Respiration Physiology (1979) **36**, 121–129

MENISCUS FORMATION IN AIRWAYS OF EXCISED RAT LUNGS

D. G. FRAZER, P. W. STENGEL and K. C. WEBER

Appalachian Laboratory for Occupational Safety and Health, National Institute for Occupational Safety and Health, Center for Disease Control, U.S. Public Health Service, and Department of Physiology and Biophysics, West Virginia University Medical Center, Morgantown, WV 26506, U.S.A.

Abstract. The object of this study was to determine the transpulmonary pressure at which menisci form in the airways of excised rat lungs. The necessary geometric requirements for meniscus formation are normally met in the airways, but a meniscus is presumably prevented from forming at large lung volumes because too little fluid is present. At lower lung volumes a meniscus could form easier since airway caliber is reduced and less fluid is required. A series of pressure–volume curves were recorded for lungs in which meniscus formation was inhibited by increasing the end expiratory pressure. Assuming the number of airways containing at least one meniscus was proportional to the amount of gas trapped in the excised rat lung, it was found that the menisci were formed in 68% of the airways between positive transpulmonary pressures of 1.4 and 3.0 cm H_2O during deflation.

Airway closure — Foam formation in lungs
Excised rat lungs — Lung pressure volume curves

It has been shown that air is trapped in excised lungs as the lungs are slowly ventilated (Hughes and Rosenweig, 1970; Faridy and Permutt, 1971; Frazer and Weber, 1976). Since a foam has been observed exuding from the trachea and has been found in the airways, many investigators (Pattle, 1958; Radford, 1964; Faridy and Permutt, 1971; Frazer and Weber, 1976; Sergysels *et al.*, 1977) have suggested that the foam is involved in trapping gas in the lungs. Recently, Frazer and Weber (1976) have shown that most of the gas is trapped in the lung during inflation since the quantity of trapped gas depends primarily upon inflation rate and is nearly independent of the deflation rate. Additional experiments (Frazer *et al.*, 1978) have shown that the majority of trapped gas enters the trapped air spaces by diffusion across a liquid film.

Accepted for publication 4 October 1978

Based on the evidence thus far, one explanation of the air trapping mechanism is that menisci described by Macklem *et al.* (1969) are formed across the airways at low lung volumes as the lungs are deflated. These menisci then form very small trapped air spaces within the lungs. The final volume of these trapped air spaces, however, is predominately determined by the amount of gas that diffuses across the meniscus walls into the trapped air spaces during lung inflation. If this explanation of the air trapping mechanism is correct, it would be necessary for menisci to form in the airways during lung deflation for air to be trapped in the lungs.

The conditions required for meniscus formation in the lungs can be analyzed theoretically using an analysis similar to that proposed by Plateau (1869) for describing the formation of emulsions. A modification of that analysis shows that the fluid in a cylinder such as an airway is in an unstable equilibrium condition when airway length (L) exceeds airway circumference ($2\pi R$). In other words when $L > 2\pi R$, an infinitely small geometric disturbance to the airway wall would cause a meniscus to form across the airway. It should be noted that the necessary conditions for meniscus formation do not depend upon the surface tension of the gas–liquid interface, although the rate at which the meniscus would form is related to the physical properties of the fluid.

A second condition required for the formation of a meniscus is that a sufficient volume of fluid must be present in the airway. Normally there may not be enough fluid in the airway at high lung volumes; but as the lung deflates, the quantity of fluid necessary to form a meniscus decreases as airway diameter decreases. Hughes *et al.* (1966), Hyatt and Flath (1966), and Sittipong and Hyatt (1974) have shown that in dog lungs airway diameter changes the greatest when the transpulmonary pressure (P_L) is less than 10 cm H_2O. It would be expected, therefore, that menisci would be more likely to form in the airways when P_L has fallen below 10 cm H_2O.

If the formation of menisci in airways is a necessary requirement for trapping gas in lungs, it follows then that inhibiting meniscus formation should result in a reduction of trapped gas. In this study lungs were ventilated at a rate that would normally trap gas, but a high positive end expiratory pressure (EEP) was used in an attempt to keep the airways patent and hinder meniscus formation. When the EEP was reduced to the point at which menisci start to form across the airways, the amount of air trapped should correspondingly increase. Lowering the positive EEP still more should enable menisci to form in additional airways and result, therefore, in a greater amount of air trapped per cycle. When the EEP is reduced to the point where all airways in which menisci are capable of forming have formed, a further reduction in the EEP should not affect the amount of air trapped per cycle.

This method of evaluating meniscus formation is based on the assumption that the amount of gas trapped in the lung is proportional to the number of airways containing at least one meniscus. By making this assumption and experimentally determining how the trapped air was affected by a given EEP, it was possible to determine the fraction of airways in which menisci had formed as a function of P_L during lung deflation.

Methods

Long Evans Hooded male rats weighing between 250 and 300 g were exsanguinated via the abdominal aorta following intraperitoneal pentobarbital injection (85 mg/kg). A bilateral pneumothorax was achieved by opening the abdominal cavity and penetrating the diaphragm. Next, the rib cage was sectioned on both sides of the midline, and the heart, lungs and diaphragm were removed *en bloc*. The lungs were then degassed in a vacuum chamber and placed in the plethysmograph shown in fig. 1. Lungs were ventilated with a positive pressure generated with the syringe type pump (Harvard Apparatus, model #901). Transpulmonary pressure was detected with a capacitance type transducer (Setra, model #233), and lung volume was measured with a minispirometer (Med Sci, model #118). Both pressure and volume were recorded on the X–Y recorder (Houston, model #2000). A constant temperature water bath was maintained at 28 °C (slightly above room temperature) with a temperature controller (Thermomix #1440) to provide temperature stability. The lung was allowed to equilibrate in the plethysmograph until there was less than a 0.1 cc change in the volume of gas surrounding the lungs over two 3-min periods as measured by the spirometer. Equilibrium was reached exponentially requiring a total time of approximately 20 min. Air entering the lungs from the syringe pump was preheated to 28 °C as it passed through a copper heat exchanger in the water bath prior to entering the trachea.

In this study a cycle was defined as a single inflation and deflation of the lung while four continuous cycles constituted one pressure–volume (P_L–V_L) curve. During

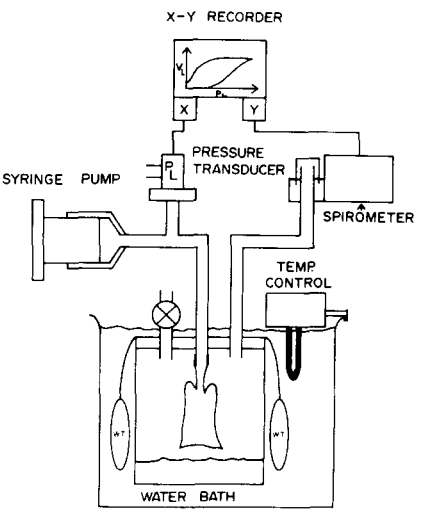

Fig. 1. Schematic diagram of the equipment used to record P_L–V_L curves of excised rat lungs. The plethysmograph was heated to 28 °C to increase the temperature stability of the system. Air entering the lungs was pre-heated to 28 °C by passing it through a heat exchanger which was also located in the water bath.

all experiments one of two different sequences of PL–VL curves was recorded for each lung. Both sequences consisted of 6 individual PL–VL curves. The initial cycle in the first sequence (seq. 1) consisted of inflating a lung from 0.0 cm H_2O to 30 cm H_2O (PL(max)) then deflating the lung to a predetermined minimum transpulmonary pressure (PL(min)). In cycles 2 and 3 the lungs were inflated from PL(min) to PL(max) and deflated from PL(max) to PL(min). On the fourth cycle the lungs were inflated from PL(min) to PL(max) then deflated from PL(max) to −5 cm H_2O. PL(min) for cycles 1, 2, 3 and 4 in curve 1 was +6 cm H_2O. PL(max) remained the same, but PL(min) was reduced for curves 2 through 6 to +4, +3, +2, 0, and −5 cm H_2O respectively.

The second sequence (seq. 2), like seq. 1, was comprised of 6 PL–VL curves. Each curve consisted of 4 inflation–deflation cycles and was recorded between the same PL(min) and PL(max) as in seq. 1. In seq. 2, however, the order in which the curves were recorded was reversed so that PL(min) was increased in order from −5 cm H_2O for curve 1 to +6 cm H_2O for curve 6.

In both seq. 1 and seq. 2 the lungs were inflated-deflated at a constant rate of 3.82 cc/min. It has been shown that when lungs are ventilated at that rate between PL equal to −5 and 30 cm H_2O for 4 cycles, they trap approximately 50% of the total lung volume (Frazer and Weber, 1976).

Results

Six typical PL–VL curves recorded for one lung during seq. 1 are shown in fig. 2. This figure illustrates that the volume of gas trapped in the lung increased as PL(min) was progressively decreased from +6 to −5 cm H_2O. A normalized index was used

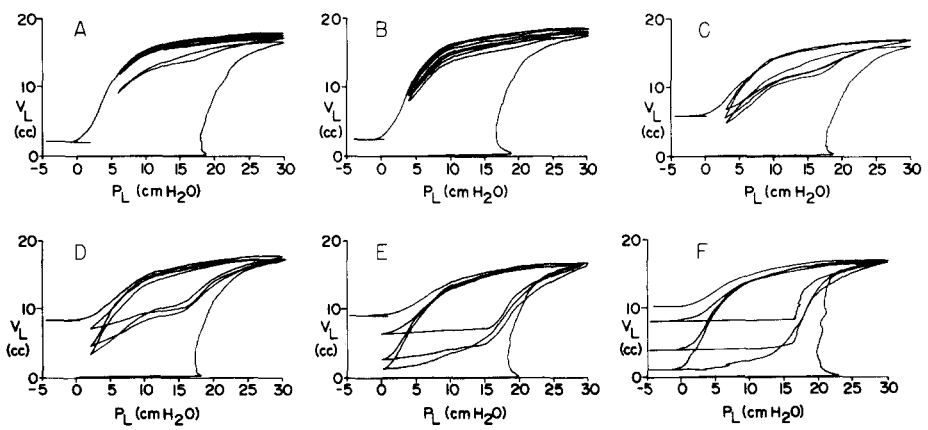

Fig. 2. Six pressure–volume curves for a typical rat lung ventilated at 3.82 cc/min showing how the amount of trapped air increased following 4 inflation–deflation cycles as the end expiratory pressure, PL(min), was progressively reduced from (A) +6 cm H_2O, to: (B), +4 cm H_2O; (C), +3 cm H_2O; (D), +2 cm H_2O; (E), 0.0 cm H_2O; and (F), −5 cm H_2O.

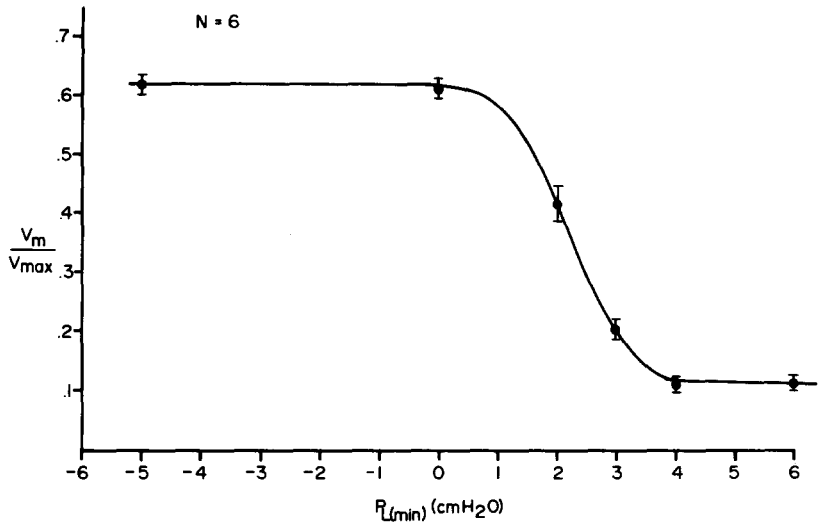

Fig. 3. The normalized amount of trapped air in the lung, Vm/Vmax, *versus* end expiratory pressure, PL(min), following 4 inflation–deflation cycles. Lungs were ventilated as shown in fig. 3 as PL(min) was decreased in order from +6, to +4, +3, +2, 0 and −5 cm H_2O. Bars represent the SEM.

to quantitate the volume of gas trapped in the lung and was obtained by dividing the volume of gas trapped after each PL–VL curve (Vm), measured at −5 cm H_2O, by the maximum lung volume (Vmax) during the fourth cycle. When Vm/Vmax was plotted versus PL(min) for 6 lungs, the curve in fig. 3 was obtained. This figure shows that Vm/Vmax increased in a nonlinear fashion as PL(min) decreased.

Results of plotting Vm/Vmax *versus* PL(min) following seq. 2 are given in fig. 4.

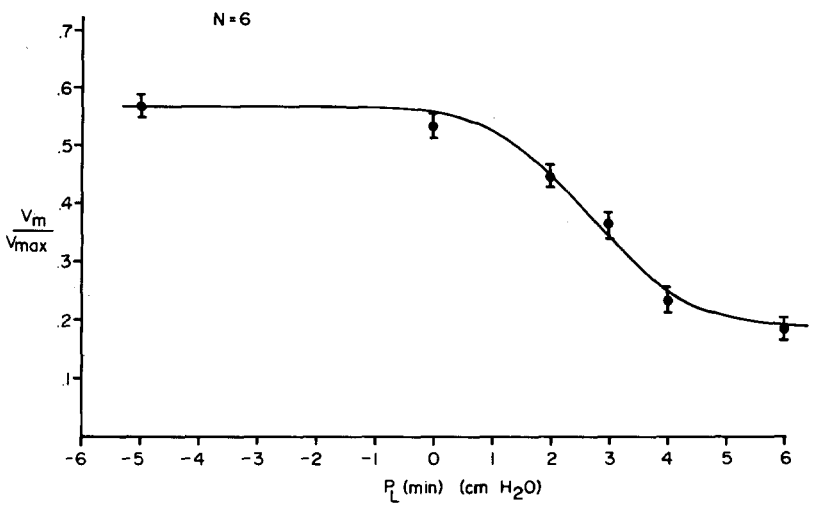

Fig. 4. The normalized amount of trapped air in the lungs, Vm/Vmax, *versus* end expiratory pressure, PL(min), as PL(min) was increased in order from −5 to 0, +2, +3, +4, and +6 cm H_2O. Bars represent the SEM.

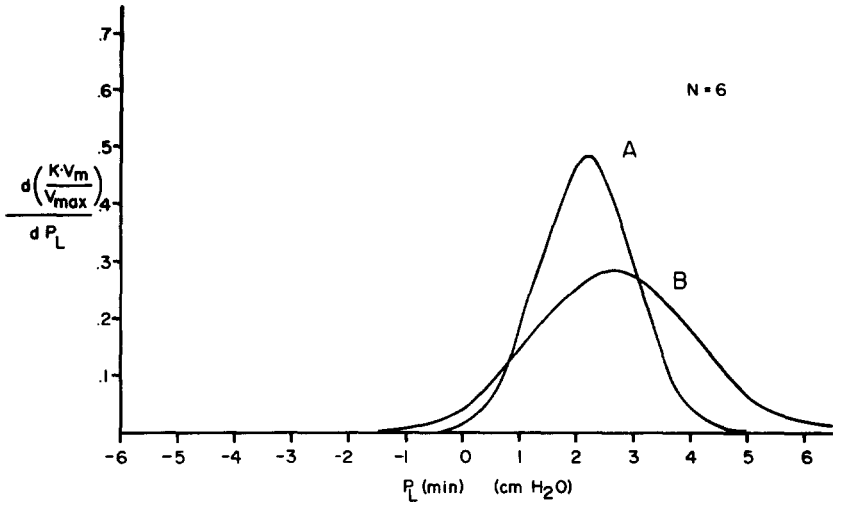

Fig. 5. Curve A is proportional to the slope of the curve in fig. 3 and represents the distribution of menisci formed in the airways as PL(min) was decreased in order from +6 to −5 cm H_2O. Curve B represents the distribution of menisci formed as PL(min) was increased from −5 to +6 cm H_2O, as shown in fig. 4.

The lung appeared to trap air at slightly higher values of PL in seq. 2. The continuous curves drawn through the points in figs. 3 and 4 represent the best least square fit of the integral of the normal curve from plus infinity to PL(min) as PL(min) approached minus infinity. If it is assumed that the amount of air trapped in the lungs is directly proportional to the fraction of airways having at least one meniscus, each value of Vm/Vmax in figs. 3 and 4 reflects the relative number of airways in which menisci have formed as the lungs were deflated from PL(max) to PL(min). The change in Vm/Vmax with respect to PL(min), or the respective slope of the curves in figs. 3 and 4, is proportional in each case to the number of airways in which menisci have formed at a given value of PL. When the slopes of both curves, which are represented by normal distributions, were plotted for seqs. 1 and 2, the results in fig. 5 were obtained. These curves indicate that during seq. 1 approximately 68% of the airways had at least one meniscus formed between a transpulmonary pressure of 3.0 and 1.4 cm H_2O (SD = 0.8), and half the airways had at least one meniscus formed during deflation prior to reaching a transpulmonary pressure of 2.2 cm H_2O. When the order of recording PL–VL curves was reversed in seq. 2, however, menisci were formed at higher values of PL; and approximately 68% of the airways had at least one meniscus formed between 4.1 and 1.3 cm H_2O (SD = 1.4), and a meniscus was formed in half the airways before reaching 2.7 cm H_2O.

Discussion

Although the rate of deflation does not seem to have an effect on the amount of trapped air, as shown by Frazer and Weber (1976), the value of PL(min) or the end expiratory pressure (EEP) during deflation does have a great effect. Our explanation of the air trapping process is that although trapped gas is increased and dependent upon lung inflation, it increases only if menisci are formed across the airways during deflation. Ventilating lungs with a positive EEP keeps airway dimensions large during deflation so that fewer menisci can develop across the airways. Reducing the number of airways having menisci formed across them with a positive EEP during deflation in turn reduces the amount of trapped gas generated during the next inflation. The net result is that less air is trapped in a lung ventilated with a positive EEP than with a zero or negative EEP.

The order in which the EEP was changed for a given lung was shown to affect the transpulmonary pressures at which the menisci form (figs. 3 and 4). As shown in fig. 5 when PL(min) changed from -5 cm H_2O to $+6$ cm H_2O (seq. 2), the menisci formed at higher PL(min). There are at least two possible explanations for these findings. The first possibility is that surfactant enhances meniscus formation in the airways allowing menisci to form sooner. There is evidence that surfactant is necessary to form foam in the lung (Faridy and Permutt, 1971), and it could be possible that when ventilating the lung first with negative or little positive EEP, as in seq. 2, more surfactant is transported up the airway than during seq. 1 (Faridy, 1976). This could result in more air being trapped at a given value of PL(min) in seq. 2 than in seq. 1. Secondly, the point at which menisci form in the lung is very dependent upon the amount of fluid in the airway, and it is possible that there was a slight shift in fluid between compartments in the excised lung as the lung was ventilated. Webb and Tierney (1974) have shown these shifts to occur in *in vivo* rat lungs. The fluid could either reduce the diameter of the airway by accumulating in the peribronchial space or could enter the lumen of the airway enabling menisci to form sooner during lung deflation.

Our results show that gas trapping occurs at positive transpulmonary pressures in agreement with Glaister *et al.* (1973). The experiments of Cavagna *et al.* (1967) and Hughes *et al.* (1970) showed that airways do not physically collapse until the transpulmonary pressure is negative (Macklem, 1971). Thus, it is likely that gas trapping is related to separately occuring events as lungs are deflated. These two events are the formation of a meniscus across the airway at positive values of PL followed by the mechanical buckling of the airway wall at negative values of PL. The first process, which we have studied, is a function of the equilibrium of surface forces associated with the film normally lining the airway walls. The point in the airway and the pressure at which the meniscus forms are both likely related to airway dimensions and the physical properties of the liquid lining layer itself. Once formed the meniscus would present a diffusional resistance for gases. The magnitude of the diffusional resistance created by the liquid across the airway would be

dependent upon the distance between the two air liquid surfaces. An interesting property of a meniscus is that it probably does not remain stationary at its point of origin but is capable of moving along the airway in response to pressure differences associated with bulk flow (Macklem *et al.*, 1969).

The formation and movement of menisci during lung deflation could be capable of transporting the surface active airway lining layer and alveolar surfactant from the small airways toward the trachea. The movement of surfactant from the lung periphery in response to ventilation has been hypothesized to occur in rats *in vivo* by Webb and Tierney (1974) and has been measured in open chested animals by Faridy (1976). These same investigators also found that a positive value of EEP was effective in preventing surfactant from leaving the periphery and escaping via the airways. If menisci are responsible for the reported transport of surfactant, a positive EEP could reduce menisci formation and explain the effectiveness of a high positive EEP in reducing the movement or removal of surface active material from the periphery of the lungs.

Acknowledgements

The authors thank N. Nehrig, M. Peterson, F. Rothlisberger, J. Riester, and B. Popovich for their excellent technical assistance.

This study was presented in part at the Fall APS Meetings in Philadelphia, Pennsylvania, August 15–20, 1976.

Brand Names are used for information only and do not constitute endorsement by the National Institute for Occupational Safety and Health.

References

Cavagna, G.E., E.J. Stemmler and A.B. DuBois (1967). Alveolar resistance to atelectasis. *J. Appl. Physiol.* 22: 441–452.

Faridy, E.E. and S. Permutt (1971). Surface forces and airway obstruction. *J. Appl. Physiol.* 30: 319–321.

Faridy, E.E. (1976). Movement of surfactant in airways. *Respir. Physiol.* 27: 323–334.

Frazer, D.G. and K.C. Weber (1976). Trapped air in ventilated excised rat lungs. *J. Appl. Physiol.* 40: 915–922.

Frazer, D.G., F. Rothlisberger and K.C. Weber (1978). Effects of gas diffusion on gas trapping in excised rat lungs. *Fed. Proc.* 37: 3640.

Glaister, D.H., R.C. Schroter, M.F. Sudlow and J. Milic-Emili (1973). Transpulmonary pressure gradient and ventilation distribution in excised lungs. *Respir. Physiol.* 17: 365–385.

Hughes, J.M.B., F.G. Hoppin, Jr. and J. Mead (1966). Effect of lung inflation on bronchial length and diameter in excised lungs. *J. Appl. Physiol.* 32: 25–35.

Hughes, J.M.B. and D.Y. Rosenzweig (1970). Factors affecting trapped gas volume in perfused dog lungs. *J. Appl. Physiol.* 29: 332–339.

Hughes, J.M.B., D.Y. Rosenzweig and P.B. Kivitz (1970). Site of airway closure in excised dog lungs: histologic demonstration. *J. Appl. Physiol.* 29: 340–344.

Hyatt, R. E. and R. E. Flath (1966). Influence of lung parenchyma on pressure-diameter behavior of dog bronchi. *J. Appl. Physiol.* 21: 1448–1452.

Macklem, P. T., D. F. Proctor and J. C. Hogg (1969). The stability of peripheral airways. *Respir. Physiol.* 8: 191–203.

Macklem, P. T. (1971). Airway obstruction and collateral ventilation. *Physiol. Rev.* 51: 368–436.

Pattle, R. E. (1958). Properties, function and origin of the alveolar lining layer. *Proc. Roy. Soc. London Series B.* 240: 148–149.

Plateau, J. (1869). Mem. Acad. Roy. Sci. Belgique 37, 11th ser. as found in Physical Surfaces, J. H. Bikerman. New York, Academic Press, 1970, pp. 141–143.

Radford, E. P. (1964). Static mechanical properties of mammalian lungs. In: Handbook of Physiology. Section 3. Respiration. Vol. 1, edited by W. O. Fenn and H. Rahn. Washington, D. C., American Physiological Society, p. 433.

Sergysels, R., R. Amyot, P. T. Macklem and R. R. Martin (1977). *In vivo* gas trapping induced by nitrous oxide. *J. Appl. Physiol.* 43: 414–420.

Sittipong, R. and R. E. Hyatt (1974). Static mechanical behavior of bronchi in excised dog lung. *J. Appl. Physiol.* 31: 201–206.

Webb, H. H. and D. F. Tierney (1974). Experimental pulmonary edema due to intermittent positive pressure ventilation with high inflation pressures. Protection by positive end-expiratory pressure. *Am. Rev. Respir. Dis.* 110: 556–565.