The Physical Properties of the Frog Lung.* (28098)

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The respiration of the frog has been studied by several investigators. Ventilatory movements have been described(1,2,3) and the diffusion of gases has been studied. Krogh(4) found that most of the carbon dioxide was eliminated through the skin while most of the oxygen was absorbed through the lungs. The lung of the frog is a simple saccular organ. Ventilation occurs under positive pressure. Air is forced into the lungs by muscular contraction of the floor of the mouth and retained in the lungs by closure of the glottis. Mechanisms by which the frog empties its lungs are not thoroughly understood although contraction of the smooth muscle in the wall of the lung is probably the major factor.

The physical properties of the frog lung have not been studied previously. An understanding of the mechanical behavior of the frog lung is of importance because of its structure. Theories of the mechanical behavior of mammalian lungs entail a consideration of forces arising from the tissue and forces arising from the air fluid interfaces throughout the lung. Data have been obtained from the lungs of frogs for interpretation in the light of these theories.

Materials and methods. Adult male frogs *(Rana pipiens)* weighing 24 to 42 g were pithed. The lungs and heart were removed and the vessels ligated. Pressure and volume were measured under static conditions with air and with liquid (Ringer's solution) filling. The lung was cannulated with a 20 cm length of polyethylene tubing type PE160. In the Ringer's solution experiments, the polyethylene tubing served as a manometer. Volume displacement inside the tubing was 100 cm/ml. Capillarity was insignificant. A calibrated tuberculin-type syringe was used to measure volume to the nearest 0.01 ml. Pressure equilibrated rapidly so all pressure measurements were made within 15 seconds following each change in volume.

The experiments with air inflation were performed with a "U" shaped glass manometer tube. The polyethylene tubing from the lung was connected to one side of the glass manometer. The other side of the manometer was open to the atmosphere. A "T" connector permitted filling and emptying of the lungs from the syringe. Volume displacement inside the glass manometer was 49 cm/ml.

A stereoscopic dissecting microscope was used to visualize the lungs. The alveoli were measured in fresh specimens inflated with air. The septae on the inner surface of the lung were counted and measured in a frog lung that had been inflated with 2.0 ml of air and dried. The total surface area of the septae was calculated from their height and frequency. The inner surface area of the lung wall was calculated from measurements of the lung.

The structure of the lung of the frog is relatively simple. The internal surface of the distended lung presents a series of incomplete septae (Fig. 1). These are principally of 3 sizes. The largest septae extend inward approximately one-fifth of the diameter of the lung cavity. They are oriented in the transverse plane of the lung and subdivide the surface area into large spaces. The second order septae are smaller and divide the lung into the spaces considered as alveoli. Another family of septae may be observed at volumes of inflation greater than one milliliter. These are the third order septae, very small and incomplete. The free edges of the second and third order septae were branched into one another affording a smooth transition between these orders.

The mean alveolar size at mid-capacity volume (1.8 ml) was 1.7 × 1.2 mm. The alveoli, as viewed through the wall of the air filled lung, uniformly increased in size during inflation and decreased in size during deflation. The internal surface area of an inflated

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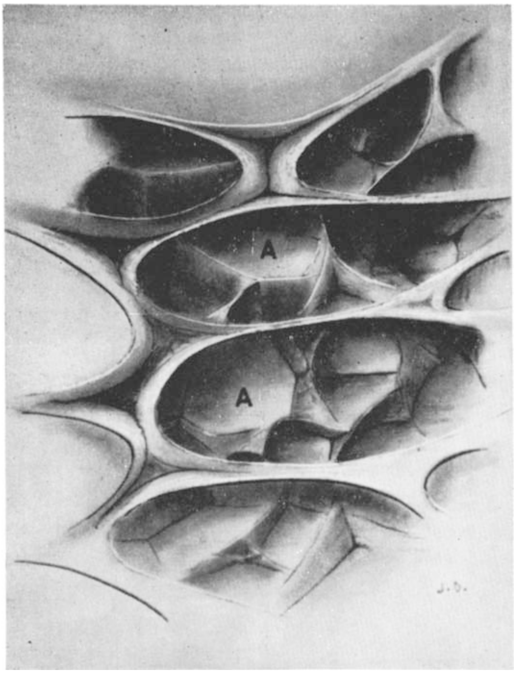

FIG. 1. Artist's sketch of internal surface of frog lung. Letter A marks an alveolus. Diameter of this space was approximately 10 times that found in human alveolus.

dried preparation was calculated to be 40 cm^2 for a single lung at 2 ml inflation. This value agrees only roughly with the data of Krogh of 98 cm^2 for both lungs at 10 ml inflation(4).

Frog lungs were removed carefully to avoid injury. These preparations were found to sustain pressure for long periods of time on inflation with air. Following introduction of Ringer's solution into the lungs, however, pressure regularly decreased with time. This decline in pressure was assumed to result from the diffusion of the solution through the wall of the lung. This finding suggested a rigid time schedule would be required to obtain reproducible results. Pulmonary mid-capacity was determined by needle aspiration of the gas within the lung in the anesthetized living frog. It has been mentioned that ventilation occurs under positive pressure in the frog so that the body can be opened without markedly disturbing pulmonary ventilation. After the lung was exposed, it was necessary only to clamp the bronchus and rapidly aspirate the contained air at the approximate end expiratory level of inflation. The volume was 1.8 ml at this level; approximate mid-capacity of the lung. Volume at the mid-point of the linear part of the pressure volume diagram was approximately 0.7 ml.

Results. Spontaneous contraction of the smooth muscle in the wall of the lung interfered with the measurements of pressure in the early experiments. It was noted that muscular contraction could be provoked by sudden introduction of air or fluid into the lungs. Electrical stimulation regularly provoked this muscular contraction when applied for 5 seconds as an induced current from a 1.5 volt dry battery. The electrically induced contraction occurred up to 3 hours, and occasionally as long as 6 hours, after the lungs had been excised. Pressures as high as 100 cm of water were observed. Muscular contraction was permanently abolished following immersion of the lungs in mercuric cyanide solution for 15 minutes. This procedure was employed routinely because it facilitated the measurement of pressure and eliminated the delays encountered awaiting spontaneous muscle relaxation. It should be emphasized that poisoning of the muscle did not alter the final results. It was demonstrated in several preparations that the pressure volume behavior of the relaxed but viable lung was similar to that obtained in the cyanide treated preparation.

Hysteresis was observed regularly with air. It was unexpected that hysteresis also occurred in the liquid filled frog lung and approximately equalled that found with air (Fig. 2). Repeated filling and emptying failed to eliminate hysteresis in the liquid filled preparations (Curve 2 of Fig. 2). This finding differs sharply from the results of experiments with liquid filling in mammalian lungs.

Satisfactory results were obtained from 19 emptying cycles in 8 lungs after filling with Ringer's solution. Mean frog weight, lung weight, and compliance are summarized in Table I. Compliance was measured in the middle linear part of the pressure volume diagram of the lung (0.7 ml) and at mid-capacity (1.8 ml). The mean transpulmonary pressure on emptying was also determined at 2 volumes of inflation (0.7 and 1.8 ml). Air

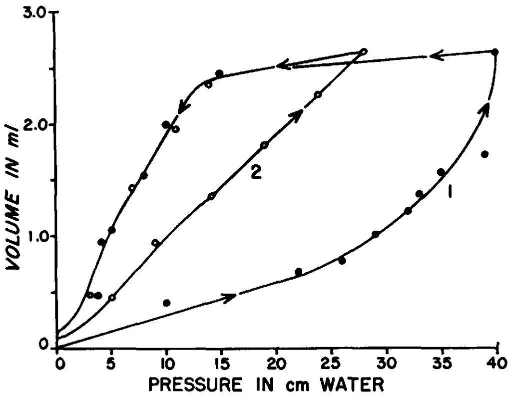

FIG. 2. Successive static pressure volume diagrams obtained with Ringer's solution filling in a single lung. Curve 1 represents initial filling from collapsed state. Curve 2 presents another filling cycle of same lung.

deflation data were satisfactory in 21 cycles on 7 lungs. Mean data are presented also in Table I. Fig. 3 presents typical pressure volume diagrams for air deflation and liquid emptying of a frog lung.

Discussion. The elastic behavior of the lung has been studied by measurement of the force which develops on distension of the lung. The resultant force-volume plot is a simple work diagram which portrays the over-all volume elastic performance of the lungs. von Neergaard(5) studied the pressure volume characteristics in air and liquid filled mammalian lungs. He found that the pressure at any given volume was much less in the liquid filled than in the air filled lung. He concluded that liquid filling abolished the contribution of surface energy to the total elastic performance of the lung by eliminating the air liquid interfaces throughout the lung. Radford(6) extended these observations to develop a method for measurement of the surface area of the lungs. He made the necessary assumption that the pressure volume behavior of the liquid filled lungs indicated the magnitude of the tissue elastic performance that occurred with air filling.

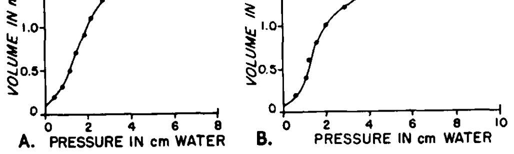

FIG. 3. Typical static pressure volume diagrams obtained on air emptying (A) and Ringer's solution emptying (B) of frog lung.

TABLE I. Mean Pressure Volume Data.

	Ringer's solution	Air
No. of lungs studied	8	7
Body wt, g	28	35
Lung wt, g	.4	.4
Pressure—cm water:		
At mid-linear volume (.7 ml)	1.8	1.7
At mid-capacity volume (1.8 ml)	10.7	8.2
Compliance—ml/mm water:		
At .7 ml	65 (36)*	66 (28)
At 1.8 ml	8 (3)	8 (2)

* Standard deviation as $\sqrt{\frac{\Sigma(d^2)}{n-1}}$ in parentheses.

The over-all elastic performance of the lungs may be considered as the summation of at least 2 major components. One is derived from the energy of the air fluid interfaces in the alveoli throughout the lung, and the other arises from the tissue. Both are complex. The air liquid interfacial contribution depends on surface tension at the interface and total surface area of the lung(6). The small size of the alveoli afford a large total surface, hence a large air liquid interfacial energy contribution. Material with high surface activity is present in the lung lining layer which permits interfacial tension to vary with variations in the volume distension of the lung. This surface active material probably plays a significant role in prevention of atelectasis but its significance to over-all lung elastic performance is less well understood. The exact role of tissue elasticity is also far from clear. It has never been proven that tissue forces behave in an identical manner with air and with liquid filling of the lungs. Interfacial tensions undoubtedly exist at cell boundaries and elsewhere in the alveolar wall. Pressure volume measurements of the lungs do not distinguish between elastic behavior arising in connective tissue fibers and that resulting from such interfacial tensions. At present no attempt is made to delineate various types of tissue factors. Tissue elastic behavior is only considered as distinct from the energy contribution of the air liquid interface.

The gas free mammalian lung fills unevenly and irregularly with air. It fills smoothly and evenly with liquid and it empties evenly with either air or liquid. This gives rise to a large amount of hysteresis during the initial air filling emptying cycle. Although hysteresis is diminished on subsequent cycles it is not abolished. The liquid filled mammalian lung exhibits very little hysteresis on the initial or on any subsequent cycle. This means that the tissue elasticity of the mammalian lung approaches ideal elastic behavior since virtually all of the energy supplied on distention is recovered on emptying. Since only the emptying portion of the cycle represents work recovered from the lung, it is significant to compare this part of the cycle. At comparable volumes of distention on the emptying cycle the liquid filled mammalian lung exhibits one-third to one-half as much pressure as does the lung filled with air. This suggests that tissue elastic forces are responsible for one-third to one-half of the total elastic work recovered on emptying the mammalian lung.

The frog lung is a simple saccular organ. The interalveolar septae fold along the wall at low levels of distention and are not apparent. With increased filling, the septae become apparent as they assume a position more nearly perpendicular to the wall of the lung. It has already been noted that the wall of the frog lung contains smooth muscle. Unlike the mammalian lung, the frog lung fills smoothly and evenly with air. Hysteresis occurs with air filling and emptying in a manner similar to that of the mammalian lung. In sharp contrast to the mammalian lung, however, hysteresis also occurs with liquid filling and emptying. This means that the tissue elastic behavior of the frog lung deviates sharply from ideal elastic performance since only a portion of the energy required for distension can be recovered on emptying. This behavior was similar in the cyanide treated lung and in the viable relaxed lung. Muscular contraction of the lung was seen to increase the intrapulmonary pressure but did not obviate hysteresis.

The emptying portions of the cycles represent work recovered from the lung as shown in Fig. 3A and 3B. The striking similarity of these curves indicates that the air liquid interfacial forces did not contribute any detectable energy to the total elastic performance of the frog lung. The failure to demonstrate an air liquid interfacial energy contribution to lung elastic performance must mean either surface tension is absent or surface area is inconsequential. Miller and Bondurant(7) studied the surface tension of frog lung extracts in a film balance and demonstrated values higher than they usually found in comparable extracts from mammalian lungs. From this it seems reasonable to conclude that the surface tension in the lung of the living frog is as great if not greater than that occurring in mammalian species. The absence of a significant surface energy contribution to lung elastic performance in the frog must therefore be attributed to the relative lack of surface area.

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