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DETERMINATION OF ALVEOLAR SURFACE AREA AND TENSION FROM *IN SITU* PRESSURE-VOLUME DATA[1]

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Abstract. Cats were anesthetized and placed, with the chest opened, inside a whole-body plethysmograph. In recording gas and liquid pressure-volume (PV) curves, 100 percent oxygen (2.9 ml/sec) and Ringer-Locke solution (1 ml/sec) were used. Gas and liquid flows were varied from 2.9–6.0 ml/sec and 0.5–5.0 ml/sec respectively, with no change in either the hysteresis or the shape of the PV curve. A constant, K, was determined by comparing the gas and liquid inflation curves at maximal lung volumes and using the equation $K = 3PV^{1/3}/2\gamma$, where γ was assumed to be 50 dyne/cm. The mean value for K in eight experiments was 1225 ± 100 SEM. At a functional residual capacity of 90 ml the alveolar surface area ($A = KV^{2/3}$) of the cat/kg body mass was 1.05 ± 0.09 m^2. Surface tension was calculated during inflation and deflation. The results indicate that surface tension approaches zero at low lung volumes or areas and is always lower during deflation than during inflation at a given volume or area.

Alveolar air-liquid interface Surface tension
Lung pressure-volume curves Surfactant

Interest in air and liquid pressure-volume (PV) curves for lungs dates back to 1929 when VON NEERGARD inflated lungs of anesthetized cats first with air and then with a gum arabic-saline solution and found that much less pressure was required to sustain a given volume with liquid than with air. He concluded that the alveolar-air interface is lined with a moist layer which exhibits surface tension and adds to the elastic recoil

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of the lung. PATTLE (1955) inferred from the stability of bubbles squeezed from lungs that an insoluble lining film covers the alveolar-air interface and acts as a surface-active agent (surfactant) to lower the surface tension. He concluded that the surface tension might be as low as zero dynes per centimeter. Others (AVERY, 1962; CLEMENTS, 1956, 1957; BONDURANT and MILLER, 1962) have investigated the alveolar surface tension by placing saline extracts of minced lungs or lung washings in a trough and measuring the surface tension with a Wilhelmy balance. The values obtained for surface tension range from almost zero at maximum compression to a limiting value of approximately 50 dynes per centimeter upon expansion. Calculations in which PV data were used to determine the alveolar surface area and surface tension of cat lungs were first made by RADFORD (1954) and later modified by BROWN (1957). These papers presented data for deflation only and did not detail the setup and experimental procedure sufficiently for other investigators to duplicate the experiments (PATTLE, 1965).

The purpose of this investigation is two-fold: firstly to determine the total alveolar surface area of the cat lung from the *in situ* PV curves for both deflation and inflation and secondly to determine the alveolar surface tension as a function of the alveolar surface area for the complete cycle from the same *in situ* gas and liquid PV data.

Methods

Mongrel cats (2.4 ± 0.1 SEM kg) were anesthetized with sodium pentobarbital (35 mg/kg) given intraperitoneally. The hair of the animal was clipped to prevent trapping of air when the animal was later immersed in saline. The trachea was cannulated with a "U"-shaped glass cannula. A bilateral pneumothorax was induced by carefully dissecting the intercostal muscles of the last two ribs and rupturing the parietal pleura. A midline incision extending from the neck to mid-abdomen was made, and the sternum was then cut beginning from the posterior aspect. The rib cage was opened widely, and the ribs were sutured to three retaining rings which circumscribed the back and sides of the animal. Any parietal pleura which might obstruct the free movement of the lungs was removed, and the two phrenic nerves were sectioned. A ligature was placed around the pericardium at the apex of the heart and tied to a retaining ring above the heart to provide support for the heart. The diaphragm was sutured to the abdominal wall to prevent expulsion of the abdominal contents into the thorax. The cat was then given an intramuscular injection of curare (0.7 mg/kg) and placed inside a whole-body plethysmograph as shown in fig. 1 (HILDEBRANDT, 1966).

The plethysmograph, which was made of leucite, had four one-quarter inch copper tubes – first to connect the plethysmograph system with the inflation/deflation systems and secondly to allow measurement of transpulmonary pressure, volume displacement and temperature inside the plethysmograph (fig. 1). An additional system not shown in fig. 1 monitored the rectal temperature of the cat. The whole-body plethysmograph was placed in a constant temperature water bath which was three degrees centigrade below the rectal temperature of the animal in order to provide a temperature gradient sufficient to dissipate the heat produced by the animal.

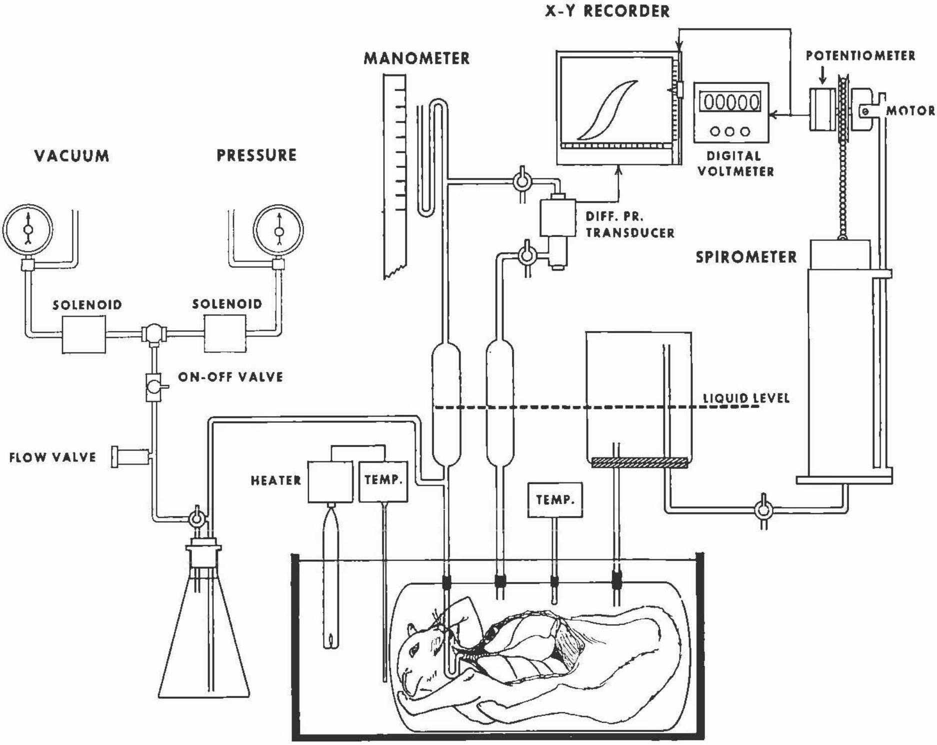

Fig. 1. Schematic representation of the experimental setup. The plethysmograph was immersed in a constant temperature water bath and had 4 one-quarter inch copper tubes connecting it with the differential pressure transducer, ambient temperature monitor, and the compensated spirometer. The potentiometer was used to produce an electrical signal so that volume measurements could be monitored with a digital voltmeter and recorded on the XY recorder, along with pressure. Inflation and deflation were initiated by activation of the appropriate solenoid. During liquid maneuvers, tubes leading to the differential pressure transducer and the compensated spirometer were filled with Ringer-Locke solution to the level indicated and the gas flow directed into the Erlenmeyer flask filled with the same solution.

A 500 ml spirometer was used to measure volume displacement of gas from the plethysmograph due to the lung volume changes. An electrical signal was produced from the potentiometer connected to the pulley of the spirometer and recorded on a digital voltmeter and the ordinate of the XY recorder as shown in fig. 1. A constant speed variable torque D.C. reversible motor (Thurst Model C-A, Princeton, Indiana) was connected to the shaft of the pulley to provide a constant tension on the bell of the spirometer to overcome the surface tension of the water on the bell. The degree of phase shift in the uncompensated spirometer was a function of the air flow into the spirometer; therefore, the higher the flow, the more torque was required of the motor. The amount of torque provided by the motor was a function of the voltage input to the motor. The motor was reversible so that torques in opposite directions were exerted during deflation and inflation. The proper voltage to be applied to the motor at a given flow was determined each day before the experiment. Compensation was adequate to eliminate any hysteresis in the spirometer. The spirometer was calibrated by injecting known amounts of air into the spirometer by means of a syringe. The output of the potentiometer of the spirometer was adjusted so that the output of the digital voltmeter was read as milliliters.

A differential pressure transducer (Statham #PM5TC) was used to measure transpulmonary pressure for both air and liquid experiments. During air maneuvers, the transpulmonary pressure was measured by connecting one side of the differential pressure transducer to the tube leading to the tracheal cannula, and opening the other side to the inside of the plethysmograph. During liquid procedures, the glass tubes connecting the plethysmograph and the tracheal cannula to the differential pressure transducer were filled with liquid to the same level as shown in fig. 1, and the air pressures above the level of the liquid were measured by the transducer.

Experimental procedure

GAS DEFLATION AND INFLATION

After the lungs had been ventilated with 100 per cent oxygen for ten or fifteen minutes, the airway leading to the tracheal cannula was clamped by means of the on-off valve

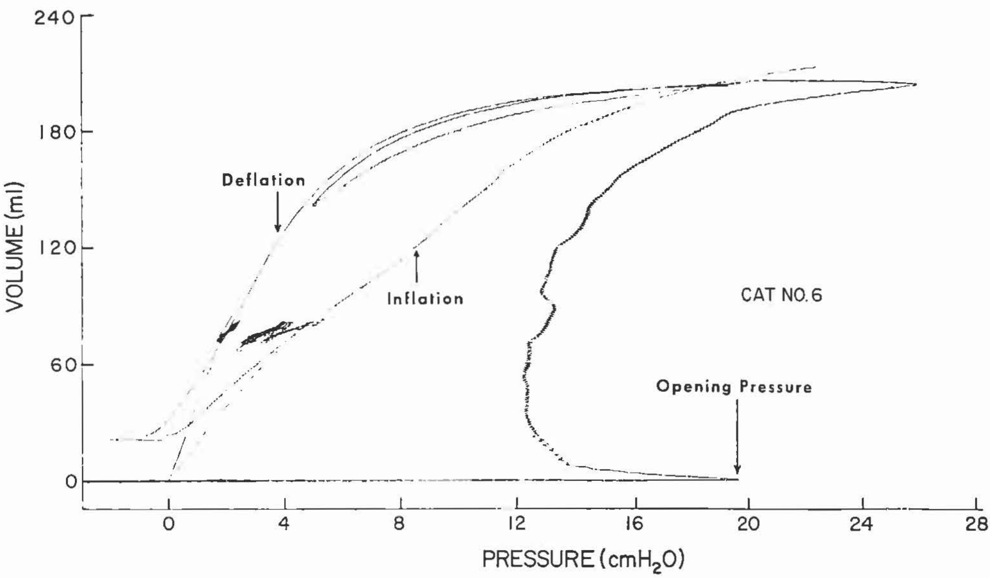

Fig. 2. A typical gas (100 per cent oxygen) pressure-volume recording obtained after atelectasis had developed. Gas flow was 2.9 ml/sec. The transpulmonary pressure was −3 cm water when atelectasis was complete. The completeness of atelectasis is shown by the high opening pressure of 19.6 cm water. Relatively strong pressure fluctuations are observed on the initial inflation curve due to pulmonary vascular pulse waves. Note the absence of an opening pressure in the second inflation and much less hysteresis than the inflation from atelectasis. Several small volume excursions between 70 and 80 ml were made to show what were considered "normal breaths."

(fig. 1) and the remaining oxygen in the alveoli was taken up by the blood. Both sides of the differential pressure transducer were opened to the atmosphere, and the zero pressure coordinate of the XY recorder was established. Atelectasis was considered complete when (1) the color of the lungs changed from the normal light pink to a deep purple, (2) cardiac arrest appeared imminent, and (3) the change of lung volume with respect to time was zero over a time interval of at least thirty seconds. When atelectasis developed, the zero volume coordinate was set on the XY recorder and the lungs were inflated at a flow of 2.9 ml/sec with 100 per cent oxygen until a transpulmonary pressure of 25 cm water was reached. The trachea was clamped for one minute to insure that all alveoli were open. The completeness of atelectasis was verified by the magnitude of the opening pressure (fig. 2). All of the experiments used in the calculations had opening pressures in excess of 17 cm water. The volume of the lungs was decreased until a pressure slightly below 20 cm water was reached and then immediately inflated to a volume which corresponded to 20 cm water. The volume at 20 cm water was defined as the initial volume for the experiments. The experimental deflation was started from this volume at a flow of 2.9 ml/sec and continued until the transpulmonary pressure became slightly negative. The lungs were then reinflated with the same flow until the initial volume was reached. This concluded a complete volume cycle for gas. In five experiments, the procedure was repeated with gas flows of 1.0, 5.0, and 6.0 ml/sec.

LIQUID DEFLATION AND INFLATION

After the gas PV curve was constructed, the lungs were deflated until the transpulmonary pressure was reduced to some small positive value. Ringer-Locke solution was introduced into the tracheal cannula at a flow sufficient to maintain the meniscus at the bottom of the "U"-shaped tracheal cannula until cardiac arrest occurred. This procedure displaced the remaining oxygen from the cannula into the alveoli so that when atelectasis was complete only Ringer-Locke solution remained in the bronchioles. The plethysmograph was then filled with Ringer-Locke solution until the fluid filled the glass tube leading to the pressure transducer to the level indicated in fig. 1. Additional fluid was added to the trachea until the same level was reached. This condition defined zero pressure and volume for the liquid maneuvers, an example of which is shown in fig. 3. The lungs were then inflated at a flow of 1.0 ml/sec until a transpulmonary pressure of 24 cm water was reached. The trachea was then clamped for one minute to insure that all alveoli were open. The volume was then decreased to about 15 ml below the initial volume used for the air deflation and reinflated until the initial volume was reached. The lungs were immediately deflated at a flow of 1.0 ml/sec until the transpulmonary pressure became slightly negative, and then reinflated to the initial volume. This concluded a complete volume cycle for liquid. In order to subtract the pressure drop due to the resistance to flow of the liquid, the flow was stopped for five seconds at approximately 30 ml intervals. The points on the graph produced at the end of the five seconds were connected by smooth lines and used as the liquid PV

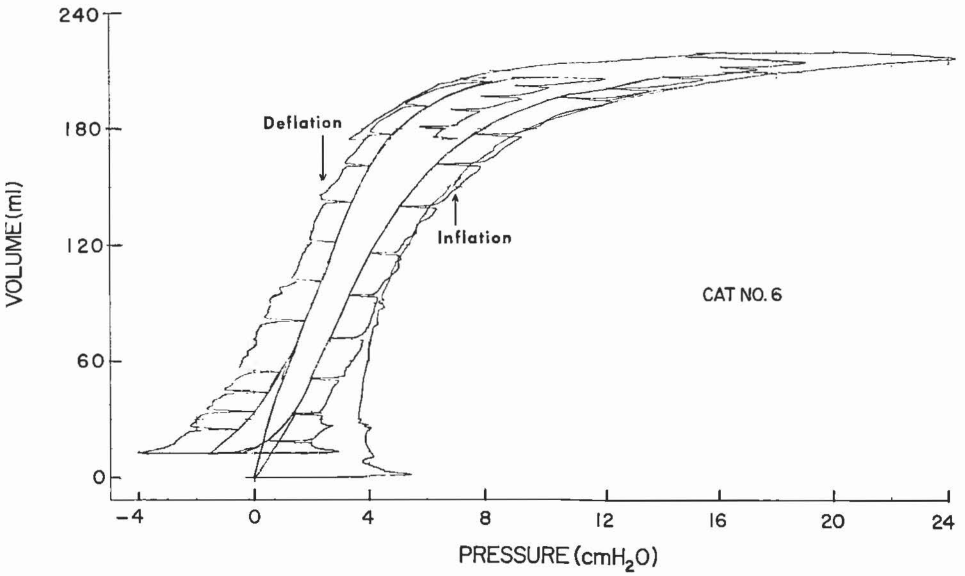

Fig. 3. Typical liquid (Ringer-Locke solution) pressure-volume recording obtained after atelectasis had developed. The inflation started from zero pressure and volume. The opening pressure was 5.4 cm water. Liquid flow (1.0 ml/sec) was interrupted for five seconds at approximately 30 ml intervals. The points on the graph produced at the end of the five seconds were connected by the smooth lines shown and used as the liquid PV curve.

curve (fig. 3). In six experiments the procedure was repeated with liquid flows of 0.5, 1.0, and 5.0 ml/sec.

Calculations

In interpreting the data, two assumptions were made: first, that the alveolar surface area is related to the volume to the two-thirds power by a constant, K (Brown, 1957; Weibel, 1963); and secondly, the surface tension occurring at maximal lung volume is 50 dyne/cm. No one has directly measured the surface tension at maximal lung volume, however, alvolar surface tension is assumed to behave similarly to surfaces formed from lung extracts. Other investigators have measured such maximal surface tensions in dynes/cm. For example, Rosenberg (1969) obtained a value of 46.9 from rat lung extracts while Clements (1957) obtained a value of 46 from rat, cat and dog lung extracts and 50 for the maximum surface tension of plasma. Avery (1959) obtained a value of 55 dyne/cm for extracts from dog lungs. The gas and liquid PV curves were extrapolated to zero pressure and volume. The zero coordinates of the gas and liquid curves were then matched and the inflation and deflation curves were transferred to separate graphs (fig. 4). The volume in the inflation curves which corresponded to the greatest pressure difference between the gas and liquid curves was chosen to represent the volume at which the surface tension reached its maximum. The volume and pressure difference and the assumed surface tension of 50 dynes per centimeter were inserted into the following equation in order to evaluate the constant K.

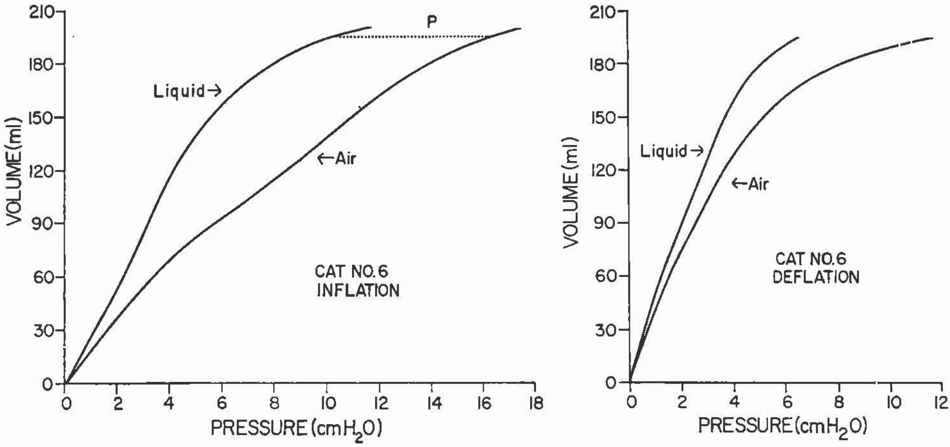

Fig. 4. Replotted data used for calculating the constant K, and surface tension as a function of volume and pressure. The left graph shows the comparison of the air and liquid inflation curves and the right graph shows the comparison of the corresponding deflation curves. Value of K is obtained by using pressure difference and corresponding volume where two curves are farthest apart (dashed line).

$$K = \frac{3}{2\gamma} PV^{\frac{1}{3}}$$

where: P = Pressure differences between the gas and liquid curves at any volume, V.
γ = The surface tension
K = The proportionality constant relating area and volume to the two-thirds power.

This equation was derived by using the definition for surface tension:

$$\gamma = \frac{dG}{dA} = \frac{PdV}{dA}$$

where: G = surface free energy and A = surface area
and area and volume were assumed to be related by the following equation:

$$A = KV^{\frac{2}{3}}$$

Differentiating we obtain: $\frac{dA}{dV} = (2/3)KV^{-\frac{1}{3}}$

Substituting: $\frac{P}{\gamma} = (2/3)KV^{-\frac{1}{3}}$

and: $K = \frac{3}{2\gamma} PV^{\frac{1}{3}}$

Results

Figure 2 shows a typical gas inflation curve starting with an atelectatic lung. The transpulmonary pressure was -3 cm H_2O when atelectasis was complete. The opening pressure was 19.6 cm H_2O. Relatively strong pressure fluctuations were noted on the initial inflation curve due to pulmonary vascular pulse waves which were initially strong due to hypoxia and hypercapnia which developed during the time needed to produce atelectasis. The volume in this lung at a pressure of 25 cm water was 204 ml. During the one-minute interval in which the trachea was clamped, the transpulmonary pressure decreased to about 20 cm water with a corresponding volume increase of three ml. The volume at 20 cm water, which was defined as the initial volume for the experiment, was 207 ml. Deflation continued until the transpulmonary pressure of -2 cm water and a volume of 21 ml were reached. The subsequent inflation showed no opening pressure and much less hysteresis than the inflation from atelectasis.

Figure 3 shows an example of a typical liquid PV curve. The transpulmonary pressure at atelectasis was zero because both the pulmonary and plethysmograph systems were opened to the atmosphere. The opening pressure was 5.4. cm water or 26.5 per cent of the opening pressure observed with the air inflation. The volume produced by

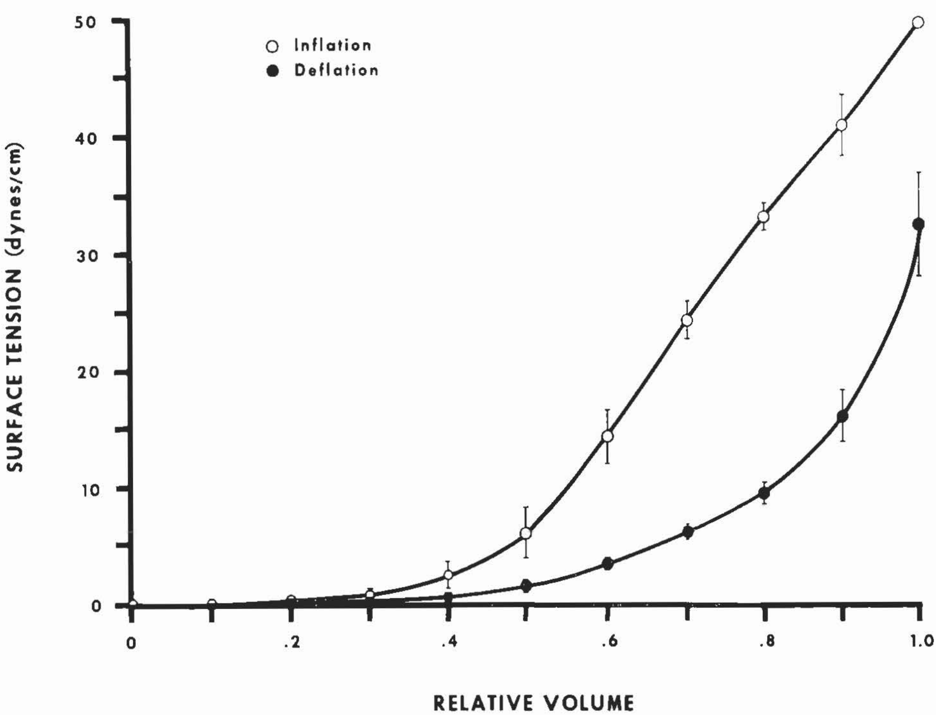

Fig. 5. Surface tension as a function of relative volume for eight experiments. The vertical lines represent ± 1 SEM. Maximal volume was the volume at which the surface tension reached a maximum (assumed to be 50 dyne/cm).

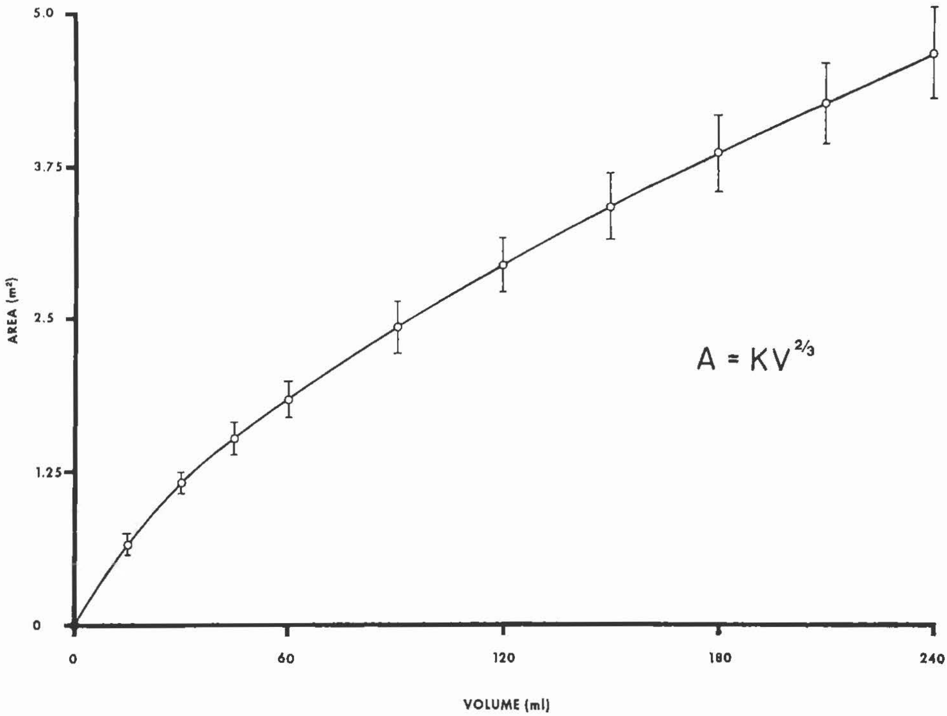

Fig. 6. Alveolar volumes were converted to area by the equation $A = KV^{\frac{2}{3}}$. The vertical lines represent $\pm$ 1 SEM, and reflect variability of K.

24 cm water was 218 ml. During the minute that the trachea was clamped, the transpulmonary pressure decreased by four cm water with a corresponding volume increase of three ml. The pressure needed to reach the initial volume of 207 ml that was observed for the air deflation was 10 cm water. Deflation was continued until the transpulmonary pressure became negative by four cm water (including pressure drop due to flow resistance). The inflation was then started and continued until the initial volume was reached. The pressure drop due to the liquid flow was approximately one cm water. In the experiments in which the gas and the liquid flows were changed, no significant change in either the shape or the hysteresis of the PV curve was found, except when the gas flow was reduced to one ml/sec or less. When a gas flow of one ml/sec was used, some atelectasis was observed to occur. In experiments in which the liquid flow was interrupted for different lengths of time, it was found that the pressure equilibrated within five seconds.

Figure 4 shows a comparison of gas and liquid inflation and deflation curves. The volume and pressure difference obtained from the inflation data where the two curves were farthest apart (dashed line) were used to obtain the values for K. The mean value of K was 1225 $\pm$ 100 SEM and the value of K/kg was 523 ± 44[1].

[1] The value reported in the preliminary note on this research (The Physiologist *12*: 226, 1969) was in error by a factor of 10.

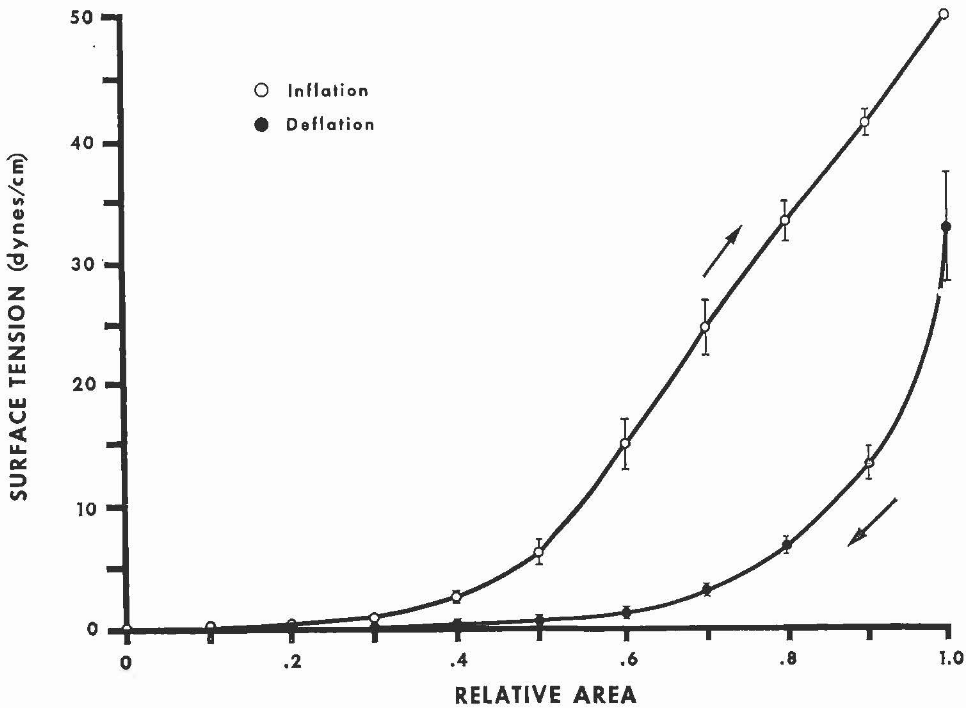

Fig. 7. Surface tension plotted as a function of relative area for eight experiments. The vertical lines represent ± 1 SEM. The maximum area was the area at which the surface tension reached a maximum (assumed to be 50 dyne/cm).

Values of surface tension at other volumes, during inflation and deflation, were determined with the same equation and the value of K which was determined for each animal. The volume at which the surface tension reached its maximum ranged from 255 to 175 ml in eight cases. In order to pool the data, the volume which corresponded to a surface tension of 50 dyne/cm was regarded as the maximum volume. Fig. 5 shows surface tension plotted as a function of relative volume. At small lung volumes the surface tension approaches zero and is always lower during deflation than during inflation for any particular volume. Alveolar volume was converted to alveolar surface area (fig. 6) by using the relationship, $A = KV^{\frac{2}{3}}$, and the value obtained earlier for the constant K. In order to pool the data, the surface area which corresponded to a surface tension of 50 dynes/cm was regarded as the maximal alveolar surface area and surface tension was then plotted as a function of the relative surface area (fig. 7). The surface tension approaches zero at small areas and is always lower during deflation than during inflation at any particular surface area.

Discussion

When gas and liquid pressure-volume curves are used to calculate the alveolar surface area, it is essential that the two curves be matched properly. In order to do this the zero references for pressure and volume must be known. The zero pressure coordinate was easily determined during gas maneuvers by using three-way stopcocks on both sides of the differential pressure transducer. The zero pressure during liquid maneuvers was established by having liquid columns of equal height above the tracheal and plethysmographic systems after atelectasis was produced. More difficult than defining zero pressure was the task of defining zero volume. During gas maneuvers, atelectasis was produced by allowing the circulatory system to absorb the oxygen from the alveoli. Since the same procedure was used to produce atelectasis for the liquid experiments, it was essential not to allow anoxia to lead to circulatory collapse. In order to insure as high a degree of atelectasis as possible three criteria were established (see experimental procedure). The most critical evaluation of atelectasis was afforded by the opening pressure. In all of the experiments used in the calculations the opening pressure was greater than 17 cm water. Before liquid measurements could be made, it was important to have replaced all of the dead space air with liquid while preventing liquid from entering the alveoli. This was facilitated by using a "U"-shaped glass cannula. By keeping the level of the liquid at the bottom of the cannula, the gas in the tracheal tree was forced into the alveoli without putting a positive pressure on the alveoli. Atelectasis was then effected by allowing the circulatory system to absorb the oxygen until cardiac arrest. Thus, as atelectasis occurred, liquid filled the bronchioles.

A gas flow of 2.9 ml/sec was chosen so that airway resistance would not become a factor in the pressure measurements. Because of the high viscosity of Ringer-Locke solution in comparison to gas, a liquid flow of 1.0 ml/sec was used to minimize the viscous resistance. The pressure drop due to resistance to liquid flow was eliminated from the pressure measurements by stopping the flow and allowing the pressure to equilibrate. Both the liquid and gas flows could be varied over an order of magnitude without affecting either the shape or the hysteresis of the PV curve. The only exception was with an air flow of 1 ml/sec where atelectasis occurred.

The liquid PV curves obtained in our experiments show a significant amount of hysteresis (fig. 3) compared with those of RADFORD (1964) which show little or no hysteresis. The amount of hysteresis probably depends on the past history of that system, the time allowed for the system to equilibrate and the rate of inflation and deflation. RADFORD (1964) began deflation at a pressure of approximately 7 cm H_2O whereas we started deflation at a pressure greater than 20 cm H_2O. Also, we felt it was imperative that the equilibration time for liquid be restricted to the time (5 sec) needed for the pressure drop due to flow resistance to become zero and that the liquid and gas flows be comparable. RADFORD (1964) allowed from 1 to 5 min for the liquid system to equilibrate, and did not state the flow used to inflate and deflate the lungs. Each of these conditions in our experiments would tend to produce more hysteresis.

The determination of alveolar surface area by RADFORD (1954) from measurements made on excised lungs differs from ours by a factor of ten. However, Radford assumed a constant surface tension of 50 dyne/cm from low lung volume to FRC. The values we obtained for surface tension over this volume range are less than 5 dyne/cm, thus accounting for the discrepancy. Later BROWN (1957) repeated the work of Radford, assuming a surface tension of 50 dyne/cm only at maximal lung inflation and determined that the surface tension reached a limiting value of 5 to 10 dyne/cm at small lung volumes. Brown's calculations were made from only deflation PV curves. We chose to use the inflation curves because the pressure differences at similar volumes were always greater during inflation than during deflation. Thus, choosing the deflation curve to evaluate K would have yielded values of surface tension greater than 50 dyne/cm at large lung volumes during inflation.

When room air was used inside the plethysmograph, each successive gas PV curve was shifted about three ml above the previous. This phenomenon was due in part to factors such as gas diffusion across the lung and body surfaces, changes in temperature of the system, or alterations in the mechanical characteristics of the lung. The temperature was monitored at various points throughout the airways, and only slight fluctuations were noted. These occurred for only a short period after the animal had been placed inside the plethysmograph. The volume drift was eliminated when the inside of the plethysmograph was flushed with 100 per cent oxygen and a carbon dioxide absorber was placed inside. However, even after the volume shift was eliminated, the compliance of the lung, at small lung volumes, during gas maneuvers, appeared to gradually decrease. The effect of this shift in volume would be a slight increase in the value obtained for K, on the order of 2 per cent.

The results of *in situ* area-tension determinations exhibit several similarities to those determinations made from surfaces formed by lung extracts in a Wilhelmy surface tension balance (CLEMENTS, 1957). In the surface tension balance, the surface tension of the lung-derived surfaces approaches zero with maximal compression and reaches a limiting value of approximately 50 dynes/cm with expansion. From the equations, it can be seen that the pressure difference between the gas and liquid curves is a major determinant of the value for surface tension. In each experiment the pressure difference was extremely small at low lung volumes and increased with larger lung volumes. However, in many experiments, including the example given in this paper, the pressure difference reached a maximum at a large lung volume and remained constant over a considerable volume interval. This suggests that the alveolar surface behaves similarly to the surface formed in the Wilhelmy balance by lung extracts, and provides evidence for assuming a surface tension of 50 dyne/cm at large lung volumes. The surface tension-area plot of the alveolar surface exhibits a large degree of hysteresis, as does the surface produced from lung extracts, indicating that neither surface is thermodynamically reversible. Another similarity is that the surface tension was always greater during inflation than during deflation at any given volume or area and approached zero at low lung volumes.

When the value of K obtained from the eight experiments is divided by mean body mass, the area per kilogram can be calculated. If a functional residual capacity (FRC) of 90 ml is assumed for the cat, the area at FRC per kg body mass is approximately one square meter. If lung area at FRC is extrapolated on the basis of body mass (BROWN, 1957), the area agrees well with the histological determinations made by WILLSON (1922) and WEIBEL (1963) for man.

The ability of the lung to modify the alveolar surface tension as a function of volume or area is important for three reasons. First, a surface tension which decreases with volume contributes to the stability of the alveoli. If the surface tension did not decrease as alveolar volume decreased, the smaller alveoli would empty into larger alveoli. It is for this reason that CLEMENTS (1957) referred to pulmonary surfactant as an "anti-atelectasis factor". Secondly, the decreased surface tension at small lung volumes reduces the pressure, and consequently the energy, needed to inflate the lung. Thirdly, reducing the surface tension reduces the tendency for transudation of fluid from the pulmonary capillaries to the alveoli.

Acknowledgements

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