Effects of Pulmonary Artery Ligation on Pulmonary Surfactant and Pressure-Volume Characteristics of Dog Lung

By Samuel T. Giammona, M.D., Isidore Mandelbaum, M.D., James Foy, B.S., and Stuart Bondurant, M.D.

The surface tension of extracts from normal lungs is extremely low owing to the presence of a specific surfactant. Experiments of Finley et al. showing an abnormally high surface tension of lung extracts obtained 12 to 16 hours after ligation of the pulmonary artery (PA) were confirmed by Long and associates. These studies suggested that the surfactant is decreased or absent after PA ligation.

The present studies were designed to correlate the pressure-volume characteristics of the lung with changes in the surface tension of lung extracts and to investigate the long term effects of PA ligation. Surface tension measurements of the lung extracts, pressure-volume curves and histological studies of lungs were made in one group of dogs four hours after the pulmonary artery was ligated and in another group of dogs two weeks after the pulmonary artery was ligated. A comparative study was also made of the results obtained by two different methods of surfactant extraction and by two different methods of surface tension measurements.

Methods

The right PA was ligated in each of 12 healthy mongrel dogs weighing 14 to 17 kg. Surgery and experimental measurements were done under thiopental (Pentothal) anesthesia. Respiration was controlled with a positive pressure respirator, administering 100% oxygen through an endotracheal tube until the PA ligation was completed (20 to 30 minutes). The dog was ventilated with room air for the remainder of each experiment. A right lateral thoracotomy was performed through the fourth intercostal space and the thoracic cavity widely exposed. Four hours after ligation of the right pulmonary artery, separate pressure-volume measurements of each lung were performed in the following manner: The indwelling endotracheal tube was connected to a three-way Luer lock stopcock with one arm leading to a calibrated 100 ml syringe and the other to a water manometer. A noncrushing clamp was applied to one main stem bronchus and the lung to be tested was inflated with air until all the lung units appeared grossly distended. The lung was then allowed to deflate passively. This was repeated two or three times and further volume-pressure studies were made using the retained lung volume at zero transpulmonary pressure (atmospheric pressure) as the base line for the volume measurements.

The lung was inflated with 100 ml increments of air and the pressure changes were recorded after stabilization of the transpulmonary pressure (15 to 20 seconds). Inflation was continued seriatim until all the lung units appeared distended. The right lung which had the PA ligated, was studied first and the maximum pressure was recorded when the right lung was distended and the pressure produced in the intact lungs was kept in a comparable range. Most lungs were completely inflated when transpulmonary pressure reached approximately 40 cm H$_2$O. Inflation was more irregular and more undistended units were observed during inflation in lungs two weeks after PA ligation than in the intact lungs. Deflation was performed by stepwise removal of 100 ml of gas from the inflated lung. The pressure changes during deflation were recorded after stabilization of the transpulmonary pressure (20 seconds). Deflation volume changes were more uniform and pressures were more...
stable than inflation volume changes and pressures in all the lungs studied.

Circulation through the control lung was unobstructed and the control lung was perfused during this study. Therefore, changes in lung volume due to gas exchange may have occurred. The volume of air recovered during deflation was nearly equal to that administered during inflation which suggested that the respiratory exchange ratio was nearly one. Since the time required to study one lung was from one to three minutes, gas exchange was not considered to be a source of important error.

In five dogs, a right thoracotomy was done initially and the main right pulmonary artery ligated. The chest was then closed and the animals were returned to their pen. Two weeks later, under Pentothal anesthesia, the thoracotomy was reopened and the pressure-volume measurements made as described.

After completion of the pressure-volume measurements, the animals were sacrificed, the lungs were excised and each lung weighed separately. The right lungs weighed about 40% more than left lungs; this difference is characteristic of normal lungs and indicates that there were no significant weight changes in the right lungs as a result of ligating their arteries.

The gas volume \( V \) in the lung at different degrees of inflation and deflation was expressed as a percentage of the air volume required for maximal inflation of the lung \( V_{\text{max}} \). This percentage \( \frac{V}{V_{\text{max}}} \times 100\% \) was related to the measured transpulmonary pressure, thus allowing a comparison to be made among the pressure-volume curves from all the lungs. During deflation, this percentage would be lower than expected if a significant number of alveoli emptied at very high transpulmonary pressure. The volume retained at 15 cm H\(_2\)O transpulmonary pressure during deflation was recorded. Johnson and co-workers have suggested that the fraction of maximum volume remaining at 15 cm H\(_2\)O transpulmonary pressure \( \frac{V_{15}}{V_{\text{max}}} \) is inversely related to the number of alveolar units prematurely closed during deflation of the lungs.

From the data relating the total volume per gram of lung, to the transpulmonary pressure, a lung stability index (LSI) was calculated by using the modified Gruenwald equation.\(^7\)

\[
\text{LSI} = \frac{2 \left( V_s \right) + \left( V_{10} \right)}{2 V_{10}}
\]

Here, \( V_s \) is the gas volume per gram of lung at 5 cm of water transpulmonary pressure and \( V_{10} \) is the volume per gram at 10 cm of water pressure; both measurements were made during deflation. The \( V_{10} \) is the maximum volume per gram on inflation. The range of possible values for LSI is from 0 to 1.5. The higher the index the greater the gas volume contained per unit distending pressure on deflation and the greater the stability of the lung during deflation.

Pulmonary surfactant was prepared within two hours after death by the foam fractionation method\(^8\) and by a minced lung technique \( (20 \text{ g of lung were minced in 50 ml of saline})^9 \). Surface tension was measured on a modified Wilhelmy balance as surface area was compressed from 100 cm\(^2\) to 20 cm\(^2\) at a frequency of 1 cycle every 90 seconds. The surface area-surface tension loop was recorded on a direct-writing X-Y recorder.

A surfactant activity index (SAI) was calculated for each extract according to the equation suggested by Clements and co-workers:\(^10\)

\[
\text{SAI} = \frac{2 \left( \text{S.T. maximum} - \text{S.T. minimum} \right)}{\left( \text{S.T. maximum} + \text{S.T. minimum} \right)}
\]

The maximum surface tension was that observed on expansion of the surface film to 100 cm\(^2\) and the minimum tension was that observed on compressing the film to 20 cm\(^2\). The range of possible values for the SAI is from 0 to 2 and Clements and co-workers have reported that lung extracts with normal surfactant have a SAI greater than 1.25 and lung extracts with loss of surface activity have a low SAI \((< 1.25)\). The SAI, therefore, serves as an index of surface compressibility. High values may indicate that alveolar lining layers are more capable of maintaining a stable surface tension-surface area relationship, thus promoting alveolar stability throughout deflation.

Surface tension was also measured with a du Noüy tensiometer.\(^11\), \(^12\) Ten milligrams of the dry foam fractionated extract were placed in 15 ml of saline and allowed to age one hour to form a stable surface film at room temperature \((22^\circ\text{C})\). Surface tension was measured by pulling a 6-cm platinum ring through the surface film and recording the force required to break the film. The ring was displaced at a constant, slow rate for each measurement and cleaned by flaming between determinations. Five measurements were made on each sample and the results averaged and corrected for the temperature coefficient.\(^12\)

**Results**

The animals tolerated the surgical procedures satisfactorily with no operative mortality. At autopsy, the gross and microscopic findings in the lungs were similar to those described by Schlaepfer in 1924.\(^13\) Neither the lungs with the PA ligated for four hours nor

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the lungs with the PA intact differed grossly or microscopically from the normal.

The dogs studied two weeks after the right PA was ligated were active without cough, evident dyspnea or signs of infection. Autopsies revealed no pleural thickening or pleural effusion, and adhesions were present only at the site of pulmonary artery ligation. The lungs with the PA ligated were cyanotic with generalized petechial hemorrhages and scattered firm, airless segments. Microscopically, alveolar exudate and hemorrhage were observed, but no alveolar hyaline membranes were identified.

Four hours after the right PA was ligated there was no difference throughout inflation or deflation in the relationship of transpulmonary pressure to the gas volume (V/V_{max}%) in the two lungs (table 1) (figs. 1 and 2). In the dogs studied two weeks after right PA ligation, there was no significant statistical difference between the inflation curves of the control lungs and that of the lungs with the PA ligated. The deflation curves were significantly different at all transpulmonary pressures and at all lung volumes (figs. 3 and 4).

The $V_{15}^*$ of the lungs with the PA intact was not significantly different from that of the lungs four hours after the PA was ligated (table 2). Two weeks after pulmonary artery ligation, the mean $V_{15}^*$ of the lungs with the PA ligated was 60 ± 14 and the mean $V_{15}^*$ of the lungs with the PA intact was 77 ± 3 (P < .05) (table 2).

There was no significant difference in the minimum or maximum surface tension of extracts from lungs with the PA intact and the lungs four hours after the PA was ligated (table 3). However, the minimum surface tension of extracts from lungs after two weeks of ligation was significantly higher than in extracts from controls. This was true whether the extracts were prepared by foaming or mincing or whether the surface tension was measured with the Wilhelmy balance or the du Noiij tensiometer. The maximum surface tension as measured by the Wilhelmy balance was unaltered by the ligation.

Four hours after right PA ligation, the surfactant activity index and the lung stability

### Table 1

<table>
<thead>
<tr>
<th>Dogs</th>
<th>Lung weight</th>
<th>Inflation pressure at 1 ml/g</th>
<th>Transpulmonary pressure at maximum inflation</th>
<th>Ratio maximum volume per g ligated/intact x 100</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intact grams</td>
<td>Intact cm H$_2$O Ligated</td>
<td>Intact cm H$_2$O Ligated</td>
<td>Intact cm H$_2$O Ligated</td>
</tr>
<tr>
<td>Four hours</td>
<td>74.1</td>
<td>7</td>
<td>9</td>
<td>40 cm H$_2$O</td>
</tr>
<tr>
<td>AP 4</td>
<td>66.0</td>
<td>3</td>
<td>6</td>
<td>35</td>
</tr>
<tr>
<td>AP 5</td>
<td>75.0</td>
<td>8</td>
<td>17</td>
<td>40 cm H$_2$O</td>
</tr>
<tr>
<td>AP 6</td>
<td>74.0</td>
<td>3</td>
<td>4</td>
<td>37</td>
</tr>
<tr>
<td>7</td>
<td>61.0</td>
<td>5</td>
<td>9</td>
<td>32</td>
</tr>
<tr>
<td>7</td>
<td>61.0</td>
<td>7</td>
<td>20</td>
<td>48</td>
</tr>
<tr>
<td>7</td>
<td>61.0</td>
<td>3</td>
<td>14</td>
<td>38</td>
</tr>
</tbody>
</table>

| Mean | 75.0 | 5 | 12 | 38 | 41 | 74 |
| Mean sd* | 12.0 | 2 | 5 | 3 | 5 | 18 |

| Two weeks | 73.0 | 4 | 13 | 37 | 47 | 57 |
| Mean sd* | 7.0 | 4 | 9 | 9 | 9 | 7 |

*sd: standard deviation.
LUNG INFLATION-FOUR HOURS AFTER PULMONARY ARTERY LIGATION

FIGURE 1

Relationship of transpulmonary pressure to per cent of maximum volume during inflation of the lung in vivo four hours after the pulmonary artery was ligated. There was no statistical difference between the curves from the lung with the ligated pulmonary artery and from the lung with the pulmonary artery intact.

index of the two lungs did not differ. Two weeks after PA ligation, both indices were reduced and there was a low correlation between the two indices \( (r = 0.48; P < 0.05) \) (table 2).

There was also a low negative correlation between \%\( V_{15} \) and minimum surface tension \( (r = 0.42; P < 0.05) \). The \%\( V_{15} \) of lungs that produced extracts with minimum surface tension of less than 15 dynes/cm was higher than that of lungs yielding extracts with minimum surface tension greater than 15 dynes/cm \( (P < 0.05) \). There was also a correlation between \%\( V_{15} \) and the surfactant activity index \( (r = 0.55; P < 0.05) \). Only one lung had a \%\( V_{15} \) greater than 70 and a surfactant activity index below 1.20. There were two lungs with a surfactant activity index greater than 1.20 and a \%\( V_{15} \) less than 70 (table 2). A comparison of the ratio of maximum volume per gram of lung in the lungs with the PA ligated and the lungs with the PA intact to both the maximum or minimum surface tension (Wilhelmy balance) was made and there was no significant correlation \( (r = 0.2; P > 0.05) \).

Lung extracts prepared by foam fractionation or by mincing lung had similar surface tensions. All the extracts from lungs with the PA intact had a surface tension less than 15 dynes/cm (Wilhelmy balance). There was a marked fall in the surface tension of saline after the addition of surfactant from the lungs.
four hours and two weeks after the pulmonary artery was ligated, indicating that a powerful surfactant agent, however, still remained in the lung.

There was a statistically significant difference \((P < 0.05)\) in the measurements of the surface tension with the du Noiiy tensiometer, between the surfactant from the control lungs \((31.4 \pm 1.1 \text{ dyne/cm})\) and the surfactant from the lungs two weeks after PA ligation. \((36.6 \pm 2.4)\). There was a high correlation between the surface tension measured with the Wilhelmy balance (table 3) \((r = 0.75; P < 0.05)\). Only one lung extract with minimum surface tension less than 15 dynes/cm measured with the Wilhelmy balance had a surface tension greater than 34 dynes/cm measured with the du Noiiy tensiometer. None of the lung extracts with minimum surface tension greater than 15 dynes/cm measured with the Wilhelmy balance had a surface tension below 34 dynes/cm measured with the du Noiiy tensiometer.

**Discussion**

The surface tension of lung extracts appears to be normal four hours after ligation of the pulmonary artery. Finley et al.\(^2\) reported changes in surfactant activity 12 hours after PA ligation. These results therefore indicated that the critical period for loss of surfactant activity is between 4 and 12 hours after liga-

![Figure 2](https://circres.ahajournals.org/)

**FIGURE 2**

Relationship of transpulmonary pressure to per cent of maximum volume during deflation of the lung in vivo four hours after the pulmonary artery was ligated. There was no statistical difference between the curves from the lung with the ligated pulmonary artery and from the lung with the pulmonary artery intact.

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tion of the pulmonary artery. Lung extracts with high surface tension were found two weeks after the PA was ligated in this study. This indicated that normal surfactant activity does not return during this period.

There are presently no techniques available for evaluating the efficiency of the various procedures which are used to extract surface active material from the lung. For this reason, it is not possible to assess completely the effect of PA ligation on extractability of surfactant. Atelectasis, which is a feature of the lung with the PA ligated, has been shown by Levine and Johnson to reduce extractability of surfactant by the mincing technique. In the present study, extracts with low surface tension were prepared four hours after the PA was ligated by mincing noninflated lung and also by foam fractionation of the inflated lung. It may be pertinent that larger pieces of lung were minced in the present study than in the study of Levine and Johnson. Since neither technique of extraction produced material with low surface tension from the lungs two weeks after PA ligation, it is not possible to evaluate the importance of altered extractability of surfactant in producing the changes in the surface tension measurements of the extract of the lungs two weeks after PA ligation. The changes in the pressure-volume characteristics which were observed, however, support the interpretation that a real change in surface forces occurred within the lung.

Johnson and co-workers have suggested that $\%V_{15}$ is an index of alveolar closure during deflation. The low mean $\%V_{15}$ of the lungs two weeks after the PA was ligated and the high surface tension in these lung extracts are consistent with the suggested causal relationship between these two phenomena. The $\%V_{15}$

\[ \text{FIGURE 3} \]

Relationship of transpulmonary pressure to per cent of maximum volume during deflation of the lung in vivo two weeks after the pulmonary artery was ligated. There was no significant difference between the curves from the lung with the ligated pulmonary artery and from the lung with the pulmonary artery intact.
in this study correlated best with the surfactant activity index.

The marked fall in the surface tension of saline after addition of the surfactant from the lungs two weeks after ligation of the pulmonary artery indicates that even in these atelectatic lungs some surface activity still remains. It is observed, however, that there is not sufficient surface activity to produce the very low surface tension values found normally upon compression on the Wilhelmy balance of the surface film formed by the surfactant. There also is a small but significant difference in the lowest surface tension measured with the du Noüy tensiometer between extracts from lung with an intact pulmonary artery and lungs two weeks after the pulmonary artery was ligated. This suggests that the cessation of pulmonary perfusion results in a definite change in the surface activity of the alveolar lining layer, conducive to alveolar instability and to pulmonary effusion.

The values for LSI in the lungs with the PA intact were lower than those reported by Gruenwald for normal dog lungs. This observation may indicate that the LSI was decreased by prolonged increased pulmonary perfusion which occurred in the dogs which had the entire cardiac output perfusing their intact lung after ligation of the PA. The lung stability indices of lungs two weeks after PA ligation were the most markedly reduced. A low but significant correlation was found between the minimum surface tension (Wilhelmy balance) and the %V_{15} and also between the LSI and SAI.

The lack of changes in the inflation curves, as compared to the significant changes in the deflation curves found in the lungs two weeks after the PA was ligated, supports the

**FIGURE 4**

Relationship of transpulmonary pressure to per cent of maximum volume during deflation of the lung in vivo two weeks after the right pulmonary artery was ligated. There was a significant difference (P <0.05) between the curves from the lung with the pulmonary artery ligated and from the lung with the pulmonary artery intact except at 100% volume.

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concept that surface forces do not play a significant role during inflation as long as the surface area increases. During deflation the decrease in surface tension extends the range of stable behavior of air spaces. A fixed elevated surface tension would result in instabil-

### Table 2

**Lung Stability Index, Surfactant Activity Index and \%V\textsubscript{15} after Pulmonary Artery Ligation**

<table>
<thead>
<tr>
<th>Dogs</th>
<th>%V\textsubscript{15}</th>
<th>Lung stability index</th>
<th>Surfactant activity index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intact</td>
<td>Ligated</td>
<td>Intact</td>
</tr>
<tr>
<td>1</td>
<td>76</td>
<td>82</td>
<td>1.00</td>
</tr>
<tr>
<td>2</td>
<td>87</td>
<td>88</td>
<td>0.95</td>
</tr>
<tr>
<td>3</td>
<td>86</td>
<td>74</td>
<td>0.91</td>
</tr>
<tr>
<td>4</td>
<td>77</td>
<td>84</td>
<td>0.55</td>
</tr>
<tr>
<td>5</td>
<td>82</td>
<td>82</td>
<td>0.61</td>
</tr>
<tr>
<td>6</td>
<td>83</td>
<td>67</td>
<td>0.69</td>
</tr>
<tr>
<td>7</td>
<td>79</td>
<td>63</td>
<td>0.76</td>
</tr>
<tr>
<td>Mean</td>
<td>81</td>
<td>77</td>
<td>0.78</td>
</tr>
<tr>
<td>sd*</td>
<td>4</td>
<td>9</td>
<td>0.16</td>
</tr>
</tbody>
</table>

*sd: standard deviation.

### Table 3

**Surface Tension of Extracts of Lung after Ligation of Pulmonary Artery**

<table>
<thead>
<tr>
<th>Dogs</th>
<th>Foamed surfactant</th>
<th>Minced extract</th>
<th>du Noüy tensiometer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lungs</td>
<td>Lungs</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Max*</td>
<td>Min†</td>
<td>Max</td>
</tr>
<tr>
<td>PA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>intact</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Four</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hours</td>
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<td></td>
</tr>
<tr>
<td>after</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ligated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sd†</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Max: maximum surface tension at 100 cm\(^2\) surface area.†Min: minimum surface tension at 20 cm\(^2\) surface area.‡sd: standard deviation.
PULMONARY SURFACTANT AND MECHANICS AFTER PULMONARY ARTERY LIGATION

ity of the lung and promote air space collapse at relatively high pressures. This was observed in the deflation curve of the lungs two weeks after PA ligation.

There are additional factors other than surface forces which may have altered the pressure-volume characteristics of the lungs after the PA was ligated. Alteration in tissue elasticity can occur, resulting in an increase in retractive forces in the lung. This, however, should chiefly affect inflation and not appreciably affect deflation. The presence of reflex closure of terminal airways cannot be excluded in this study and this reflex closure would result in undistensible lung units. The geometric arrangement of the alveoli as outlined by Mead may be altered after pulmonary artery ligation resulting in alveolar instability at low volumes during deflation.

The causes of the changes in surfactant were not examined in this study. Clements has postulated that reduced perfusion of the alveolar cells after the PA is ligated may result in interference with synthesis of pulmonary surfactant by the alveolar cell mitochondria.

Summary

The effects of pulmonary artery ligation upon pulmonary surfactant and upon the pressure-volume characteristics of the canine lung were studied. Four hours after ligation of one pulmonary artery the two lungs had similar deflation pressure-volume curves and extracts from these lungs had similar surface tension. Two weeks after ligation, the lungs with a ligated PA differed from the contralateral lungs in having a smaller fraction of total volume retained at each pressure during deflation, and extracts with a higher minimal surface tension, and a lower surfactant activity index. Low but significant correlations were observed between the fractional volume at 15 cm H$_2$O transpulmonary pressure and the minimal surface tension of the lung extracts (Wilhelmy balance), between the fractional volume of 15 cm H$_2$O transpulmonary pressure and the surfactant activity index, and between the surfactant activity index and the lung stability index. These data suggest a close relationship between altered surface forces and the pressure-volume characteristics of the lung after pulmonary artery ligation.

References

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