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# THE EFFECT OF PULMONARY EDEMA ON GAS TRAPPING IN EXCISED RAT LUNGS

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**Abstract.** The object of this study was to determine how pulmonary edema affects the volume of gas trapped in excised lungs as they are slowly ventilated. In this study trapped gas is defined as the volume of gas that cannot be removed from the lungs with a negative transpulmonary pressure of $-5$ cm $H_2O$ (Frazer and Weber, 1976). Experimental pulmonary edema was produced by ventilating rats using high inspiratory positive pressure breathing (HIPPB) as described by Webb and Tierney (1974). The amount of gas trapped in both edematous and control lungs was then compared as the lungs were inflated-deflated under identical conditions in 6 different 4 cycle sequences. During each sequence the lungs were deflated to an end expiratory pressure of either $+6$, $+4$, $+3$, $+2$, 0.0, or $-5$ cm $H_2O$. It was found that gas became trapped at more positive values of end expiratory pressure in lungs having pulmonary edema than in control lungs. These results were interpreted as evidence that the airways close sooner, at more positive transpulmonary pressures, in edematous lungs than in control lungs.

Airway closure
Excised rat lungs
Foam in lungs
Lung pressure–volume curves
Meniscus formation in airways

In a recent literature review Staub (1974) noted that lung function tests, including various measurements of lung volumes, have had limited success in detecting pulmonary edema. Most investigators have not found consistent changes in lung compliance that could be related to an increase in measured lung water content (Guyton and Lindsey, 1959; Levine *et al.*, 1965; Staub *et al.*, 1967). There are studies using closing volume measurements which report, however, that airways close sooner at low lung volumes as interstitial fluid accumulates around the airway (Laws *et al.*, 1975). Premature airway closure presumably occurs because the blood

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vessels and airways are crowded during the initial stages of lung edema as the interstitial connective tissue compartment becomes filled with fluid (Hughes *et al.*, 1970; Staub *et al.*, 1967). Other evidence of the effect of edema on small airways has been presented by Hoss *et al.* (1972). These investigators measured the distribution of airway resistance with developing pulmonary edema and found an initial increase in the peripheral resistance of the lung which coincided with the competition for space of the arteries and small airways in the peribronchial sheath.

Recently, still another method has been described for detecting airway closure in excised rat lungs (Frazer *et al.*, 1979a). This method is based on the assumption that, as airways close, menisci are formed across their lumens. The menisci, in turn, are thought to be responsible for trapping the gas in the lungs during subsequent lung inflation (Frazer and Weber, 1976). If this is true, it should be possible to predict the relative number of airways that closed during lung deflation by examining the amount of gas trapped in the lungs during the following inflation–deflation cycle. The transpulmonary pressure at which airways begin to close can be determined by initially ventilating the lungs at high end expiratory pressures (above 6 cm $H_2O$) so that the airways are prevented from closing. In this case, no air is trapped in the lungs. The end expiratory pressure (EEP) can then be reduced in steps until gas begins to become trapped in the lungs. The transpulmonary pressure at which gas starts to become trapped can be interpreted as the pressure at which airways start to close in the lungs. Airway closure is a process that is likely related to airway diameter, the quantity of fluid in and around the airway wall, and the physical properties of the liquid lining the airway lumen. There is evidence that all these properties are altered by pulmonary edema in a manner that would cause premature airway closure.

The first objective of this investigation was to detect the effect of pulmonary edema on gas trapping in lungs ventilated at several end expiratory pressures. Then the amount of gas trapped at each EEP was used to determine how edema affects airway closure. The second objective of this study was to simplify the gas trapping method so that it could be used to more easily determine if a given lung has edema.

## Methods

A comparison was made between the minimum transpulmonary pressure and the amount of gas trapped in normally ventilated excised rat lungs ($n = 6$) and rat lungs having experimentally induced pulmonary edema ($n = 6$). Both groups were composed of Long Evans hooded male rats weighing between 250 and 300 g. The rats were anesthetised with pentabarbitol (85 mg/kg) injected intraperitoneally. In the experimental group perivascular edema was produced with HIPPB as described by Webb and Tierney (1974). Following a tracheotomy, the lungs were inflated to a positive pressure of +30 cm $H_2O$ and deflated to 0.0 cm $H_2O$ at a rate of

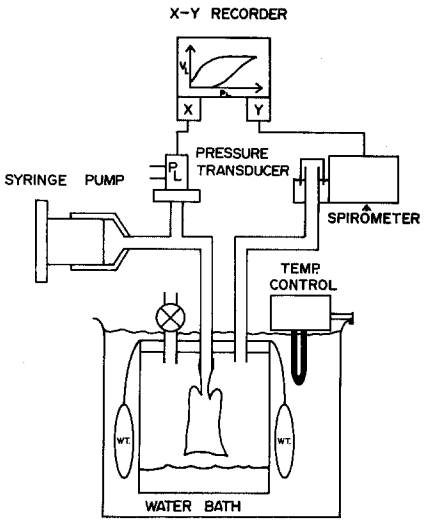


Fig. 1. Schematic diagram of the equipment used to record PL–VL curves of excised rat lungs. The plethysmograph was heated to 28 °C to increase its temperature stability. Air entering the lungs was pre-heated to 28 °C by passing it through a copper coil in the water bath.

30 breaths per min for 60 min. Webb and Tierney (1974) showed that lungs ventilated under these conditions have moderate perivascular edema with little alveolar edema. Once edema was produced, the edematous and normal lungs were studied under identical conditions. The lungs were removed by opening the abdominal cavity and penetrating the diaphragm to produce a bilateral pneumothorax. The rat was then exsanguinated via the abdominal aorta. 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 inflated-deflated 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 with a temperature controller (Thermomix, model 1440) to provide temperature stability. The lung was allowed to equilibrate in the plethysmograph until there was less than a 0.1 ml change in the volume of gas surrounding the lungs over two 3-min periods as measured by the spirometer. Temperature equilibrium was reached exponentially and required a total time of approximately 20 min. Air entering the lungs from the syringe pump was preheated to plethysmograph temperature as it passed through a copper heat exchanger in the water bath prior to entering the trachea.

Six pressure–volume, PL–VL, curves were recorded for both the normal and edematous lungs. Prior to recording each curve, the lungs were degassed in a vacuum chamber to assure that they always had the same volume history. Each curve

consisted of 4 inflation–deflation cycles. During the initial cycle lungs were inflated from 0.0 cm $H_2O$ to 30 cm $H_2O$ (PL (max)) then deflated to predetermined end expiratory pressures (PL (min)). In cycles 2 and 3, lungs were inflated-deflated between PL (min) and PL (max). In the fourth cycle the lungs were once again inflated from PL (min) to PL (max) then deflated back to $-5$ cm $H_2O$. PL (min) was $+6$ cm $H_2O$ for the first PL–VL curve then reduced in order to $+4$, $+3$, $+2$, 0, and $-5$ cm $H_2O$ as the second through sixth PL–VL curves were recorded. Frazer *et al.* (1979a) have shown that the order in which the curves are recorded affects the results. The order used in recording the curves in this study is the least likely to disturb the surfactant or fluid distribution in the airways (Faridy, 1976; Frazer *et al.*, 1979a).

A variable, proportional to the fraction of gas trapped in the lungs following a 4 cycle PL–VL curve, was calculated by dividing the amount of gas trapped in the lungs at $-5$ cm $H_2O$ (Vm) following the fourth inflation–deflation cycle by the maximum lung volume during the same cycle (Vmax). The variable (Vm/Vmax) was equal to the normalized minimum volume of the lungs. By recording a series of 4 cycle PL–VL curves for each lung in which PL (min) was held at different values, it is possible to determine the relationship between PL (min) and Vm/Vmax for that lung.

In order to assess qualitatively the amount of edema in the lungs, the wet/dry wt. ratio was determined for normal and edematous lungs after each experiment.

## Results

After six 4 cycle PL–VL curves were recorded for both control and edematous lungs, Vm/Vmax was calculated and plotted versus the respective value of PL (min) for each group. The results are shown in figs. 2A and 2B. The average values of Vm/Vmax $\pm$ the standard error of the mean (SEM) are given. The continuous curve drawn through the points represents the best fit of the integral of the normal curve to the data using least squares analysis. It can be seen that lungs with pulmonary edema tend to trap gas at more positive values of EEP than control lungs. The slopes of the curves in fig. 2 are represented by normal curves, and the slopes have been plotted in figs. 3A and 3B respectively. These figures show that as the EEP was reduced there was a distribution of transpulmonary pressures over which there was a change in the amount of gas trapped in the lung. The mean value and standard deviation of the distribution of transpulmonary pressures for the control lung (fig. 3) was $1.9 \pm 1.2$ cm $H_2O$, while the mean and standard deviation for the edematous lung was $3.4 \pm 1.3$ cm $H_2O$.

When the wet/dry wt. ratios were computed for both the normal and edematous lungs, it was found that these ratios were significantly different ($P > 0.01$) and were equal to $4.9 \pm 0.2$ (SEM) and $6.5 \pm 0.5$ (SEM) respectively.

The second objective of this study was to determine if the trapped gas detection

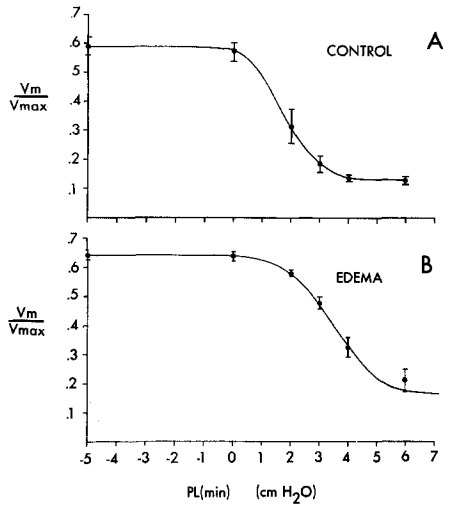


Fig. 2. The normalized minimum volume of the lung, Vm/Vmax ± SE, plotted as a function of the end expiratory pressure, PL (min), following 4 inflation–deflation cycles for: (A) normal rat lungs (n = 6), and (B) edematous rat lungs (n = 6).

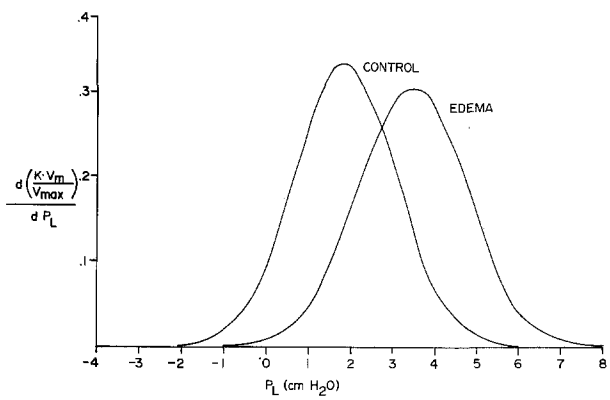


Fig. 3. Distributions showing the change in Vm/Vmax between different end expiratory pressures for both control rats and rats having pulmonary edema.

method for evaluating edema formation could be simplified. The next step, therefore, was to determine if a single 4 cycle PL–VL curve with an appropriate value of PL (min) could be used to detect edema in the lung. A series of experiments was conducted with PL (min) held at + 4, + 3, and – 5 cm $H_2O$, and the normalized minimum volumes were calculated and plotted *versus* the lungs wet/dry wt. ratio. The results are shown in fig. 4. Table 1 shows that when PL (min) was held at either + 4 or + 3 cm $H_2O$, there was a significant difference in Vm/Vmax between edematous and normal lungs. The results indicate, therefore, that recording single PL–VL curves

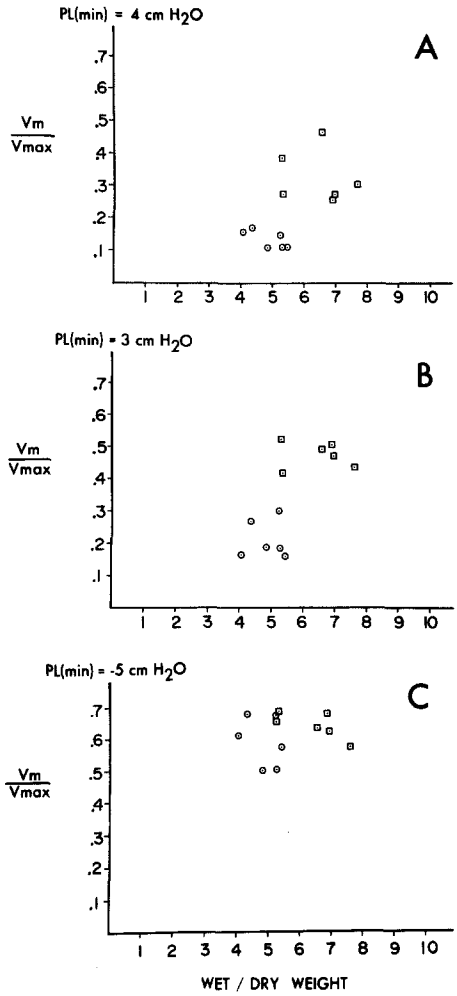


Fig. 4. The normalized minimum volume of the lung after 4 inflation–deflation cycles plotted *versus* the wet/dry wt. ratios for normal and edematous lungs is shown for different end expiratory pressures $P_L$ (min): (A) $P_L$ (min) = +4 cm $H_2O$, (B) $P_L$ (min) = +3 cm $H_2O$, and (C) $P_L$ (min) = 5 cm $H_2O$.

TABLE 1

Fraction of gas trapped (Vm/Vmax) in control and edematous lungs after 4 inflation-deflation cycles for different values of $P_L$ (min)

| | Vm/Vmax | | |
|---|---|---|---|
| $P_L$ (min) (cm $H_2O$) | Control<br>Mean ± SEM<br>(n = 6) | Edema<br>Mean ± SEM<br>(n = 6) | *P* value |
| +4 | 0.13 ± 0.01 | 0.32 ± 0.03 | < 0.001 |
| +3 | 0.20 ± 0.03 | 0.48 ± 0.02 | < 0.001 |
| −5 | 0.59 ± 0.03 | 0.64 ± 0.02 | < 0.20 |

with PL (min) equal to either + 3 or + 4 cm $H_2O$ could be used as a very sensitive test to identify lungs which are in the early stages of edema. In addition, Fig. 4C and table 1 indicate that a typical PL–VL curve recorded between – 5 and + 30 cm $H_2O$ is not a good method for detecting changes in the mechanical properties of lungs caused by pulmonary edema.

## Discussion

It has been reported (Hughes *et al.*, 1970; Hogg *et al.*, 1972) that the diameter of the small airways is reduced during the early stages of pulmonary edema as a result of fluid accumulating in the peribronchial sheath. This reduction in airway cross-section has been shown to be responsible for the increase in peripheral airway resistance (Hogg *et al.*, 1972). Even though this increase in resistance has been difficult to detect when measuring total airway resistance (Milic-Emili and Ruff, 1971), the fact that the resistance does increase illustrates that alterations in airway radius and stability are important parameters to examine when determining the initial effects of pulmonary edema. This study shows that the quantity of gas trapped in excised rat lungs ventilated for 4 cycles at positive end expiratory pressures can be increased by pulmonary edema. We feel that the increase in gas trapping is due to unstable airways closing at more positive transpulmonary pressures during lung deflation. There is evidence that airway closure is composed of two related but separately occurring events. The two events are the formation of menisci across the airways at positive transpulmonary pressures (Macklem *et al.*, 1969) followed by the physical collapse of the airway walls at negative transpulmonary pressures (Cavagna *et al.*, 1969). Since the gas trapping process is likely related to meniscus formation, the increase in trapped gas at positive EEP's due to edema likely results from premature meniscus formation in the airways. The point at which a meniscus forms across an airway during lung deflation is determined by: (1) the caliber and geometry of the airways; (2) the amount of fluid in the airways; and (3) the integrity of the surfactant in the airways (Frazer and Khoshnood, 1979b). It could be predicted that menisci would form differently in edematous lungs since edema could affect all three of the above variables.

The method we have used to detect trapped gas has been shown to be both simple to apply and sensitive. In order to determine if a lung has edema, it is necessary to record only one PL–VL curve, hold the EEP at + 3 or + 4 cm $H_2O$ for three cycles, and then determine Vm/Vmax on the fourth cycle. Our results also show that this method is more sensitive for detecting lungs having edema induced by HIPPB than the method of measuring the W/D wt. ratio (see fig. 4A, 4B, and table 1).

Another important observation of this study is that Vm/Vmax for control and edematous lungs recorded between + 30 and – 5 cm $H_2O$ had very nearly the same value. This is in agreement with Faridy (1973) who reported that an increase in

lung water content did not increase the residual volume of the lung after 4 inflation–deflation cycles. In addition, this result may also help explain the conclusions of other investigators who in the past found little correlation between edema and changes in the lungs mechanical properties (Guyton and Lindsey, 1959; Levine *et al.*, 1965; Staub *et al.*, 1967).

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