Effects of atelectasis on pulmonary surfactant and quasi-static lung mechanics

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LEVINE, BERNARD E., AND RUDOLPH P. JOHNSON. Effects of atelectasis on pulmonary surfactant and quasi-static lung mechanics. J. Appl. Physiol. 20(5): 859-864. 1965.-The effects of unilateral atelectasis on pulmonary surfactant activity and lung mechanics have been studied in rabbits. Total left lung collapse was produced by pneumothorax and maintained from 90 min to 8 days. Quasi-static pressure-volume relationships were determined with air and saline after varying intervals. Surface activity of minced lung extracts and of dried foam obtained by tracheal washings was measured on a surface balance. A marked decrease in lung inflatability progressing with duration of collapse was noted during air and saline pressure-volume studies. However, deflation characteristics of all atelectatic lungs were normal. Extracts from minced, in vitro reinflated atelectatic lung showed a variable but significant loss of surface activity compared to the right (normal) lung of the same animal. However, highly surface-active material could be demonstrated in the collapsed lungs by the more efficient extraction procedure of tracheal foaming. Correlation exists therefore between normal volume stability of alveoli after initial inflation and presence of surfactant activity in atelectatic lungs.

METHODS

Pneumothorax procedure. Albino rabbits weighing 5-6 lb. were selected. Using local anesthesia, a 70- to 90-ml pneumothorax was induced on the left side under fluoroscopic control. Animals were checked daily by fluoroscopy and 10- to 30-ml added to the pleural cavity as necessary to maintain total collapse. No air was added on the day of sacrifice. Intrapleural pressure was studied in a number of animals during and soon after injection. The pressure rose as high as +9 cm H$_2$O during the procedure but fell to -2 cm H$_2$O a few hours later. After lung collapse of from 90 min to 8 days animals were sacrificed rapidly with intravenous pentobarbital and studied immediately after death.

Pressure-volume studies. Only those left lungs which were completely airless or had very small subpleural areas of inflation at autopsy (see RESULTS) were used. Lungs were carefully removed from the thorax, the trachea was cannulated, and the right lung bronchus tied off. The cannula was attached to an air-filled 50-ml syringe in parallel with a water manometer.

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Stepwise inflation and deflation of lungs was carried out with a 2-min equilibration period between steps. In most cases lungs were inflated until airway pressure after equilibration equaled between 29 and 31 cm H$_2$O pressure and then deflation was begun. A number of saline pressure-volume studies were performed after varying periods of collapse using the method of Radford (22).

Most determinations were made during the initial air or saline inflation from the atelectatic state. Repeated pressure-volume studies were also done on individual lungs to determine effects of prior inflation and to compare deflation characteristics after inflation to higher maximum pressures. The majority of studies were done at room temperature (22 ± 4 C) but some lungs were studied at 37 C.

Pulmonary surfactant activity. Only lung tissue which had been totally collapsed in vivo was utilized. Initially, atelectatic lung was reinflated as well as possible and then 2 g of tissue were minced in saline and studied on the surface balance. In each case the right lung served as a control for the atelectatic left lung. If extracts of atelectatic lungs sustained low surface tensions, then the rate of rise of tension after maximum compression of the surface was studied and compared to that of the control extract.

Because minced-lung data were variable and appeared to be related to success of lung inflation, a more effective extraction method was felt necessary. Lungs were cannulated and were washed with air and saline so that alveolar material returned as a foam. The saline substrate of the foam was aspirated and the foam dried overnight in air. Weighed aliquots of dried foam from right and left lungs were spread on clean saline to compare surface activity. Studies were also done on foam before drying and were in agreement with those using dry material. Rate-of-rise determinations were performed on varying weights of powder from atelectatic and control lungs.

Surface activity determinations were done on the modified Wilhelmy-Langmuir balance (7) previously described. Maximum and minimum surface tensions during 5-min cycles were recorded for all specimens and stability indices (8) were calculated.

**Histology.** All lungs studied were formalin fixed and stained with hematoxylin and eosin. A few lungs were removed from the study because of parenchymal murine pneumonia. Some specimens were also stained with Masson's trichrome in order to evaluate any connective tissue changes.

**RESULTS**

**General.** Fluoroscopic evidence of complete collapse throughout the experimental period correlated well with total atelectasis at autopsy. Atelectatic specimens were grossly and microscopically identical to in vitro degassed lungs, appeared “liver-like,” and sank in water. In some animals a small area of subpleural inflation was present in the upper lobe. No surfactant study was performed on this tissue and pressure-volume studies were done only if the inflated area was estimated to be less than 2% of the surface area of the collapsed lung.

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or saline, no areas of gross pathology were noted. All left lungs weighed between 3 and 4 g and no relationship between weight and duration of collapse was present. A distinct pallor of the reinflated atelectatic lung as compared to its control right lung was a constant finding and was felt to be due to decreased blood content of the experimental lung.

Pressure-volume studies. The most striking alteration in mechanical properties of atelectatic lung was a roughly progressive decrease in "inflatability" with duration of collapse. An increase in airway pressure at any inflation volume as well as a decrease in total volume at maximum inflation pressure were seen.

These findings are illustrated by representative quasi-static pressure-volume diagrams after 3, 24, and 144 hr of collapse (Fig. 1). Atelectatic lungs always appeared fully inflated at 30 cm H2O pressure but were smaller than control left lungs. No significant differences were noted between characteristics of specimens degassed in vitro and of those atelectatic up to 3 hr, all of which were therefore considered control specimens.

Figure 2 summarizes the equilibrium pressures obtained during the steep slope of the initial air inflation in 19 animals. This portion of the pressure-volume diagram represents the opening pressure for most of the alveoli. This marked alteration in opening pressure with duration of atelectasis is also seen during saline inflation of atelectatic lungs as shown in Fig. 3.

When repeated pressure-volume determinations were made on individual lungs, successive air or saline inflation required less pressure. This finding is in accord with prior work on lung mechanics (23). However, differences in compliance between lungs, related to duration of atelectasis, were maintained on repeated inflations, and approximate correlation between air and saline differences was a consistent finding.

Because of the altered inflation characteristics caused by atelectasis, direct comparison of the deflation portion of the pressure-volume diagram was difficult (Fig. 1). To determine if deflation characteristics were altered by atelectasis, the volume which was in the lung at an equilibrium pressure of approximately 30 cm H2O was considered as 100% and deflation volumes graphed as fractions of this. Figure 4 shows deflation limbs from seven lungs which had been collapsed over periods up to 8 days. All lungs show a high degree of alveolar stability and little difference can be detected between control and atelectatic lungs. In other words, those alveoli which are inflated act as if they possessed normal surfactant activity. Studies of lungs inflated to lower (20 cm H2O) or higher (40 cm H2O) maximum pressure showed very similar deflation curves. Pressure-volume determinations at 37 C done on degassed normal lung and on lungs collapsed 72 and 96 hr also showed no marked alteration in deflation characteristics. The deflation pressure-volume curve of saline-filled control lungs was very similar to that for inflation, i.e., there was little

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FIG. 3. Pressure during steep slope of initial saline inflation after atelectasis. Dotted line with * from study of degassed, normal lung. Other dotted line obtained on a lung after 2 hr pneumothorax collapse.

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FIG. 4. Deflation limbs of initial air pressure-volume diagrams after atelectasis. Volumes calculated as percentages of total volume at 30 cm H2O pressure.
from control and atelectatic lungs

Studies on minced lung extracts which show a loss in pressure-volume relationships noted after collapse was due to causes other than alveolar surfactant.

**DISCUSSION**

If the surface activity of the phospholipid-protein complex at the alveolar air-fluid interface is altered, a correlated change in quasi-static lung mechanics should also be observed. Such correlation is present in isolated lungs after destruction of the surfactant by enzymes (14) or its displacement by detergents (22). It has been shown in the intact animal following pulmonary artery occlusion (12), cardiopulmonary bypass (11), and in vivo fluid filling of lung (13). Hyaline membrane disease of the newborn also demonstrates related change in surfactant activity and pressure-volume relationships (8). It is important to note, however, that simple dilution of surfactant by repeatedly washing normal excised lungs does not alter mechanical stability (manuscript in preparation). It appears that the chemical complex at the alveolar surface must be changed qualitatively to alter lung mechanics.

The normal deflation limbs of pressure-volume diagrams from atelectatic lungs show that after in vitro reinflation the alveoli act as if they were lined by normal surfactant. Surface-balance studies, however, suggest a quantitative decrease in material extractable from these lungs. Whether this decrease is due to a real chemical alteration at the alveolar surface or to extraction difficulties secondary to altered lung inflatability can only be solved by careful biochemical study.

**TABLE 2. Rise of surface tension after 30 min at maximum compression-mined lung extracts**

<table>
<thead>
<tr>
<th>Duration of atelectasis, hr</th>
<th>Atelectatic (Left)</th>
<th>Control (Right)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Minimum $\gamma_{s}$, dynes/cm</td>
<td>Change in $\gamma$ after 30 min</td>
</tr>
<tr>
<td>19</td>
<td>4</td>
<td>13</td>
</tr>
<tr>
<td>48</td>
<td>6</td>
<td>16</td>
</tr>
<tr>
<td>72</td>
<td>4</td>
<td>18</td>
</tr>
<tr>
<td>72</td>
<td>7</td>
<td>18</td>
</tr>
<tr>
<td>96</td>
<td>5</td>
<td>16.5</td>
</tr>
</tbody>
</table>

Numbers in parentheses are numbers of animals. Values are means ± standard deviation.

"Hysteresis" noted. Saline filled atelectatic lungs demonstrated increased hysteresis, suggesting that the alteration in pressure-volume relationships noted after collapse was due to causes other than alveolar surfactant.

**Surface-tension studies** Table 1 summarizes the data from studies on minced lung extracts which show a loss of surface activity after 24 hr of collapse. All right lung extracts and those from left lungs collapsed less than 24 hr achieved low minimum tensions and had high stability indices. While the alteration in minimum tension after 24-hr atelectasis was statistically significant, it was variable and appeared to be related more to degree of inflation prior to extraction than to time of collapse. Those atelectatic extracts which achieved low tensions exhibited a rapid rate of rise of surface tension at maximum compression, as is shown in Table 2. While during the 1st hr rise of tension was faster for atelectatic than control extracts, equilibrium tensions finally reached were very similar.

Studies using dried foam helped to clarify the differences in surface activity noted with minced lungs. In Table 3 data are tabulated from six animals in which 1 mg of powder was added from right and left lung were spread on clean saline. Differences in activity very similar to those obtained with minced lung are apparent. After observing the activity of 1 mg of dried foam, powder was then added in millgram increments until no change in stable minimum tension was observed with further addition of powder. In all cases minimum tension was read at the eighth cycle after addition of powder. Table 4 illustrates the final surface tension, stability indices, and the amount of powder needed. Dried foam from atelectatic lung always formed a surface layer on saline with activity comparable to the control lung, but about twice as much material was needed. This suggests a quantitative alteration in surfactant either secondary to extraction difficulties or to an actual change in surface material. The rate of rise of surface tension became less with successive additions of powder from control lungs indicating that this is a quantitative phenomenon in these lungs. Rate-of-rise comparisons between control and atelectatic lungs showed differences that were similar to but less marked than those seen with minced lung, depending again on quantity of powder used.

**Pathology.** Careful examination of the microscopic sections revealed no differences in alveolar histology between normal and atelectatic lung reinflated in vitro. There was no evidence of hyaline membranes, lung edema, or inspissated bronchial mucus.
been shown to be less efficient when the lung is airless (16).

Differences in pulmonary arterial flow in atelectasis due to pneumothorax and bronchial occlusion may account for varying data also. Finley et al. (11) report a loss of surface activity after bronchial occlusion of 48 hr duration in dogs and relate this not to atelectasis, but to total absence of pulmonary arterial flow to the collapsed lung. Other studies of blood flow after bronchial occlusion have demonstrated changes which might be responsible for decreased inflatability. Postmortem angiography after lung collapse by pneumothorax of 1 week duration demonstrated persistent contraction of muscular arteries after inflation with barium-gelatin mixture to 30 mm Hg pressure (1). Changes in saline pressure-volume characteristics similar to those seen after atelectasis were obtained by Radford (23) following administration of bronchoconstrictor agents. Moreover, histologic studies of lung after collapse of 1 week show changes primarily in pattern, morphology, and staining of elastic fibers (1, 18). These observations, along with the fact that saline and air inflation changes are parallel, point to altered tissue characteristics as the most likely factor responsible for the progressive decrease in lung inflatability observed by us.

The actual physical state of the surfactant during atelectasis is unknown; however, Pattle (19) has estimated that a normal surface layer forms very rapidly after re-inflation of collapsed lung. This is in keeping with our observation that once atelectatic alveoli are opened by an initial inflation, they demonstrate normal volume stability and are lined by highly surface-active material.

The authors thank Dr. Mary Ellen Avery for her advice and encouragement, Dr. Charles Kuhn for reviewing the histologic studies of lung, and Kenneth Wilson for reviewing the manuscript.

### TABLE 3. Surface activity of 1-mg samples of dried foam from control and atelectatic lungs

<table>
<thead>
<tr>
<th>Duration of Atelectasis, hr</th>
<th>Right Lung, Control</th>
<th>Left Lung</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum γ, dynes/cm</td>
<td>Minimum γ, dynes/cm</td>
<td>S</td>
</tr>
<tr>
<td>24</td>
<td>49</td>
<td>9</td>
</tr>
<tr>
<td>48</td>
<td>53</td>
<td>5</td>
</tr>
<tr>
<td>48</td>
<td>47</td>
<td>2</td>
</tr>
<tr>
<td>66</td>
<td>48</td>
<td>5</td>
</tr>
<tr>
<td>144</td>
<td>51</td>
<td>2</td>
</tr>
<tr>
<td>144</td>
<td>44</td>
<td>1</td>
</tr>
</tbody>
</table>

* Values are means ± standard deviation.

### TABLE 4. Maximum surface activity of dried foam from control and atelectatic lungs and amount of dried foam needed to achieve maximum activity

<table>
<thead>
<tr>
<th>Right Lung</th>
<th>Minimum γ, dynes/cm</th>
<th>Maximum γ, dynes/cm</th>
<th>Powder Needed, mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trachea</td>
<td>3.6±1.8</td>
<td>4.7±2.9</td>
<td>1.73±1.3</td>
</tr>
<tr>
<td>Left lung</td>
<td>3.1±1.0</td>
<td>4.3±2.1</td>
<td>1.7±0.1</td>
</tr>
<tr>
<td>1-4 hr collapse</td>
<td>4.3±2.1</td>
<td>1.7±0.1</td>
<td>1.0</td>
</tr>
<tr>
<td>Left lung</td>
<td>5.0±0.7</td>
<td>6.4±1.0</td>
<td>1.7±0.1</td>
</tr>
<tr>
<td>1-4 hr collapse</td>
<td>4.7±1.0</td>
<td>1.7±0.1</td>
<td>1.0</td>
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<td>5.0±0.7</td>
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<tr>
<td>1-4 hr collapse</td>
<td>4.7±1.0</td>
<td>1.7±0.1</td>
<td>1.0</td>
</tr>
<tr>
<td>Right lung</td>
<td>4.8±2.6</td>
<td>3.8±1.8</td>
<td>1.72±0.1</td>
</tr>
<tr>
<td>1-4 hr collapse</td>
<td>4.6±2.6</td>
<td>1.7±0.1</td>
<td>1.0</td>
</tr>
<tr>
<td>Left lung</td>
<td>5.1±1.9</td>
<td>6.0±2.0</td>
<td>1.64±1.3</td>
</tr>
<tr>
<td>1-4 hr collapse</td>
<td>4.9±1.8</td>
<td>1.6±0.1</td>
<td>1.0</td>
</tr>
</tbody>
</table>

* Values are means ± standard deviation.

Numbers in parentheses are numbers of animals. Values are means ± standard deviation.

“sticking together” collapsed small bronchioles. However, this would not explain the changes in compliance related to duration of collapse or the persisting decreased compliance during successive inflations of the collapsed lung. Other studies have demonstrated changes which might be responsible for decreased inflatability. Postmortem angiography after lung collapse by pneumothorax of 1 week duration demonstrated persistent contraction of muscular arteries after inflation with barium-gelatin mixture to 30 mm Hg pressure (1). Changes in saline pressure-volume characteristics similar to those seen after atelectasis were obtained by Radford (23) following administration of bronchoconstrictor agents. Moreover, histologic studies of lung after collapse of 1 week show changes primarily in pattern, morphology, and staining of elastic fibers (1, 18). These observations, along with the fact that saline and air inflation changes are parallel, point to altered tissue characteristics as the most likely factor responsible for the progressive decrease in lung inflatability observed by us.
REFERENCES


