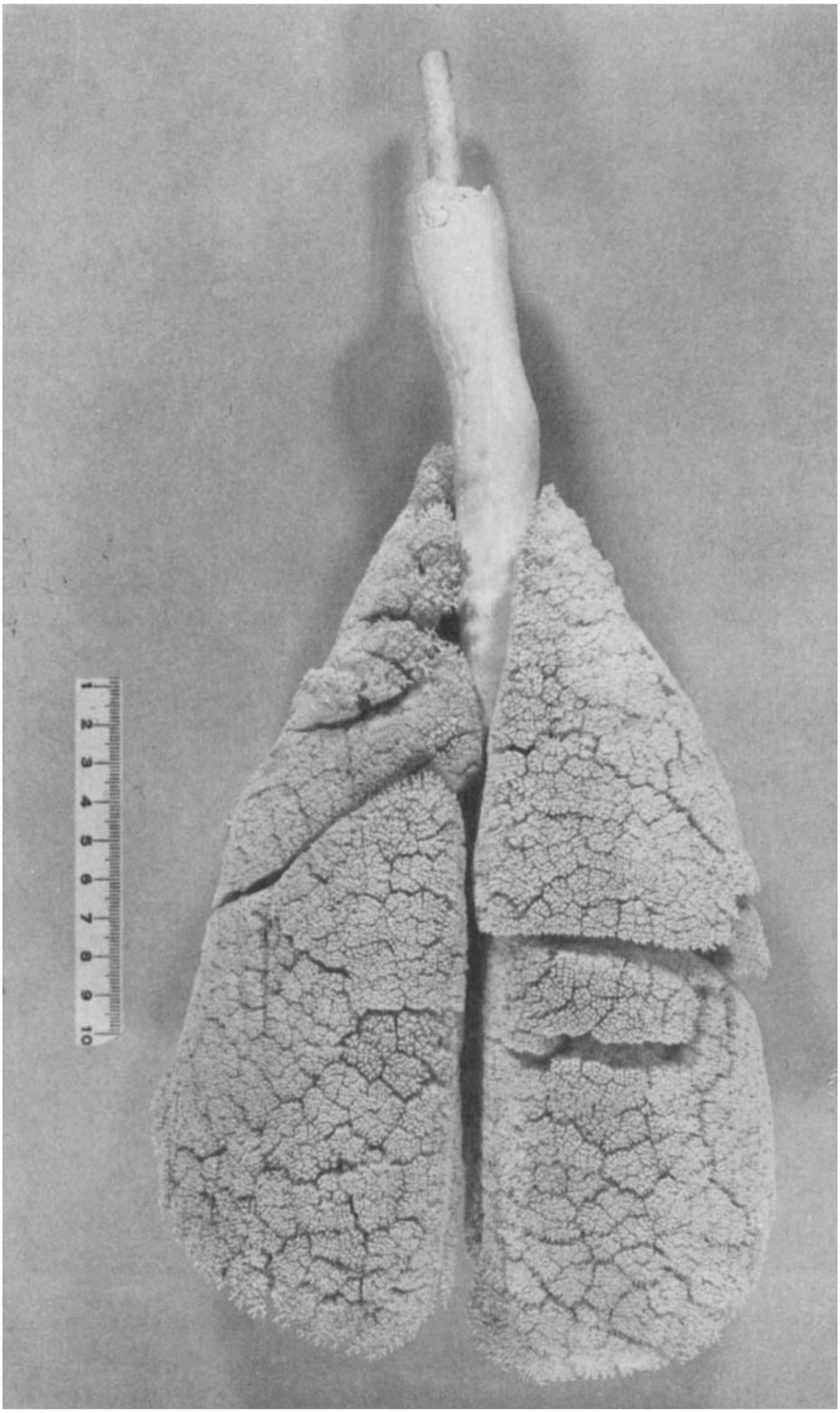


Fig. 6 *In-situ* cast of the dog lung after digestion of lung tissue.

dogs and humans, respectively. A filling time for the lungs of about one hour is considered optimal. This avoids excessive pressures within the lungs and is within the working time of the casting material. Saline in the lungs diffuses freely out of the lungs and through the pleural membrane as it is displaced by the casting material. The injected casting material is metered and the injection stopped when filling corresponds to a predetermined lung volume.

Casts are made to volumes that correspond to lung volumes occurring during normal breathing. Three methods are used for determining the filling volume of the lungs. Whenever possible, measurements of functional residual capacity and tidal volume made on unanesthetized animals in our pulmonary physiology laboratory (Mauderly, '72) are used. These measurements are always performed on our dogs. When such data are not available, as in the cases of the rodent or human, published values of lung volumes for the appropriate species, size, sex and age are used (Comroe et al., '62; Tenney and Remmers, '63; Weibel, '63; Crosfill and Widdicombe, '61). In those cases where the lung volume values from the literature are inappropriate, molding compound is injected until the lungs appear to be well-filled and just fill the available space in the thorax without distention of the chest wall. Ideally, 12 hours at room temperature are allowed for curing of the cast before it is removed from the thorax. The cast lungs are then carefully excised from the thorax and placed in a fresh one molar NaOH solution for about 24 hours at room temperature for tissue digestion. A final washing with a weak acid solution may be used to provide a more durable cast.

DISCUSSION

Although lung casts of excised lungs have been prepared by many investigators, (Eisman, '70; Frank and Yoder, '66; Liebow et al., '47; Narat et al., '36; Nowell et al., '72; Parker et al., '71; Rahn and Ross, '57; Tucker, Jr. and Krementz, '57) the *in-situ* method was developed to provide casts of the lungs in their normal anatomical configuration. Gross lung shape is determined by the interplay of the lungs' intrinsic structural properties and constraints imposed by the thoracic walls, diaphragm, heart, major vessels, and other tissues within the thoracic cavity. When detailed airway geometry, including branch angles, must be replicated, these shape constraints become important.

Casts of the lungs of female rats (280–330 gm body weight) made by three techniques, (1) excision — air drying — vacuum filling (Rahn and Ross, '57); (2) excision — saline replacement (Tompsett, '70); and (3) *in-situ* — saline replacement, as herein described, were compared to a cast of the rat thoracic cavity (fig. 3). The overall shape of the cast made *in-situ* best approximated that of the thoracic cavity. The lobes of excised lungs had a tendency to overinflate and move relative to one another, thus changing the gross lung shape and the branching angles of the major bronchi. Also, hanging the excised lungs from the tracheas during casting caused the tracheas to be oriented to the lungs at abnormal angles in the excised lung casts. The *in-situ* method minimizes these and other obvious distortions caused by the lack of physiological anatomical support during casting of excised lungs.

The *in-situ* technique has three basic drawbacks: (1) the technique requires a relatively fresh and complete animal or human body which, in many instances, may not be available; (2) it requires more time and more careful attention than excised lung techniques; and (3) artifacts may be produced by filling the lungs with 100% CO_2 or saline. For example, flushing the lungs with CO_2 gas causes a condition of lowered pH that may lead to constriction of the smooth muscle of the airways. Upon close examination, however, the casts show no evidence of significant muscular constriction. Alveoli with slightly angular shapes are clearly seen upon microscopic examination of the casts (fig. 4) and the transition of conducting airways to respiratory bronchioles is clearly evident (fig. 5).

The *in-situ* technique, which has been applied successfully to hamster, rat, monkey, Beagle dog and human, has provided our laboratory with lung casts suitable for detailed airway morphometry. A

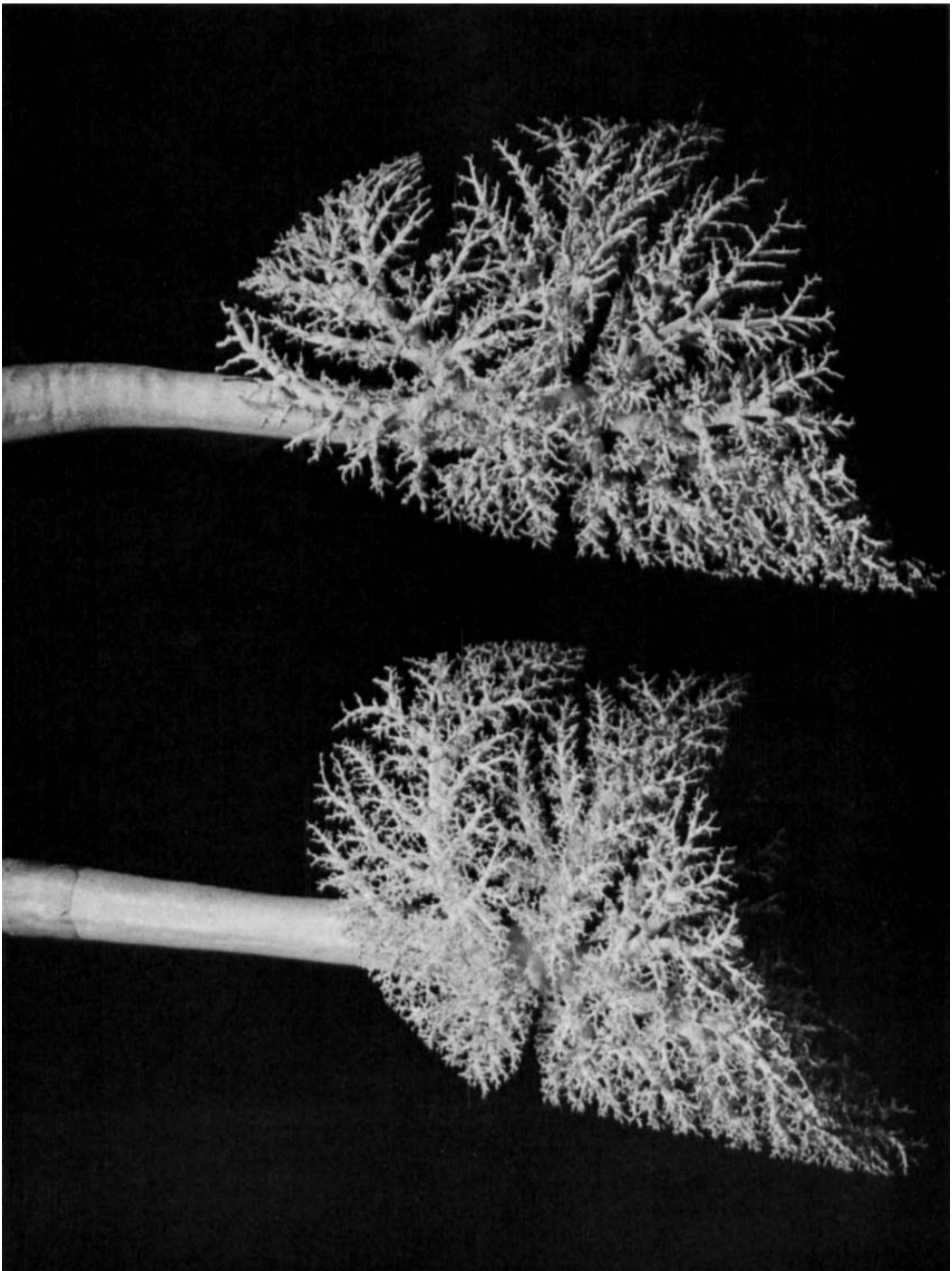


Fig. 7 Trimmed casts of the Beagle dog lung. Cast on the left was made from an excised lung. Cast on the right was made *in-situ*. Dog weights were 11.3 kg and 11.6 kg, respectively.

complete *in-situ* cast of a Beagle dog lung is shown in figure 6. When the casts of dog lungs are trimmed to show the major bronchioles, the more anatomically correct orientation of the airway of casts prepared by the *in-situ* method becomes more apparent. In figure 7, a cast prepared by the excision-saline replacement method shows spreading of lobes not found in the *in-situ* cast. Also, the gross artifacts previously noted in excised lung casts are seen.

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