Lung degassing: an evaluation of two methods

P. W. STENGEL, D. G. FRAZER, AND K. C. WEBER
Division of Respiratory Disease Studies, Appalachian Laboratory for Occupational Safety and Health, National Institute for Occupational Safety and Health, Center for Disease Control; and Department of Physiology and Biophysics, West Virginia University Medical Center, Morgantown, West Virginia 26506

STENGEL, P. W., D. G. FRAZER, AND K. C. WEBER. *Lung degassing: an evaluation of two methods.* J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 48(2): 370–375, 1980.—This study compares the effectiveness of the oxygen absorption and vacuum degassing methods for removing trapped gas from lungs. In addition, the effects of changing vacuum pressure, number of times to degas, and lung orientation during the vacuum degassing procedure were evaluated. To evaluate the two methods, a capacitance spirometer was designed and constructed to record lung volume as lungs were vacuum degassed. When lungs containing trapped gas were degassed in a vacuum chamber, they initially expanded, then slightly decreased in volume until the vacuum was released. Lung volume rapidly decreased as the pressure in the vacuum chamber returned to ambient pressure. The results showed that oxygen absorption atelectasis was more effective in removing gas from the lungs than vacuum degassing the lungs. When vacuum degassing was used, it was found to be most effective when the pressure in the chamber was reduced to the vaporization pressure of H_2O and when the lungs were degassed twice. Degassing the lungs more than twice did not significantly remove more gas from the lungs. Lung orientation did not affect the removal of gas during vacuum degassing.

O_2 absorption atelectasis; vacuum degassing of lungs; trapped gas in lungs; capacitance spirometer

IN STUDIES that examine pressure-volume (PL-VL) curves of excised lungs, it is important that the lungs initially be gas free, since the mechanical behavior of the lungs is a function of their volume history (16). It has been shown that lungs tend to trap gas when they are ventilated slowly after the thorax has been opened (10). Even when lungs are quickly removed from the animal following the opening of the thorax, they contain a small amount of trapped gas (14). When excised lungs are artificially ventilated at slow rates, the trapped gas can accumulate until it reaches approximately 75% total lung capacity (TLC) (12).

The two methods for removing trapped gas from the lungs have been O_2 absorption and vacuum degassing. When the O_2 absorption technique is used, the lungs are ventilated with 100% O_2 for at least 10 min after which the trachea is clamped. All the remaining O_2 in the air spaces of the lungs is presumably absorbed by the blood, causing the lungs to become atelectatic (6, 10, 20). Vacuum degassing, on the other hand, results from placing excised lungs in a vacuum chamber and then reducing the pressure in the chamber. Some investigators (4, 8, 17, 18) have suggested that when the pressure surrounding the lungs during vacuum degassing is reduced to the vapor pressure of H_2O, all gases other than H_2O vapor are displaced from the chamber. When the vacuum is released, the H_2O vapor condenses, and the lungs are essentially gas free. Previous investigators agreed that it was necessary to reach H_2O vapor pressure but disagreed upon how long the vacuum should be applied. Tierney (21) subjected excised rat lungs to vacuum degassing for 10–20 s; Edmunds and Huber (7) vacuum degassed dog lungs for 10 min after H_2O vapor pressure was reached; and Levine and Johnson (15) vacuum degassed rabbit lungs four times for 40 min. Other investigators (9, 11, 12, 19) feel that menisci or a foam are responsible for trapped gas in the lungs. These thin film structures would expand in the presence of a vacuum until either the walls of the trapped air spaces rupture, the recoil pressure of the lungs balances the outward pressure of the gas, or the trapped gas diffuses from the spaces.

If lung volume could be measured while the lungs are being degassed, it should be possible to predict the relative amount of gas trapped in the lungs and determine the optimum conditions necessary for removal of the gas. One way of measuring lung volume is to use a capacitance spirometer, which has the advantage of being unaffected by large pressure and temperature changes that occur during the degassing process. Various types of capacitance spirometers have been used in the past for determining lung volume changes in laboratory animals and man (1–3, 5, 13). In this study, however, a new design of the capacitance spirometer was developed to meet the necessary requirements for measuring lung volume as the lungs were being degassed in a vacuum chamber. The objectives of this study were *1*) to determine which method is best for degassing lungs, and *2*) to obtain more information concerning the processes that occur in the lungs as they are vacuum degassed under various conditions.

Downloaded from journals.physiology.org/journal/jappl at CDC Information Center (158.111.236.068) on August 24, 2020.

METHODS

Equipment. In this study P_L-V_L curves were recorded and lungs were degassed in the system shown in Fig. 1. Transpulmonary pressure (P_L) was measured with a pressure transducer (Setra model 236 ± 0.5 psid), and lung volume (V_L) was detected with both a minispirometer (V_{LS}) (Med-Science Electronics model 118) and a capacitance spirometer (V_{LC}). When the lungs were ventilated, P_L-V_L curves were recorded on an *X-Y* recorder (A) (Hewlett-Packard model 118). V_{LS} versus V_{LC} was plotted on the other *X-Y* recorder (B) (Houston model 2000) and was used to calibrate the capacitance spirometer for each lung. The capacitance spirometer was subsequently used to measure lung volume as the lungs were being degassed. In Fig. 1, three valves had to be opened or closed when ventilating or vacuum degassing the lungs. As the lungs were ventilated, valve 1 (V_1) was opened between the minispirometer and the plethysmograph but was closed when the lungs were vacuum degassed. Valve 2 (V_2) was a two-way valve that connected the syringe pump to the trachea of the lungs when they were ventilated. During vacuum degassing, V_2 was opened so that lung pleural and tracheal pressures were equal. Valve 3 (V_3) was closed when the lungs were ventilated but opened to vacuum degas the lungs.

The electronic circuitry for the capacitance spirometer (V_{LC} in Fig. 1) is shown in Fig. 2. A copper screen was placed in the plethysmograph and surrounded the lungs while serving as the fixed plate of the capacitor. In this configuration, the screen was grounded and acted as a shield to reduce noise (Fig. 2). The surface of the lungs served as the second plate of the variable capacitor. Changes in capacitance between the lungs and the plate were detected with a circuit that consisted of operational amplifiers (A and B) and a demodulator. The trachea of the lungs was connected electrically to the negative input of amplifier A, while the positive input was connected to an oscillator, Vs (Kronhite model 5100A). The oscillator provided a sinusoidal carrier frequency of 25 kHz. In the second stage of the detector, amplifier B, the carrier frequency was subtracted from the output of A and

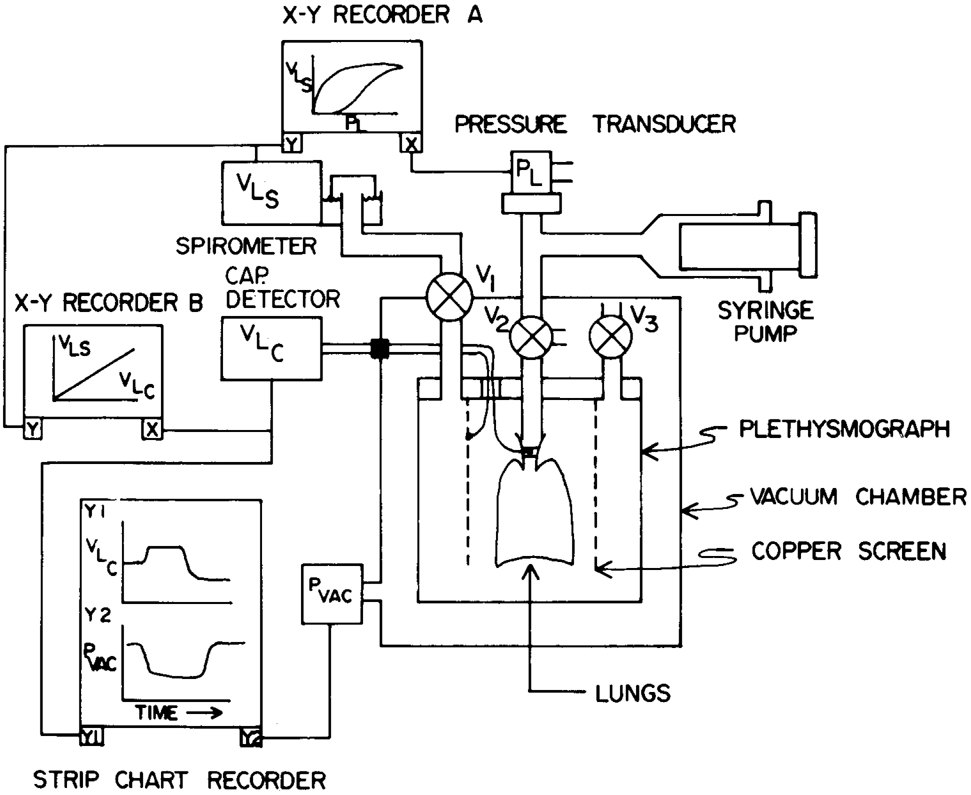

FIG. 1. Schematic diagram of lung plethysmograph and recording apparatus. Capacitance detector was used to measure lung volume while lung was being inflated-deflated and during vacuum degassing. For explanation of valve (V_1-V_3) positions, see text.

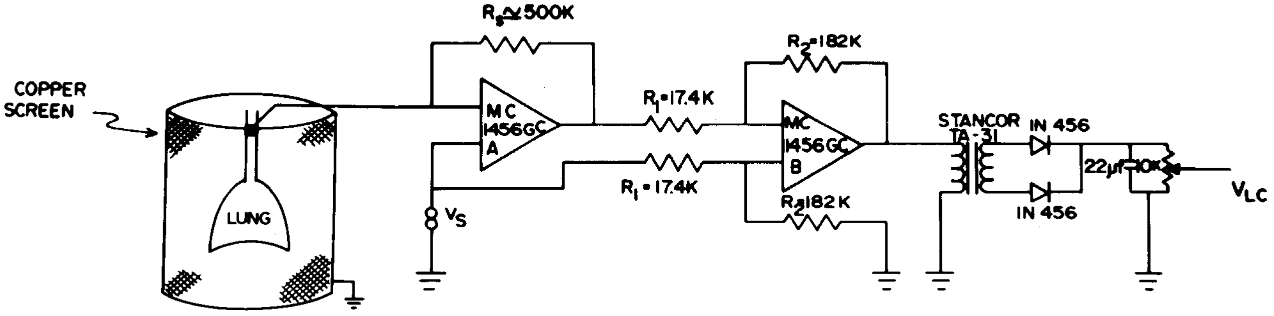

FIG. 2. Schematic diagram of the capacitance spirometer (capacitance detector of Fig. 1).

Downloaded from journals.physiology.org/journal/jappl at CDC Information Center (158.111.236.068) on August 24, 2020.

amplified. The signal from amplifier B was directly proportional to the capacitance between the lungs and the screen and was demodulated and plotted on an *X-Y* recorder (B) and a strip-chart recorder (Hewlett-Packard model 7702B) as shown in Fig. 1.

Experimental procedures. Long-Evans hooded male rats of approximately 250–300 g were anesthetized with pentobarbital sodium administered intraperitoneally (85 mg/kg body wt). A tracheostomy was performed and the rats were endotracheally intubated with a cannula that fit tightly over a 15-gauge needle. To produce O_2 absorption atelectasis, the rats were ventilated with 100% O_2 for 10 min using a small-animal respirator (Harvard apparatus model 665). At the beginning of the 10-min period, a bilateral pneumothorax was produced by opening the abdominal cavity and sectioning the diaphragm. After the 10-min period was over, the trachea was clamped and the O_2 remaining in the lungs was absorbed by the blood. The rat was then exsanguinated by severing the axillary artery. The rib cage was cut away and the heart and lungs were removed en bloc. The dissection took approximately 20 min. The lungs were prevented from drying during vacuum degassing by keeping the external surface moist with saline and by adding H_2O to the lung plethysmograph.

During a typical experiment the lungs were first weighed on an analytical balance (Mettler model H51AR) in a specially constructed basket that enabled the lungs to be weighed both in air and submerged in saline. The lungs were promptly removed from the balance and placed in the plethysmograph that was placed inside a larger Plexiglas container that served as a vacuum chamber (Fig. 1).

The lungs were immediately vacuum degassed twice to determine the changes in volume of the atelectatic lungs to a reduction in pressure. They were then inflated-deflated between a minimum and maximum transpulmonary pressure of −5 and +30 cmH_2O for either two or five cycles at a rate of 3.82 ml/min. As they were ventilated, air became trapped in the lungs as described by Frazer and Weber (12). The transpulmonary pressure transducer and minispirometer were then disconnected and the appropriate valve settings again made to degas the lungs. Care was taken not to disturb the orientation of the lungs in the chamber to ensure that the calibration of the capacitance spirometer remained unchanged. During the degassing procedure the output of the capacitance spirometer was plotted along with vacuum chamber pressure versus time on a strip-chart recorder (Fig. 1). Approximately 10 s after the minimum pressure in the chamber (Pvac) was reached, approximately 1.5 min after the application of the vacuum, the pressure was released. This degassing sequence was repeated twice. Finally, the lungs were removed from the plethysmograph and weighed a second time in saline.

The amount of gas that could not be removed from the lungs (VR) by vacuum degassing was determined using two different methods. The first was by directly recording the output of the capacitance spirometer. The second was to determine VR using the H_2O displacement (weight) method where the amount of air trapped in the lungs was calculated by the following equation

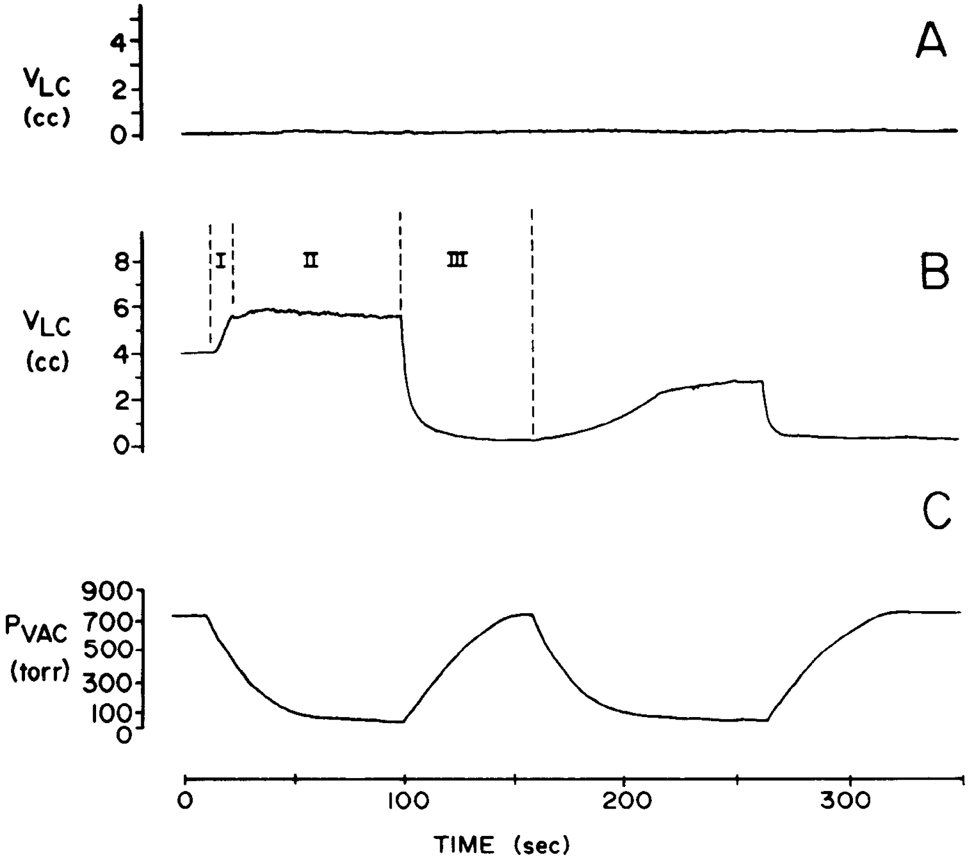

FIG. 3. Results of measuring lung volume using capacitance spirometer during two consecutive vacuum degassings. *A*: lung had been made atelectatic by O_2 absorption. Note lung volume did not change detectably during vacuum degassing. *B*: lung volume changes during vacuum degassing when lung had previously been inflated-deflated to trap gas. Note initial increase in lung volume as vacuum pressure (Pvac) was applied. *C*: vacuum pressure (Pvac) as it was applied to the lung plethysmograph.

Downloaded from journals.physiology.org/journal/jappl at CDC Information Center (158.111.236.068) on August 24, 2020.

$$V_R = (V_{L_i} - V_{L_f})$$

where V_{L_i} is the initial volume of the lungs (ml) and V_{L_f} the final volume of the lungs (ml). V_{L_i} and V_{L_f} were determined by weighing the lungs in air and saline and assuming lung density to be 1.06 g/ml.

Three additional series of experiments were performed to determine the best procedure for degassing lungs in a vacuum chamber. First, the effect of the vacuum pressure (Pvac) on the efficiency of degassing lungs was examined. Lungs were inflated-deflated for either two or five cycles to trap gas. Then they were degassed in a vacuum chamber two times with Pvac held at values ranging between 70 and 20 Torr (the vapor pressure of H_2O). The amount of gas removed as the lungs were vacuum degassed at a given pressure was determined. Second, the number of times the lungs should be degassed was examined. In these experiments, the lungs were degassed three consecutive times with Pvac equal to the vapor pressure of H_2O. The amount of gas removed each of the three times the lungs were degassed was measured with the capacitance spirometer. The third study was performed to determine if the orientation (hanging vertically or lying horizontally) of the lungs affected the amount of gas that could be removed from the lungs when they were vacuum degassed. Lungs were degassed twice as they hung vertically in a chamber with Pvac equal to the vapor pressure of H_2O. The amount of gas removed from these lungs was compared with the amount of gas removed from lungs degassed under identical conditions but with the lungs lying horizontally. The amount of gas removed from the lungs lying on their sides was determined using the H_2O displacement method.

RESULTS

When lungs that had been degassed using the O_2 absorption method were degassed twice in a vacuum chamber, no change in lung volume could be detected as shown in Fig. 3*A*. That is, the amount of gas trapped in the lungs was not sufficient to produce detectable lung volume changes. The changes that occurred in the vacuum pressure (Pvac) during degassing are shown in Fig. 3*C*.

After the two initial degassings, the lungs were inflated-deflated for either two or five cycles. The trapped gas volume averaged 31% TLC after two PL-VL cycles and 55% TLC after five cycles. A typical example of a five-cycle PL-VL curve is shown in Fig. 4*A*. At the same time the PL-VL curves were recorded, the outputs from the minispirometer and the capacitance spirometer were plotted on another *X-Y* recorder to obtain the calibration factor for the capacitance spirometer. A typical calibration curve is shown in Fig. 4*B*. The linear relationship illustrates that the volume measured by each spirometer was very nearly identical. The output of the minispirometer is linear over the volume range used in these studies. The capacitance spirometer was also linear over the same volume range. This was not an expected finding because the capacitance and volume of the lung are presumably not linearly related. However, the geometry involved in our system, i.e., size of screen and lung volume changes, resulted in only small, if any, nonlinearities.

A typical curve showing lung volume changes measured with the capacitance spirometer and Pvac as the lungs containing trapped gas were degassed two consecutive times is shown in Fig. 3, *B* and *C*. It can be seen that lung volume increased significantly as the vacuum was applied, then decreased rapidly as the vacuum was released. During the second degassing, lung volume increased a comparable amount even though the amount of gas trapped was only 5% of that trapped before the first degassing. The quantity of gas removed from the lung was much greater during the first degassing than during the second degassing.

The amount of gas trapped in the lungs was determined using the H_2O displacement (weight) method and was compared to the results obtained using the capacitance spirometer. The weight method showed 99 ± 1.2% (mean ± SE) removal while the capacitance method showed 97 ± 2.3% removal for a series of six lungs. There

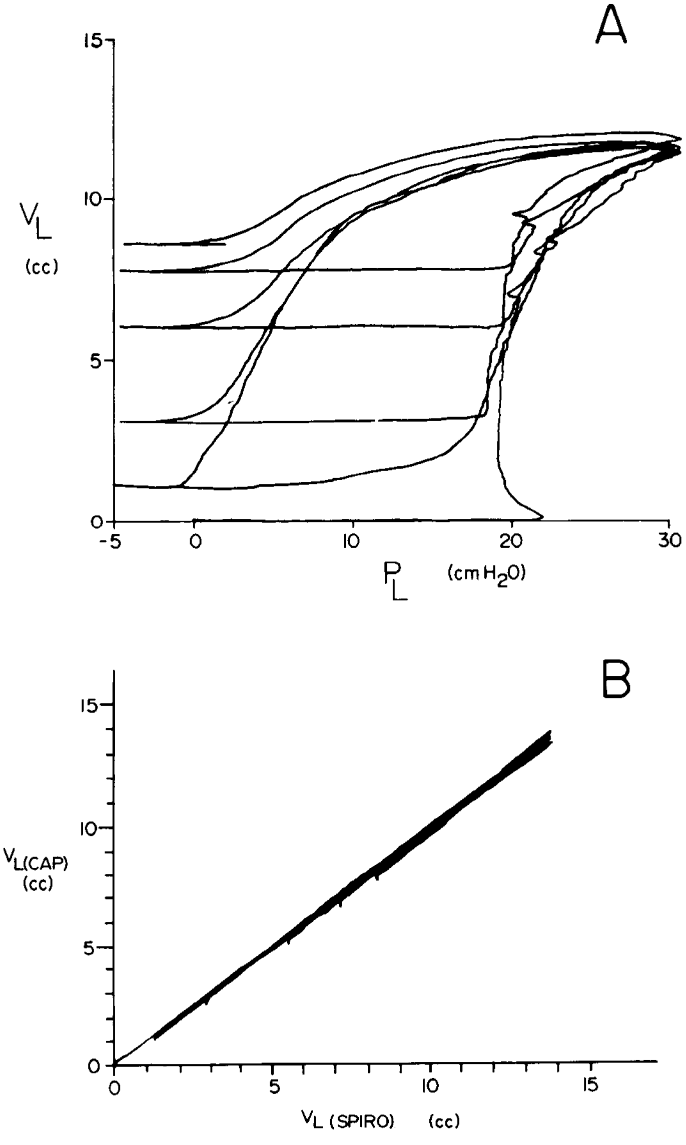

FIG. 4. *A*: typical 5-cycle pressure-volume curve. Inflation-deflation rate was 3.28 ml/min. *B*: plot of output of capacitance spirometer vs. output of minispirometer during 5-cycle pressure-volume curve shown in *A*. Line of identity (*B*) is linear and shows very little drift indicating stability of capacitance spirometer with time.

Downloaded from journals.physiology.org/journal/jappl at CDC Information Center (158.111.236.068) on August 24, 2020.

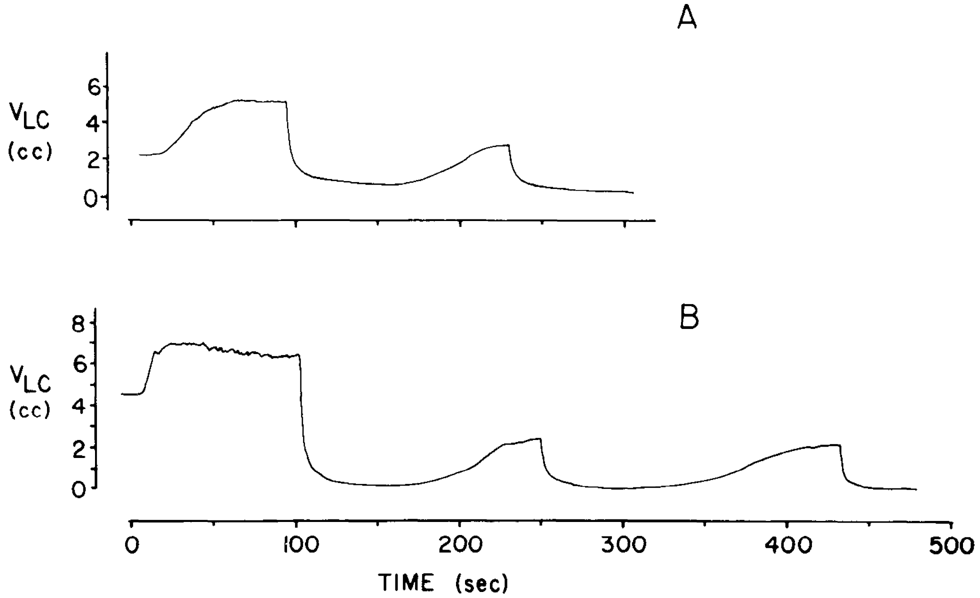

FIG. 5. *A*: typical example of lung volume changes during two vacuum degassings when Pvac = 70 Torr. *B*: typical example of lung volume changes when lung is degassed 3 times with Pvac = 20 Torr. Time course of applying and releasing the vacuum was equivalent to that shown in Fig. 3*C*.

was no significant difference between the two results showing that the capacitance spirometer was both sensitive and stable enough to adequately determine the trapped gas volume.

A typical example of lung volume changes during two sucessive degassings with Pvac equal to 70 Torr is shown in Fig. 5*A*. Fig. 5*B* shows an example of a lung degassed three times with Pvac equal to 20 Torr (H_2O vapor pressure). The vacuum was applied and released over a similar time period as shown in Fig. 3*C*.

The amount of gas removed from the lungs when they were degassed in a vacuum chamber was a function of Pvac as shown in Fig. 6. The percentage of trapped gas removed from the lungs decreased from 97 to 82% as Pvac increased from 20 to 70 Torr. In each case the lungs were degassed twice with the crosshatched area representing the amount of gas removed with the second degassing. When Pvac was 20 Torr and the lungs were degassed three times, the percentage of gas removed, as measured by the capacitance spirometer, is shown in Fig. 7. Approximately 95% of the gas was removed during the first degassing whereas only an additional 1% was removed during the third degassing.

Six experiments were performed where the position of the lungs was changed from vertical (hanging via the trachea) to horizontal (lying on their side). There was no significant difference in the results with approximately 99% of the gas removed in each case.

DISCUSSION

When lungs containing trapped gas are degassed in a vacuum chamber several events occur between the time the vacuum is applied and the time the vacuum has been fully released. Consider Fig. 3, *B* and *C*, showing typical lung volume and chamber pressure changes (Pvac) as the lungs are being degassed. During phase I when the vacuum was initially applied, lung volume increased. The decrease in pressure surrounding the trapped air spaces caused them to expand; and as these spaces increased in size, lung volume increased accordingly. Most of the air removed from the lungs during the degassing procedure probably leaves during phase I. This can be shown by considering Boyle's law in terms of the trapped air space volume. When a vacuum is applied to reduce the chamber pressure to 20 Torr, an infinitely compliant trapped air space would expand to more than 35 times its original volume. If the trapped air spaces had the same compliances as the lungs themselves, they would increase in volume by approximately 16 times. Since the lungs only expand a few milliliters, most of the air must leave the lungs during the initial portion of phase I. During phase II lung volume is elevated and small volume oscillations are observed. Average lung volume appears to decrease slightly with time in most cases. This small decrease represents less than 0.1% of the total gas removed. Visual examination of the lung surface during phase II suggests

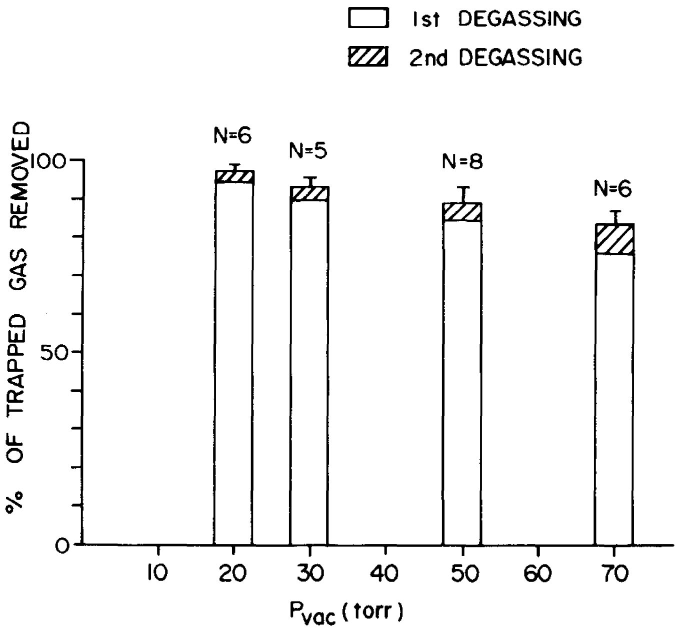

FIG. 6. Percent of trapped gas removed is decreased as minimum pressure (Pvac) during vacuum degassing is increased from 20 to 70 Torr. *Open bars*, percent removed with first degassing; *crosshatched bars*, percent removed with second degassing. SEMs are also shown.

Downloaded from journals.physiology.org/journal/jappl at CDC Information Center (158.111.236.068) on August 24, 2020.

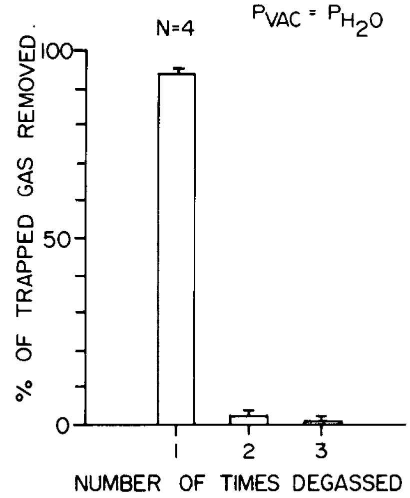

FIG. 7. Percent of gas removed as lung was degassed 3 times. *Bars*, mean ± SE. Approximately 98% of gas is removed after two degassings if Pvac = P_{H_2O}.

that some foam or bubbles may be breaking. These oscillations are much reduced in magnitude and frequency for the second and subsequent degassings. When the vacuum is released during phase III, lung volume decreases abruptly. The decrease in lung volume below the initial level (before degassing) is equal to the quantity of gas removed from the lungs during the degassing procedure (see Figs. 3*B* and 5, *A* and *B*).

If the magnitude of the increase in lung volume during vacuum degassing is used as an index of the amount of air trapped in the lungs, the results show that the O_2 absorption method leaves the lungs essentially gas free (see Fig. 3*A*). Vacuum degassing, on the other hand, leaves a small amount of gas in the lungs. Our speculation is that a lattice of stable films are present within the lung periphery which prevent the last small amount of gas from escaping.

In many studies that use excised lungs, however, it is not possible to use the O_2 absorption method, and lungs must be vacuum degassed. To obtain the most complete degassing of the lungs the vacuum should be reduced to the vaporization pressure of H_2O for the maximum removal of trapped gas. Approximately 98% of the gas will be removed with two vacuum degassings if the pressure is reduced to the vaporization pressure as shown in Figs. 6 and 7. During the initial cycle 95% of the trapped gas is removed from the lungs and when the procedure is repeated an additional 3% of the gas was removed. When lungs were degassed a third time, only an additional 1% was removed from the lungs. The number of degassings required will depend on the effects, if any, of the remaining gas on the succeeding experiments an investigator might want to do.

Finally, the orientation of the lungs, hanging vertically or lying horizontally, does not alter the amount of gas removed from the lungs during the degassing procedure.

Received 26 February 1979; accepted in final form 7 September 1979.

REFERENCES

1. BARROW, R. E., AND F. J. COLGAN. A noninvasive method for measuring newborn respiration. *Respir. Care* 18: 412-414, 1973.
2. BARROW, R. E., A. J. VORWALD, AND E. DOMEIER. Capacitance respirometry. *Arch. Environ. Health* 19: 579-585, 1969.
3. BARROW, R. E., A. J. VORWALD, AND E. DOMEIER. The measurement of small animal respiratory volumes by capacitance respirometry. *Am. Ind. Hyg. Assoc. J.* 32: 593-598, 1971.
4. CLEMENTS, J. A., R. F. HUSTEAD, R. P. JOHNSON, AND I. GRIBETZ. Pulmonary surface tension and alveolar stability. *J. Appl. Physiol.* 16: 444-450, 1961.
5. COLGAN, F. J., J. Q. LIANG, AND R. E. BARROW. Noninvasive assessment by capacitance respirometry of respiration before and after extubation. *Anesth. Analg. Cleveland* 54: 807-813, 1975.
6. DALE, W. A., AND H. RAHN. Rate of gas absorption during atelectasis. *Am. J. Physiol.* 170: 606-615, 1952.
7. EDMUNDS, L. H., AND G. L. HUBER. Pulmonary artery occlusion. I. Volume-pressure relationships and alveolar bubble stability. *J. Appl. Physiol.* 22: 990-1001, 1967.
8. FARIDY, E. E. Effect of hydration and dehydration on elastic behavior of excised dogs' lungs. *J. Appl. Physiol.* 34: 597-605, 1973.
9. FARIDY, E. E., AND S. PERMUTT. Surface forces and airway obstruction. *J. Appl. Physiol.* 30: 319-321, 1971.
10. FISHER, M. J., M. F. WILSON, AND K. C. WEBER. Determination of alveolar surface area and tension from *in situ* pressure-volume data. *Respir. Physiol.* 10: 159-171, 1970.
11. FRAZER, D. G., P. W. STENGEL, AND K. C. WEBER. Meniscus formation in airways of excised rat lungs. *Respir. Physiol.* 36: 121-129, 1979.
12. FRAZER, D. G., AND K. C. WEBER. Trapped air in ventilated excised rat lungs. *J. Appl. Physiol* 40: 915-922, 1976.
13. KERFOOT, E., AND E. DOMEIER. Pulmonary function measurements of large animals using the capacitance respirometer. *Lab. Anim. Sci.* 22: 854-859, 1972.
14. KLEINMAN, L. I., D. A. POULOS, AND A. A. SIEBENS. Minimal air in dogs. *J. Appl. Physiol.* 19: 204-206, 1964.
15. LEVINE, B. E., AND R. F. JOHNSON. Surface activity of saline extracts from inflated and degassed normal lungs. *J. Appl. Physiol.* 19: 333-335, 1964.
16. MEAD, J., AND C. COLLIER. Relation of volume history of lungs to respiratory mechanics in anesthetized dogs. *J. Appl. Physiol.* 14: 669-678, 1959.
17. PATTLE, R. E. Properties, function, and origin of the alveolar lining layer. *Proc. R. Soc. London Ser. B* 148: 217-240, 1958.
18. PATTLE, R. E. Surface lining of lung alveoli. *Physiol. Rev.* 45: 48-79, 1965.
19. RADFORD, E. P., JR. Static mechanical properties of mammalian lungs. In: *Handbook of Physiology. Respiration.* Washington, DC: Am. Physiol. Soc., 1964, sect. 3, vol. I, chapt. 15, p. 429-449.
20. STEMMLER, E. J., AND A. B. DUBOIS. Pulmonary tissue and surface elastic forces at low lung volumes in rabbits. *J. Appl. Physiol.* 25: 473-478, 1968.
21. TIERNEY, D. F. Pulmonary surfactant in health and disease. *Dis. Chest* 47: 247-253, 1965.

Downloaded from journals.physiology.org/journal/jappl at CDC Information Center (158.111.236.068) on August 24, 2020.