Measurement of Pulmonary Edema

By Morton Lee Pearce, M.D., Joe Yamashita, B.A., and James Beazell, B.A.

Although it has been suggested recently that an increase of airway pressure with negligible change in compliance is a useful method for the serial measurement of the volume of pulmonary edema in guinea pigs, the quantification of pulmonary edema has depended largely on postmortem methods. These techniques usually compare lung and body weights or the wet and dry weights of the lungs, and permit only one measurement to be made after death. An indicator dilution method suggested by Chinard and others permits serial in vivo measurements to be made in human subjects as well as animals. With this technique, indicator dilution curves are made by the simultaneous injection of two labels: one for total water estimation, such as tritiated water (THO), and the other for plasma volume, such as tagged human albumin (RISA). The pulmonary extravascular water volume (PEWV) is computed from the difference in mean transit times of the two indicators multiplied by cardiac output.

The experiments reported here were done to evaluate this indicator dilution method for measuring PEWV by comparing it with a postmortem method in which the water in the pulmonary blood and the pulmonary extravascular space are estimated separately. Four groups of dogs were studied: (a) controls, (b) a group with pulmonary edema induced by alloxan with little reduction of PaO₂ during breathing of 100% oxygen, (c) a group with alloxan-induced pulmonary edema with marked reduction of PaO₂, and (d) a group with pulmonary edema caused by elevation of pulmonary venous pressure.

Although opening the chest and applying positive pressure breathing may alter the course of pulmonary edema, our desire to compare the two methods as nearly simultaneously as possible made this maneuver necessary. The dynamics and localization of pulmonary edema are important problems which are the subject of another report.

Methods

Mongrel dogs weighing 15 to 25 kg were anesthetized with morphine (1 mg/kg sc), followed in one-half to one hour by chloralose (100 mg/kg iv). Additional increments of chloralose were given as needed. A cuffed endotracheal tube was inserted and artificial respiration with 100% oxygen was provided by a respiratory pump. Paralytic drugs were not used. Respiratory rate and tidal volume were maintained so that the arterial PCO₂ was kept in a range of 30 to 40 mm Hg. A catheter for isotope injection was inserted into the right atrium. Another catheter with a dead space of 1.9 ml was inserted into the aorta to monitor arterial pressure and to sample arterial blood for the measurement of PO₂, PCO₂, and pH. Samples required for the indicator dilution curves were also collected from this catheter. The dogs' chests were opened with sternal splitting incisions. Atelectasis was avoided by increased inflation of the lungs every five minutes. Heavy ligatures were placed around the lung hila. As soon as the indicator dilution curve was completed, the ligatures were tightened and the lungs were removed, weighed, and inspected. In the experiments in which pulmonary edema was produced by elevating pulmonary venous pressure, another catheter was sewn into the left atrium and the pressure monitored through a strain gauge.

Total lung water was measured and its partition between blood and pulmonary tissue was determined by a variant of a technique described by Hemingway. A measured amount of water was added to the whole lungs, which were then homogenized in a Waring blender.
Aliquots were weighed and evaporated to constant weight in a heated sand bath at 85°C. Other aliquots were centrifuged in an International no. 2 centrifuge at 2500 rpm for 30 minutes. Samples of this fluid were pipetted and weighed. Initially, these solutions remained turbid despite repeated centrifuging. It was found that sodium lauryl sulfate added in a final concentration of 1% completely cleared the solutions.* Furthermore, the spectrophotometric properties of oxyhemoglobin and cyanmethemoglobin were not changed, and added hemoglobin could be recovered completely. Hemoglobin was measured in a Beckman DU spectrophotometer on the cleared supernatant fluid both as oxyhemoglobin and cyanmethemoglobin. A blood specimen taken at the time of the indicator dilution curve was also measured for hemoglobin concentration and blood density and wet and dry weights were determined on this systemic blood sample. In other experiments, total pulmonary hematocrit was measured in normal dogs and in dogs with alloxan-induced pulmonary edema by simultaneously injecting Cr²⁺ tagged red cells and I²¹² tagged human albumin. These experiments confirmed the work of Rapaport et al.¹⁰ and a factor of 1.055 was used to correct for the difference between systemic large vessel and lung hematocrit. The calculations employed are as follows:

(1) Wt of lung water + added water =
\[
\frac{(\text{wt of lungs + added water}) \times \text{wet wt} - \text{dry wt}}{\text{wet wt}}
\]

(2) Wt of blood in lungs =
\[
\frac{\text{concentration of homogenate Hgb}}{\text{concentration of blood Hgb}} \times \frac{(\text{wt of lungs + added water}) \times \text{sp. gr. blood}}{\text{sp. gr. homogenate} \times \text{pulmonary hematocrit correction}}
\]

(3) Water in blood =
\[
(\text{wt of lungs + added water}) \times \text{lung water - blood water}
\]

Following a method suggested by Ramsey et al., cardiac output and the mean transit time of the intravascular and water tags were measured by the rapid (<1 second) injection of a mixture of 5 µc of I²¹² tagged human albumin and 50 µc of tritiated water into the right atrium. Serial arterial blood specimens were collected from the aortic catheter in one of two fraction collectors* at 1.5-second intervals. Blood flow during the collections in the first collector ranged from 1.5 to 3.0 ml per 1.5-second sample, but were equal in individual runs. Blood flow was set at 2.0 ml per 1.5-second sample in the second collector. Aliquots of 0.5 ml were pipetted into test tubes and, together with standards made by diluting the injectate with the animals' blood, were counted twice for three minutes in a Packard auto-gamma counter. Ten ml of absolute ethyl alcohol were then added to each of these tubes and they were shaken vigorously in a vortex shaker for three minutes and centrifuged. Two ml of the clear supernatant fluid were pipetted into 10 ml of a scintillation mixture and these samples were counted twice for 10 minutes in a Packard tri-carb scintillation counter. The counts were corrected for background and normalized as fraction of injectate per liter of blood by dividing the corrected count obtained for each specimen by the injectate value. These values were then plotted on semilogarithmic paper to correct for recirculation by extrapolation. The more obvious onset of recirculation of RISA was used to determine the onset of recirculation of THO. Cardiac output and mean transit time were determined for each of the two curves by the method of Hamilton et al.¹² The RISA curve was used for the flow measurement in the computation of mean transit time. However, the THO and the RISA cardiac outputs were almost the same. When we injected the two isotopes into the left atrium, the aortic dilution curves were the same, suggesting that the longer mean transit time of THO, with respect to RISA, is due to its passage through the pulmonary capillary bed (see also Ramsey et al.¹¹)

Four groups of dogs were studied. The control group of six dogs was maintained with chests open for 15 to 40 minutes before the indicator dilution curve was obtained and the lung hila were ligated. Two groups of dogs were studied after pulmonary edema had been produced by the intra-

*Suggested by Dr. Norman Simmons, Department of Nuclear Medicine, University of California at Los Angeles.

Circulation Research, Vol. XVI, May 1965

*Two collectors were employed. In the first,¹¹ the blood flowed freely. The second, constructed by F&H Machine Works, Culver City, California, drew measured anaerobic samples into a series of 2-ml syringes attached to a 30-sample manifold.

14 g 2,5-diphenyloxazole and 0.15 g 1, 4-bis-2-(5 phenyloxazo)-benzene in one liter of toluene.
Pulmonary Extravascular Water Volumes (PEWV) and Related Parameters in Pulmonary Edema (means ± se)

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of dogs</th>
<th>Cardiac output ml/min/kg*</th>
<th>Indicator dilution PEWV ml/kg*</th>
<th>THO recovered as per cent of RISA %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>57 ± 5.0</td>
<td>2.78 ± 0.22</td>
<td>97.7 ± 1.2</td>
</tr>
<tr>
<td>High pressure edema</td>
<td>6</td>
<td>233 ± 15.54</td>
<td>4.75 ± 1.36</td>
<td>98.3 ± 2.20</td>
</tr>
<tr>
<td>Alloxan edema without marked shunting</td>
<td>6</td>
<td>69 ± 6.51</td>
<td>3.12 ± 0.35</td>
<td>97.4 ± 3.50</td>
</tr>
<tr>
<td>Alloxan edema with marked shunting</td>
<td>5</td>
<td>63 ± 9.10</td>
<td>2.71 ± 0.62</td>
<td>86.3 ± 2.1*</td>
</tr>
</tbody>
</table>

Significance of differences of means from control group by t test: °P > 0.2. *0.2 < P > 0.1. †0.1 < P > 0.05. ‡0.05 < P > 0.01. §P < 0.01.

*kg implies per kilogram body weight.
†Pulmonary extravascular water volume.

venous injection of alloxan. The studies were done 10 to 40 minutes after giving the alloxan. The first group of six animals was given 50 mg/kg, while a second group of five was given 75 or 100 mg/kg. The second group showed marked reductions of PaO₂ despite 100% oxygen breathing (PaO₂ less than 100 mm Hg), as compared with their control values. At autopsy dependent, firm, congested areas in the lungs were prominent. These areas were not examined histologically and therefore it cannot be stated that they indicated atelectasis.

In another group of six dogs, pulmonary edema was produced by the intravenous infusion of one liter of 6% dextran, followed by 0.002% solution of epinephrine at a rate which elevated the mean left atrial pressure to 20 to 25 mm Hg and systemic pressure into the range of 250 to 350/190 to 250 mm Hg. Indicator dilution determinations were made approximately 10 minutes after the beginning of the epinephrine infusion. Arterial pressure was monitored in all experiments and PaO₂, PaCO₂, and pH were checked at frequent intervals. Except for the second or "severe" alloxan group, the animals were maintained in good condition throughout the experiments.

Results

The results of the four groups of experiments are presented in tables 1 and 2 and in figures 1 and 2. Using RISA as a reference, we accounted for approximately 98% of the injected THO in the controls, in the high pressure pulmonary edema group and in the first or "mild" alloxan group, but we accounted for only 86% of the injected THO in the "severe" alloxan group.

Using the postmortem determination of PEWV as a reference, the isotope dilution method accounted for 71% of the PEWV in controls, 94% in high pressure edema, and 57% in the first group of alloxan edema. In the "severe" alloxan group, only 31% of the gravimetrically measured PEWV was detected by the isotope dilution method. These differences are statistically significant.

Cardiac output averaged 57 ml/min/kg in controls, 233 ml/min/kg in high pressure edema, and 69 ml/min/kg in "mild" alloxan edema. In the "severe" alloxan group, cardiac output averaged 63 ml/min/kg. The increase of output in the high pressure group is significant.

The lungs and their contained blood weighed 1.41% of total body weight in the controls, 1.92% in high pressure edema, 1.71% in the first group of alloxan edema and 2.01% in the "severe" alloxan group. As compared with the control group, the increases are significant in all the edema groups.

The postmortem water content of the lungs averaged 78.9% in controls, 83.1% in high pressure pulmonary edema, 80.9% in the first alloxan group and 83.3% in the "severe" alloxan group. The standard error of this measurement is small and the differences of

Circulation Research, Vol. XVI, May 1965
MEASUREMENT OF PULMONARY EDEMA

Lung weight

<table>
<thead>
<tr>
<th>Group</th>
<th>Lung weight (g/kg)</th>
<th>Lung blood (ml/kg)</th>
<th>Per cent lung weight as water</th>
<th>Postmortem PEWV (ml/kg)</th>
<th>Ratio of indicator PEWV to postmortem PEWV × 100%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14.1 ± 0.7</td>
<td>8.51 ± 0.66</td>
<td>78.8 ± 0.6</td>
<td>3.93 ± 0.25</td>
<td>70.5 ± 4.8</td>
</tr>
<tr>
<td>High pressure edema</td>
<td>19.2 ± 1.87a</td>
<td>12.10 ± 1.36a</td>
<td>83.1 ± 0.524</td>
<td>4.98 ± 0.581</td>
<td>94.0 ± 5.44</td>
</tr>
<tr>
<td>Alloxan edema without marked shunting</td>
<td>17.1 ± 1.03a</td>
<td>9.64 ± 1.039</td>
<td>80.9 ± 0.34</td>
<td>5.49 ± 0.493</td>
<td>56.5 ± 3.03</td>
</tr>
<tr>
<td>&quot;severe&quot; alloxan group</td>
<td>20.1 ± 1.44</td>
<td>8.57 ± 0.349</td>
<td>83.3 ± 0.344</td>
<td>9.39 ± 0.974</td>
<td>31.4 ± 9.14</td>
</tr>
</tbody>
</table>

The edema groups from the control group are highly significant. The pulmonary blood volume measured after death averaged 8.21 ml/kg in controls, 12.1 ml/kg in high pressure edema, 9.64 ml/kg in the "mild" alloxan group, and 8.57 ml/kg in the "severe" alloxan group. Only the increase in the high pressure group is significant.

In figure 1 the PEWV per kg body weight determined by the postmortem method is plotted against the isotope dilution values. The linear regression of the postmortem PEWV on the isotope PEWV was computed by least squares. The regression coefficients are presented in table 2. The intercepts are all significantly greater than zero. The intercepts of the two edema groups are not significantly different from the control group. The slope of the "mild" alloxan edema group is significantly different from the control slope (P < 0.05). Figure 2 presents results from the "severe" alloxan group.

TABLE 2
Least Squares Regression of the Postmortem Extravascular Water Volume on the Isotope Dilution Measurement. (means ± se)

<table>
<thead>
<tr>
<th>Group</th>
<th>Slope</th>
<th>Intercept</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.69 ± 0.35</td>
<td>2.02 ± 1.01</td>
</tr>
<tr>
<td>High pressure edema</td>
<td>0.75 ± 0.14</td>
<td>1.41 ± 0.88</td>
</tr>
<tr>
<td>Alloxan edema without marked shunting</td>
<td>1.29 ± 0.28</td>
<td>1.48 ± 0.90</td>
</tr>
</tbody>
</table>

Discussion

The validity of flow and compartment measurements by indicator dilution techniques has been discussed extensively. The recovery of 98% of injected THO as compared with RISA in the experiments reported here (except for the "severe" alloxan group) suggests that equilibration with the measured pulmonary extravascular water space is virtually complete and that THO diffusion out of the pulmonary capillaries and back is rapid. Whether the residual 2% is due to pulmonary lymphatic flow or equilibration with a second space with a much slower time constant is not clear. The diffusion constant of O18 tagged water in water at 37°C is 4.05 X 10^-6 cm² per second.14 Assuming that there is no restriction to diffusion at the alveolar membrane, it can be calculated that the injected THO will come into 99% equilibrium with the pulmonary extravascular water in less than one second after its arrival in the pulmonary capillary bed if the longest path from the capillary blood is not greater than 0.2 mm. This is well within the range of alveolar size, even if the alveoli are completely filled with edema fluid. Parts of the alveolar ducts could also be equilibrated.

The volume measured by this technique in normal dogs is proportionally smaller than that measured by Cander and Forster15 in human beings by means of acetylene or nitrous oxide and various periods of breath.
holding. However, their technique measures exchanges through the surface layers of the conducting airways in addition to those of the alveoli.

Since the isotope dilution method cannot be expected to measure water which is a part of the larger airways, blood vessels, and perivascular lymphatics, it is suggested that a large fraction of the total extravascular water of the lungs is in the alveolar walls or close to them. The observation that the fraction of total water measured in the alloxan group is less than the others suggests that perfusion of parts of the lungs is diminished in alloxan edema as compared with the controls, or possibly that the lymphatic volume is increased. Perfusion of edematous portions must have been particularly poor in the "severe" alloxan group. On the other hand, the observation that there is less unmeasured water in high pressure pulmonary edema suggests that there may have been better perfusion of all parts of the lungs in this group than in the others.

The preparation we employed is far from physiological; the dogs were supine, they had open chests and positive pressure breathing was employed. It is possible that the results obtained here may not even apply to dogs with closed chests, let alone to human subjects. This is particularly a cause for concern in the alloxan group. The values for PEWV obtained in the open-thorax dogs by the isotope dilution technique are smaller than those which were obtained in another set of experiments in which pulmonary edema was also produced by alloxan, but in closed chest, spontaneously breathing dogs.7 Preliminary experiments* with intravenous radioactive xenon suggest that the uppermost portions of the lungs are poorly perfused in closed chest supine dogs breathing spontaneously. However, even with these reservations, the data in figure 1 suggest that the isotope dilution method has value in the serial quantification of pulmonary edema,

---

* N. C. Staub, and M. L. Pearce (unpublished data).

Circulation Research, Vol. XVI, May 1965
particularly when the latter is due to elevated pulmonary venous pressure.

When the isotope derived values are plotted against the postmortem ones, there is a slope-intercept of 1.56 ml/kg. This value represents probably the portion of the lungs which cannot be equilibrated with the isotope in a single transit, while the variations of slope in the different groups represent varying degrees of completeness of perfusion of tissues in close proximity to the alveolar capillaries.

The postmortem technique for measuring the total blood and extravascular water in the lungs seems to be reliable, although the partition into these two volumes requires some justification. The technique which we employed for tissue hemoglobin depends upon the clearing effect of sodium lauryl sulfate which is due presumably to the dispersion of large molecular aggregates. The objection to the use of a systemic blood sample as a reference for the concentration of hemoglobin in the pulmonary blood is met by a correction for pulmonary hematocrit (a relatively small correction in any case). In another group of experiments, our values for pulmonary hematocrit in normal dogs and in dogs with pulmonary edema were similar to those reported by Rapaport et al. Since the partition of lung water depends on the measurement of pulmonary blood water content, it follows that our method for determining the fraction of lung water which is outside of the pulmonary blood volume is also acceptable.

The data for pulmonary blood volume as determined by the postmortem method are interesting. The mean value of our controls, 8.2 ml/kg, is similar to the values reported by Kuno and deBurgh Daly who also employed postmortem methods involving the extraction and measurement of lung hemoglobin. On the other hand, McGaff and Milnor found a mean value for pulmonary blood volume in the dog of 11.1 ml/kg, but they used a method in which the difference in mean transit time between the pulmonary artery and the femoral artery was multiplied by cardiac output. Their method should give larger values because: (1) it measures blood in the left atrium and main pulmonary artery, (2) their dogs were breathing spontaneously with closed chests and negative intrapleural pressure and (3) cardiac outputs in their experiments were higher than the outputs in ours.

The pulmonary blood volume was elevated in our high pressure edema group. In previous experiments on spontaneously breathing dogs with closed chests, pulmonary capillary blood volume and central blood volume were also elevated.

Our postmortem values for pulmonary blood volume show no significant change in alloxan edema. This result is not in agreement with that of Aviado and Schmidt who found an increase of pulmonary blood volume when using a technique in which P tagged red blood cells were allowed to equilibrate in the animal's blood volume and were then counted in a lung segment. Although these differences may be due to our use of pressure breathing in animals with open chests, Staub et al. demonstrated reductions of pulmonary capillary blood volume (Vp) and central blood volume in alloxan pulmonary edema in dogs breathing spontaneously.

The values presented here for lung weight as a percentage of total body weight, and for lung water content, are similar to those presented in the literature. Although the differences in the means are small, the calculation of lung water content as a percentage of lung weight provides a better measure of pulmonary edema than does the lung weight: body weight ratio.

**Summary**

Two methods for the measurement of pulmonary edema are compared. The first method, which permits serial measurements in vivo, depends on the difference in mean transit time of a water label and an intravascular label. The second method is a postmortem
one in which total lung water is measured and allocated to the pulmonary blood or to pulmonary extravascular water.

Three groups of six dogs each were studied: controls, a group with pulmonary edema produced by elevating pulmonary venous pressure, and a group with pulmonary edema produced by alloxan. The isotopic as compared with the postmortem method accounted for 71% of the pulmonary extravascular water found in the controls, 94% in high pressure edema and 57% in alloxan edema. Shunting, as measured by a large fall in PaO₂ despite 100% oxygen breathing, became marked in a second group of five alloxan dogs and only 31% of the postmortem pulmonary extravascular water was measured by the isotope method.

It is concluded that the isotope transit time method is a useful one for the serial measurement of pulmonary edema in vivo. The edema water is determined more accurately when pulmonary edema is due to elevated pulmonary venous pressure than when due to increased vascular permeability.

Acknowledgment
We thank Mr. Clifton Davenport, Jr., for help with the surgery. We are grateful to Dr. Norman C. Staub for his critical reading of the manuscript.

References
Measurement of Pulmonary Edema
MORTON LEE PEARCE, JOE YAMASHITA and JAMES BEAZELL

Circ Res. 1965;16:482-488
doi: 10.1161/01.RES.16.5.482

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/16/5/482

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/