Effects of Acid Aspiration on Pulmonary Alveolar Epithelial Membrane Permeability*

Frederick L. Glauser, M.D., F.C.C.P.; J. Eugene Millen, B.S.; and Randall Falls, B.S.

Employing a modification of the in vivo model of a liquid-filled canine lung, we measured the movement of substances of specific sizes (albumin, 69,000 daltons with a molecular radius of 35 Å; and dextran with a molecular weight of 150,000 to 170,000 and an approximate molecular radius of 100 Å) from the pulmonary capillary blood to the liquid-filled lung. A solution with a specific pH (1.5 to 4.5) was instilled into the left lung of the animals at a dosage of 3 to 5 ml/kg of body weight. For both albumin and dextran with a molecular weight of 150,000 to 170,000, the time for 50 percent equilibration between the specific substance in the blood and the same substance in the pulmonary liquid decreased significantly with instillation of pulmonary liquid with a pH of 1.5 and 2.5 but did not with a pH of 3.5 or above (P < 0.05). In addition, since histamine has been implicated as a possible humoral mediator leading to increased permeability of alveolar membranes, the levels of histamine were measured in pulmonary liquids and blood in all groups. Levels of histamine in the pulmonary liquid (but not blood) were significantly higher in animals with instillation of liquids with a pH of 1.5 and 2.5 compared to all other groups.

The aspiration of gastric contents with a low pH (2.5 or less) may lead to disruption of the alveolar capillary membrane, with concomitant exudation of intravascular liquid and protein into terminal air-exchanging units. Electrophoresis and electron-microscopic analysis of this alveolar liquid reveals it to have a similar distribution of proteins as is found in serum. To our knowledge, quantitative evaluation of changes in the permeability of the alveolar capillary membrane in response to substances of various molecular sizes and weights has not been determined for aspiration of acid. It would be advantageous to possess an experimental model in which the movement of molecular substances of specific sizes from the pulmonary capillary to the alveolus could be measured, for in this way the value of such controversial therapy as steroids in the treatment of aspiration of acid could be more accurately determined. We therefore have established an in vivo model of a liquid-filled canine lung and have followed the movement of specific molecular species from the pulmonary capillaries to the alveolus. In addition, since histamine has been implicated as a possible mediator of the increased permeability of the alveolar capillary membrane that is found with noncardiogenic pulmonary edema (adult respiratory distress syndrome), we measured this substance in the blood and pulmonary liquid.

MATERIALS AND METHODS
The in vivo transalveolar movement of endogenous albumin (molecular weight of 69,000 daltons and molecular radius of 35 Å) and exogenous dextrans (molecular weight of 150,000 to 170,000 and molecular radius of approximately 100 Å) was measured in the saline-filled segment of the canine lungs according to a previously established method. Briefly, mongrel dogs weighing between 12 and 15 kg (26 to 33 lb) were anesthetized with intravenous administration of 50 mg of pentobarbital per kilogram of body weight, were intubated, and were ventilated with a respirator (Harvard) at a fractional concentration of oxygen in the inspired gas of 1.0, a tidal volume of 15 ml/kg, and a respiratory rate of 12 breaths per minute. The kidneys were externalized, and the renal pedicles were ligated to ensure a "closed" vascular compartment with no subsequent urinary loss of intravascular tracers (as discussed subsequently). Tracheotomy was performed, and a Carlens endotracheal tube was inserted to separate the lungs. In selected animals a Swan-Ganz catheter was placed into the pulmonary artery under pressure monitoring. All animals had femoral arterial blood pressure monitors continuously.

Following these experimental procedures, 10 gm of dextran with a molecular weight of 150,000 to 170,000 in 50 ml of a 0.9 percent solution of sodium chloride was injected intravenously, and one hour was allowed for a constant level to be achieved in the blood. Animals were then tilted to the 30° to 35° semiprьnt position; the left arm of the Carlens tube was clamped at the end of expiration, and 15 minutes later (after atelectasis had occurred), a solution with a specific pH (discussed subsequently) was instilled at a dosage of 3 to 5 ml/kg into the left lung via a No. 5 Swan-Ganz catheter placed intrabronchially as far distal as possible (60 to 70 cm). The final airway pressure was atmospheric. Simultaneous samples of the pulmonary liquid (1 to 2 ml) and arterial blood were obtained every 15 to 20 minutes for four to six hours for...
determination of levels of albumin, dextran, and histamine. This amount of pulmonary liquid was not replaced, and no "tidal breathing" occurred.

Dogs were grouped as follows: (1) a 0.9 percent solution of sodium chloride with hydrochloric acid added to yield a solution with a pH of 1.5 was instilled into the left lung in 12 dogs (group 1); (2) a 0.9 percent solution of sodium chloride with hydrochloric acid added to yield a solution with a pH of 2.5 was instilled into the left lung in 8 dogs (group 2); (3) a 0.9 percent solution of sodium chloride with hydrochloric acid added to yield a solution with a pH of 3.5 was instilled into the left lung in 8 dogs (group 3); and (4) a 0.9 percent solution of sodium chloride with a pH of 4.5 or greater (control) was instilled into the left lung in 11 dogs (group 4).

Specific Method

Systemic, pulmonary arterial, and pulmonary wedge pressures were monitored using a transducer (Statham) and a physiologic recorder (Electronics for Medicine VR-6). The levels of albumin in the pulmonary liquid and blood were determined by a fluorometric method and the levels of dextran by the method using anthrone. Levels of histamine were determined by a modification of the fluorometric method of Shore et al. Shunt (QS/QT) was determined by the standard formula employing samples of arterial and mixed venous blood. Hematocrit readings were determined in duplicate on a microhematocrit centrifuge.

Calculations

The problems involved in the calculation of the time for 50 percent equilibration between a specific substance in the blood and the same substance in the pulmonary liquid are discussed by Theodore et al and Fischer et al. Formulas for calculating this time for 50 percent equilibration and for apparent permeability, assuming a single compartment of constant volume exchanging with a large volume (plasma) of constant composition, are as follows:

\[ dQ_a = Va \frac{dC_a}{dt} = p'(A'C_b - C_a) \]

where \( dQ_a \) is the amount of a given molecular species in the alveolar liquid (in moles), \( Va \) is the volume of the compartment (in milliliters), \( C_a \) is the concentration of a given molecular species in the alveolar liquid (in moles per liter), \( p' \) is the permeability constant (in centimeters per second), \( A \) is the surface area, and \( C_b \) is the constant concentration of the molecular species in the plasma (in moles per liter).

By integrating this equation,

\[ \ln \frac{C_a - C_b}{C_b} = \frac{(Ap')t}{Va} \]

By plotting \( \log \left( \frac{C_a - C_b}{C_b} \right) \) against time, the time for 50 percent equilibration (T%) can be determined.

At that time,

\[ p' = \frac{(0.693)(Va)}{(T\%)(A)} \]

Least-squares regression analysis was performed on a programmable calculator (Monroe 1860). Any individual experiments in which the coefficient of correlation for T% was less than 0.9 were discarded. Because the calculation of permeability constant (p') depends on several assumptions, we report all results in values for the time for 50 percent equilibration.

RESULTS

Hemodynamic and Physiologic Data

Hematocrit readings, systemic blood pressure, mean pulmonary arterial pressures, and pulmonary wedge pressures (Table 1) remained relatively constant over time in all groups, although in group 1, the systolic blood pressure slowly decreased over the course of the experiment. In addition, hematocrit readings in group 2 were higher than the other groups but did not change over time. As expected,

<table>
<thead>
<tr>
<th>Group and Time</th>
<th>Hematocrit Reading, percent</th>
<th>Blood Pressure, mm Hg</th>
<th>Mean Pulmonary Arterial Pressure, mm Hg</th>
<th>Pulmonary Wedge Pressure, mm Hg</th>
<th>QS/Qr, percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (pH of 1.5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>42±2</td>
<td>177±30</td>
<td>125±20</td>
<td>18±2</td>
<td>11±3</td>
</tr>
<tr>
<td>30-60 minutes after instillation of acid</td>
<td>41±2</td>
<td>167±35</td>
<td>114±22</td>
<td>20±2</td>
<td>10±2</td>
</tr>
<tr>
<td>End of experiment</td>
<td>41±2</td>
<td>163±32</td>
<td>121±18</td>
<td>23±2</td>
<td>8±3</td>
</tr>
<tr>
<td>Group 2 (pH of 2.5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>49±1</td>
<td>150±5</td>
<td>90±5</td>
<td>14±2</td>
<td>7±3</td>
</tr>
<tr>
<td>30-60 minutes after instillation of acid</td>
<td>50±1</td>
<td>150±5</td>
<td>100±5</td>
<td>22±2</td>
<td>9±2</td>
</tr>
<tr>
<td>End of experiment</td>
<td>50±1</td>
<td>157±15</td>
<td>90±7</td>
<td>25±2</td>
<td>8±4</td>
</tr>
<tr>
<td>Group 3 (pH of 3.5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>36±4</td>
<td>150±5</td>
<td>100±5</td>
<td>18±2</td>
<td>6±2</td>
</tr>
<tr>
<td>30-60 minutes after instillation of acid</td>
<td>35±4</td>
<td>153±7</td>
<td>88±9</td>
<td>18±3</td>
<td>9±3</td>
</tr>
<tr>
<td>End of experiment</td>
<td>37±6</td>
<td>158±26</td>
<td>90±8</td>
<td>20±4</td>
<td>12±4</td>
</tr>
<tr>
<td>Group 4 (control; pH ≥ 4.5)</td>
<td>35±4</td>
<td>160±5</td>
<td>100±7</td>
<td>17±2</td>
<td>6±2</td>
</tr>
<tr>
<td>Baseline</td>
<td>35±4</td>
<td>162±10</td>
<td>90±10</td>
<td>18±2</td>
<td>7±2</td>
</tr>
<tr>
<td>30-60 minutes after instillation</td>
<td>35±4</td>
<td>156±7</td>
<td>92±6</td>
<td>18±3</td>
<td>6±3</td>
</tr>
</tbody>
</table>

*Table values are means ± SD.

202 GLAUSER, MILLEN, FALLS

CHEST, 76: 2, AUGUST, 1979
the arterial oxygen pressure decreased significantly following instillation of pulmonary liquid in all groups; this was reflected by an increase in shunt from an average of 8 ± 2 percent to 51 ± 4 percent (all groups combined at 30 to 60 minutes after instillation of pulmonary liquid). Just prior to termination of the experiments, the shunt (for all groups combined) had increased to 54 ± 5 percent (not significant).

**Time for 50 Percent Equilibration**

Values for the time for 50 percent equilibration of albumin between the blood and pulmonary liquid (Table 2) decreased from 3,653 ± 753 minutes in the control group 4 to 527 ± 90 minutes in group 1 and 861 ± 154 minutes in group 2 (all P < 0.05); in group 3 (pH of 3.5), the values did not change significantly compared to control values. Times for 50 percent equilibration of dextran paralleled those for albumin, as there was a significant decrease for groups 1 and 2, compared to control. Interestingly, the times for 50 percent equilibration were longer for all groups (except group 3) for dextran, compared to control. Since it could be argued that instillation of such large dosages of an acidic solution (3 to 5 ml/kg) into a small area of the lung may not be clinically relevant and could decrease the applicability of this method to clinical problems, we performed three additional studies as follows: Animals were prepared in the usual manner (as discussed previously), and following the induction of atelectasis of the left lung, a 0.9 percent solution of sodium chloride with a pH of 1.5 was infused into the right lung at a dosage of 3 to 5 ml/kg. Thirty minutes later, 80 to 90 ml of a 0.9 percent solution of sodium chloride with a pH of 4.5 or greater was also infused into this lung, and samples of liquid and blood were taken every 30 minutes for determination of times for 50 percent equilibration of albumin and dextran with a molecular weight of 150,000 to 170,000. This variation in our model was studied to confirm that lower, more clinically relevant dosages of acidic aspirate would induce the same change as the larger dosages (3 to 5 ml/kg). The time for 50 percent equilibration of albumin was 364 ± 147 minutes and for dextran was 427 ± 107 minutes. Neither value is significantly different from group 1 (discussed previously). We thus conclude that instillation of pulmonary liquid at a dosage of 3 to 5 ml/kg causes the same changes in permeability as smaller (1% ml/kg), more clinically relevant dosages.

**Levels of Histamine**

Levels of histamine in the blood (Table 3) were comparable in all groups (although tending to be higher [not significantly] in group 2) and did not change significantly following instillation of pulmonary liquid. Levels of histamine in the pulmonary liquid in group 1 (pH of 1.5) were significantly increased at all times, when compared to all other groups (P < 0.01). Levels of histamine in the pulmonary liquid in group 2 (pH of 2.5) were lower than in group 1 but higher (P < 0.05) than in groups 3 and 4. When the remaining groups were compared with regard to levels of histamine in the pulmonary liquid at comparable times, there was no significant difference between groups 3 and 4.

**DISCUSSION**

The physiologic consequences of aspiration of acid were studied to confirm that lower, more clinically relevant dosages of acidic aspirate would induce the same change as the larger dosages (3 to 5 ml/kg). The time for 50 percent equilibration of albumin was 364 ± 147 minutes and for dextran was 427 ± 107 minutes. Neither value is significantly different from group 1 (discussed previously). We thus conclude that instillation of pulmonary liquid at a dosage of 3 to 5 ml/kg causes the same changes in permeability as smaller (1% ml/kg), more clinically relevant dosages.

**Levels of Histamine**

Levels of histamine in the blood (Table 3) were comparable in all groups (although tending to be higher [not significantly] in group 2) and did not change significantly following instillation of pulmonary liquid. Levels of histamine in the pulmonary liquid in group 1 (pH of 1.5) were significantly increased at all times, when compared to all other groups (P < 0.01). Levels of histamine in the pulmonary liquid in group 2 (pH of 2.5) were lower than in group 1 but higher (P < 0.05) than in groups 3 and 4. When the remaining groups were compared with regard to levels of histamine in the pulmonary liquid at comparable times, there was no significant difference between groups 3 and 4.

**Table 2—Times for 50 Percent Equilibration between Levels in Blood and in Pulmonary Fluid**

<table>
<thead>
<tr>
<th>Group and Time</th>
<th>Time for 50 Percent Equilibration, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>527 ± 90**</td>
</tr>
<tr>
<td>Group 2</td>
<td>861 ± 154**</td>
</tr>
<tr>
<td>Group 3</td>
<td>3,466 ± 1,364</td>
</tr>
<tr>
<td>Group 4</td>
<td>3,653 ± 753</td>
</tr>
</tbody>
</table>

*Table values are means ± SE.
**P < 0.05, compared to control (group 4).

**Table 3—Levels of Histamine at Various Times after Instillation of Acid**

<table>
<thead>
<tr>
<th>Group and Time</th>
<th>Group and Time</th>
<th>Blood</th>
<th>Pulmonary Liquid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>0.30 ± 0.13</td>
<td>0.09 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>30-75 min</td>
<td>0.17 ± 0.02</td>
<td>1.80 ± 0.54**</td>
<td></td>
</tr>
<tr>
<td>90 min</td>
<td>0.16 ± 0.02</td>
<td>1.60 ± 0.40**</td>
<td></td>
</tr>
<tr>
<td>120-150 min</td>
<td>0.21 ± 0.03</td>
<td>0.22 ± 0.05</td>
<td></td>
</tr>
</tbody>
</table>

*Table values are means ± SE.
**P < 0.05, compared to control (group 4).
acid in the experimental animals have been well described and include (depending on the dosage of aspirate and the experimental design) transient apnea, rapid onset and progression of hypoxemia, hypocapnia, decreased compliance, hemoconcentration, decreased cardiac output, and metabolic acidosis. Pathologic evaluations of aspiration of acid at various times following the insult has revealed evidence of alveolar collapse, intra-alveolar edema, destruction of type-I alveolar lining cells, bronchial epithelial degeneration, exudation of fibrin, and intracapillary polymorphonuclear trapping.1,2,11-14

From a combination of these physiologic and pathologic changes, it has been postulated that aspiration of acid leads to disruption of the pulmonary alveolar capillary membrane, with exudation of plasma from the pulmonary microvasculature. Quantitative evaluation related to the molecular weight or size of substances which flow from the pulmonary capillaries has not been reported, except for the study of Awe et al,2 who found that the composition of the bronchiolar secretion in response to the aspiration of hydrochloric acid (pH of 1.0), when evaluated by electrophoresis, paralleled the levels of albumin and globulin that were found in the plasma. Their results indicate that between 1 and 45 minutes after aspiration of acid, the levels of albumin and globulin have already reached maximum in the bronchiolar secretions. Our results would tend to confirm and extend these results in a quantitative manner, in that we found a marked decrease in the time for 50 percent equilibration (an increase in alveolar epithelial permeability) for substances with molecular weights up to 170,000 daltons (molecular radius of approximately 100 A) with pulmonary aspiration of liquids with a pH of 1.5 and 2.5.

As noted, the time for 50 percent equilibration for both albumin (molecular weight of 69,000 daltons and molecular radius of 35 A) and dextran with a molecular weight of 170,000 decreased progressively from a pH of 3.5 to a pH of 1.5. In addition, in animals from group 3 that had an aspirate with a pH of 3.5, there was no significant difference in the time for 50 percent equilibrations, as compared to control values (groups 4), for either albumin or dextran with a molecular weight of 170,000. When the times for 50 percent equilibration of albumin were compared to those for dextran with a molecular weight of 170,000 in each group, the times tended to be longer for the substances with larger molecular radii (Table 2). Thus, it can be seen that with progressive decreases in pH to 2.5 or below, there are decreases in the time for 50 percent equilibration that are significant compared to baseline values.

The cause of this increased alveolar epithelial permeability associated with aspiration of acid would seem to be a direct burn injury to the alveolar epithelium;11 however, it is possible that chemical mediators such as histamine could play a role in increasing permeability.3 To test this hypothesis, we measured levels of histamine in the pulmonary liquid in all groups and found a marked increase in levels of histamine in group 1 (pH of 1.5) (and a lesser increase in group 2, with a pH of 2.5), when compared to all other groups (Table 3). It is therefore possible that the release of histamine that is associated with aspiration of acid may play a role in the increased alveolar epithelial permeability. Lerner et al15 found that with aspiration of acid (3 mg of a 0.1N solution of hydrochloric acid per kilogram of body weight in dogs), there were transient increases in the levels of histamine base in the blood, but pretreatment of the animals with H1 or H2 histamine-receptor antagonists had no effect on the physiologic changes associated with aspiration of acid. Although we could not find any increase in the levels of histamine in the blood, our fluorometric method may not be sensitive enough to detect such small changes.

We thus conclude that our experimental model reproducibly measures increased alveolar epithelial permeability associated with aspiration of acid (pH of 2.5 or less) and may be useful in measuring the effects of steroids in the treatment of aspiration of acid. In addition, the role of pulmonary histamine in inducing the increased alveolar epithelial permeability needs to be further elucidated.

ACKNOWLEDGMENT: Keith Wright, B.S. provided technical assistance.

ADDENDUM

Since acceptance of this article for publication, it has been reported that in the unanesthetized rabbit, acid aspiration with a pH of less than 2.5 causes an increase in alveolar capillary membrane permeability to small molecular weight substances when traced from the lung liquid to the blood. These investigators found no increased permeability with pH of approximately 3 (Jones JG, Berry M, Huland GH, et al: The time course and degree of change in alveolar-capillary membrane permeability induced by aspiration of hydrochloric acid and hypotonic saline. Am Rev Respir Dis 118:1007-1013, 1978).

REFERENCES

2 Awe WC, Fletcher WS, Jacob SW: Pathophysiology of aspiration pneumonia. Surgery 60:232, 1966

204 GLAUSER, MILLEN, FALLS

CHEST, 76: 2, AUGUST, 1979

SECOND PAN AMERICAN CONGRESS ON DISEASES OF THE CHEST
Sponsored by the Brazil Chapters of the International Academy of Chest Physicians and Surgeons affiliated with the American College of Chest Physicians
Hotel Nacional, April 19-23, 1980 Rio de Janeiro—Brazil

PRELIMINARY SCIENTIFIC PROGRAM

SYMPOSIA
Chagas' Disease
Cardiomyopathies
Surgical Indications and Late
Results in Heart Diseases
Progress in Cardiology
Short Course Chemotherapy for
Tuberculosis
Bronchial Asthma
Nonembolic Pulmonary Vascular
Diseases
Occupational Lung Diseases
Trauma of the Thorax
Surgery for Acquired Heart Disease
Cancer of the Lung
Surgery for Congenital Heart Disease

MAJOR LECTURES
A special address on the most important advances in chest diseases by a renowned guest lecturer will be held on each of the four working days.

POSTGRADUATE COURSES
PULMONARY MEDICINE
Clues of Lung Mechanics to the
Practicing Chest Physician
Scientific Basis of Physical Therapy
in Chest Medicine
Updating Physical Diagnosis in
Chest Medicine
Progress in Pulmonary Radiology and
Radioisotope Scanning

CARDIOTHORACIC SURGERY
Technological Advances in
Cardiovascular Surgery
Direct Diagnostic Procedures in
Chest Diseases
Controversies in Cardiac Surgery
Technical Refinements in General
Thoracic Surgery

Each of the 12 subjects listed above will include five lectures, given as ORIGINAL INVESTIGATIONS.

A daily continuing forum will provide a review of current work being done in respiration, circulation and allied diseases.

Send four copies of 200-word abstract to Dr. Stans Murad-Netto, Chairman, Scientific Program, Send Pan American Congress on Diseases of the Chest, Caixa Postal 370, Rio de Janeiro, R.J., 20000, Brazil.

SIMULTANEOUS TRANSLATION WILL BE OFFERED IN ENGLISH, SPANISH AND PORTUGUESE.

For information concerning the congress write Segundo Congresso Pan Americano de Doencas do Torax, Caixa Postal 370, 20000 Rio de Janeiro, R. J., Brazil.

CHEST, 76: 2, AUGUST, 1979

EFFECTS OF ACID ASPIRATION 205