Regional Edema Formation in Isolated Perfused Dog Lungs

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SUMMARY Studies using gravimetric analysis of lungs of frozen animals have suggested that the differences in pulmonary microvascular pressure between non-dependent and dependent lung do not influence the formation of regional pulmonary edema. We wondered if the inability to detect variation in regional extravascular lung water (EVLW) was due to the slow freezing process and, therefore, reassessed the distribution of EVLW in vertically suspended isolated perfused dog lungs with a radioisotopic technique that does not require freezing. Total lung water (TLW), blood or intravascular lung water (IVLW), and EVLW were measured in absolute quantities using a positron camera and the positron-emitting isotopes C\(^{14}\)O as a blood label and H\(_2\)\(^{15}\)O as a total lung water label. Mean isotopic TLW in 17 lungs that were normal or moderately edematous (wet:dry ratio < 7) was 142 ± 9 (SE) ml compared to the gravimetric estimate of 148 ± 7 ml (r = 0.92) and isotopic EVLW was 64 ± 6 ml compared to the gravimetric estimate of 70 ± 6 ml (r = 0.8). Analysis of the distribution of regional isotopically measured EVLW in the 17 lungs in various states of spontaneous edema formation revealed a small non-dependent to dependent, gravity-related increase in percent regional EVLW compared to percent regional TLW, which did not vary with the degree of edema in the lung. Serial measurements of absolute regional EVLW in four lungs during spontaneously developing edema also failed to show a disproportionate increase in accumulation of EVLW in any lung zone. Thus, despite the wide variation in microvascular hydrostatic pressure between top and bottom of the vertical isolated lung, edema formation seems to be uniform. Circ Res 48: 121-127, 1981

THERE are large differences in microcirculatory perfusion and pressure between non-dependent and dependent lung zones due to the influence of gravity. The usual assumption is that vascular hydrostatic pressure increases down the lung without a corresponding increase in perimicrovascular pressure so that a gradient develops for enhanced outward movement of fluid from basal lung vessels. Clinically, this seems the case, with edema formation most prominent in dependent parts of the lung of patients with high pulmonary vascular pressures. However, recent data for animals on the location of pulmonary extravascular water in normal lungs have not corroborated this observation (Naimark et al., 1971; Baile et al., 1979; Flick et al., 1979). These data have been accumulated using whole lung or animal freezing techniques with subsequent analysis of the wet-to-dry weight ratio of lung slices taken perpendicular to the gravity gradient. The wet-to-dry weight ratio, although persenting some problems, has been the gold standard of assessing lung water (Staub, 1974). Freezing the lung to allow it to be sectioned for determination of regional lung water is new, however, and has several potential problems. The freezing process is slow, must be done on a non-perfused lung, and the freezing itself is destructive to the lung. These features might confuse the resulting calculations of regional extravascular lung water (EVLW) and prevent recognition of a gravity gradient. Difficulties in correcting for regional intravascular lung water already have been found in frozen lungs and have resulted in correction of original results which showed greatest accumulation of EVLW in non-dependent lung (Baile, et al., 1976) to results now suggesting a rather uniform presence of EVLW (Baile et al., 1979; Flick et al., 1979).

Recently, a steady state radioactive tracer technique has been developed to assess qualitatively regional extravascular lung water volume and regional lung blood volume (Jones et al., 1976). This technique uses the positron-emitting isotopes, C\(^{15}\)O and H\(_2\)\(^{15}\)O, respectively, to label blood and lung water. In contrast to the freezing technique, they reported an increase in regional isotopic extravascular lung water in dependent vs. non-dependent lung. However, their distribution of regional extravascular lung water also correlated strongly with the distribution of perfusion. These investigators were unable to quantify total or regional lung isotopic extravascular water or blood and, thus, suspected they may have underestimated extravascular lung water in non-dependent lung where regional perfusion was poorest.

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Because of (1) the inherent appeal of the radioisotopic tracer technique with its non-invasive, non-destructive nature, which makes it of potential value in studies on humans; (2) the conflicting results about location of regional pulmonary extravascular lung water; and (3) uncertainty about the effect of freezing on regional lung water, we have reassessed the steady state dual isotope studies but with the addition of a positron camera. The use of a positron camera coupled to the steady state double isotope method allows quantification of the isotopically determined water values, since the camera images the entire lung at once and, through the use of coincidence detection, corrects for differential \( \gamma \)-ray absorption in various thicknesses of lung (Burnham and Brownell, 1972). Thus, we can evaluate directly the steady state double isotope values for lung water as compared to the wet-to-dry weight estimate. If the comparison is favorable, then analysis of regional distribution of isotopic lung water would allow an accurate examination of lung water regionally by a method not dependent on freezing the lung.

**Methods**

**Imaging System**

The positron camera consists of two banks of 127 NaI (TI) crystals that are mapped by 72 photomultiplier tubes and cover a field of 10" x 12". Each face has honeycomb lead collimators that limit the solid angle of one crystal on the reference side in such a way that it can view only 25 crystals of the opposite face.

A MODCOMP computer system was used to collect and process the scintigrams. Other details of the positron camera and the computer system have been described elsewhere (Burnham and Brownell, 1972; Alpert et al., 1975). A positron emitter gives rise to two back-to-back \( \gamma \) rays of energy 511 keV, and the positron camera imaging is based on coincidence counting. Because of the coincidence imaging, all depths of lung tissue are imaged with equal sensitivity.

The ability of the camera to respond equally to all regions of the field of view was tested with the use of a uniform field source. This source, which was 1 cm thick and exceeded the size of the camera's field of view, was filled with 2 mCi \(^{68}\)GaEDTA in saline. Images were taken with the uniform source placed parallel to the two detector arrays on a plane at 40% and 60% of the 60-cm distance between the detectors. The computer then was directed to quantify counts from multiple 6 cm x 6 cm subsections of the image varying from top to bottom and generally conforming to the area normally occupied by the isolated perfused lung. Mean count rate per second varied by less than 5% among any of the 6 cm x 6 cm subsections and was random, with no consistent effect due to location of the subsection on the plane or to depth of the plane between the two camera detectors.

**Isolated Lung Preparation**

The isolated lung preparation used has been described in detail in another publication from this laboratory (Schmidt-Nowara et al., 1973). Mongrel dogs weighing 17-20 kg were anesthetized with intravenous sodium pentothal (30 mg/kg, iv), intubated, ventilated with room air with a volume-cycled animal respirator, infused with 0.5 liter of 6% dextran, and killed by exsanguination. The heart and lungs were removed rapidly from the chest. The lymphatics were cut and drained freely. The left lung was ligated at the hilum. A left atrial pouch was constructed while the remainder of the heart was discarded. Rigid glass cannulas of known volume per unit length were inserted into the right pulmonary artery and the atrial pouch serving as arterial in-flow and venous out-flow lines, respectively. The lungs were suspended in a Lucite box by the trachea so that the long axis of the lungs was vertical to the ground. The box and lungs were positioned between the two positron camera faces. The in-flow cannula was placed directly above the out-flow cannula and both cannulas were positioned parallel to the camera faces for purposes of imaging. The trachea and non-ligated right lung were ventilated with room air by a constant volume respirator with a tidal volume of 7.5 ml/kg of dog body weight at a frequency of 15 breaths per minute. The end expiratory pressure outlet was placed under 3 cm of water.

The isolated right lung was perfused at 0.16 ml/g per sec by the dog's own heparinized blood using a Pemco roller pump (model 1100-7). A gas mixture of \( O_2 \) and \( CO_2 \) was directed through the disc exchanging so as to maintain the \( PO_2 \) and \( PCO_2 \) of the blood returning to the lung in normal mixed venous range. Temperature of the exchanger was maintained thermostatically at 38°C by the water circulation. Ports in the in-flow and out-flow cannulas permitted blood sampling and pressure measurements. Vascular pressures were measured with zero reference at the lung hilum. A Statham pressure transducer and an Electronics for Medicine DR 8 recorder were used for monitoring and recording of pressures. The atrial pouch pressure was maintained between 2 and 4 mm Hg in all experiments. In these preparations the mean pulmonary arterial pressure was 17 ± 9.6 (sd) mm Hg, the mean pulmonary venous pressure was 3 ± 1.0 mm Hg. Mean peak tracheal pressure was 6 ± 1.5 mm Hg. With time, all the isolated lungs developed spontaneous edema. Prior to all studies, the lung was inflated transiently to 30 cm \( H_2O \) by occlusion of the expiratory port.

**Total Lung Water and Blood Volume Scintigrams**

Total lung water scintigrams were obtained by the addition of 5 mCi of oxygen-15 labeled water (\( H_2^{15}O \)) as the diffusible label into the perfusion circuit. Thirty-second scintigrams of the isolated
lung including in-flow and out-flow cannulas were taken sequentially for 6 minutes. The positron camera is capable of producing both the total counts in any image and the counts from any designated subsegment of the image. To do the latter, an electronic cursor is used to delineate the areas or regions of interest on the image from which the camera is to quantify counts. In each image, a region of interest was placed over the arterial line, the venous line, and the total lung. Figure 1 shows the decay-corrected time activity recorded by the positron camera from regions of interest over the arterial in-flow, venous out-flow and the whole lung after injection of H$_2^{15}$O into the in-flow line. The curves for these three sites are parallel after 3–4 minutes of circulation of the radioactive perfusate, indicating equilibration of H$_2^{15}$O in the system at that time. Samples of the exhaled gas were collected; the activity of the exhaled sample was less than 0.1% of the H$_2^{15}$O activity in the total lung image, and, therefore, activity in the alveolar air spaces was ignored. The volume covered by the region of interest placed on the arterial and venous lines was calibrated independently and was equal to 3 ml. At 6 minutes the activity in the in-flow and out-flow cannulas was within ±3% of each other in all instances.

Thus, the small regions of interest over the cannulas represent known volumes for which counts per unit volume or count rate density can be calculated. Since the count rate density is the same throughout the pool after equilibration has been reached, counts in any area in the H$_2^{15}$O scintigram can be converted to the volume or weight of the total lung water pool. Typically, the lung was subdivided into four zones of equal height from apex to base in order to quantify regional water formation in the lung. After decay of the H$_2^{15}$O radioisotope, blood volume similarly was obtained from the positron scintigrams after equilibration with 5–10 ml of blood labeled with C$^{15}$O. C$^{15}$O binds to hemoglobin and is an effective blood pool label (Glass et al., 1968). Extravascular lung water volume was calculated by subtracting the measured C$^{15}$O blood volume from the total lung water (H$_2^{15}$O) volume. This procedure is valid since the positron emission in both images is identical in energy and since counts are obtained with equal geometrical efficiency in both the lung water and blood volume image. The final intravascular, extravascular, and total lung water volumes were compared with the corresponding values obtained from wet:dry weight measurements of the perfused lung.

In four lungs on which sequential studies were performed, wet:dry measurements also were performed on the ligated left lung, which was assumed to have the same quantity of lung water as the perfused right lung at the beginning of the experiment.

When possible, Student's t-test for paired values was applied (Bahn, 1972).

**Postmortem Lung Water**

At the end of each experiment, the filled arterial and venous lines connected to the isolated lung were clamped at the point at which they entered the field of view of the camera. The preparation was removed from the Lucite box and blood was drained passively from the lung and cannulas. The perfused lungs were separated and weighed. The wet lungs were dried to a constant weight in an oven at 80°C to obtain the wet-to-dry weight ratio. In six lungs, correction for residual blood volume in the lung was made by the technique of Hemingway (1950) and Pearce et al. (1965). A measured amount of water was added to the whole lungs and homogenized in a blender. Aliquots were weighed and evaporated completely. Other aliquots were centrifuged at 2500 rpm for 30 minutes. Hemoglobin content of the supernatant was measured with a spectrophotometer. Hemoglobin content, hematocrit, and wet-to-dry weight ratios were also determined for drained blood. The amount of blood in the lungs and the volume of intravascular and ex-
travascular lung water were measured using the calculations of Pearce et al. (1965). These results were compared with those obtained by the radioisotope equilibration technique. In the six lungs in which intravascular blood volume was determined by the technique of Pearce et al. (1965), the correlation with the isotopic technique was excellent ($r = 0.93, P < 0.001$) (Fig. 2). Consequently, in the remaining 11 lungs, only the isotopic blood volume measurement was obtained and this value was used to calculate the extravascular water content of the lungs either by the gravimetric or isotopic method. Residual blood volume in the drained wet lung was calculated as the isotopic total blood volume of the lung minus blood drained from the lung prior to obtaining its wet weight.

**Results**

The range of lung wet-to-bloodless dry weight ratios studied was 3.3 to 11.1 g/g. Below a wet-to-dry weight ratio of 7, the correlation between the gravimetric technique and the isotope technique for total lung water was excellent ($r = 0.92, P < 0.001$) with an average error of 4% underestimation by the isotopic technique (Fig. 3). In eight lungs above a wet-to-dry weight ratio of 7, however, the average error between the two techniques was 28%, with the isotopic technique tending to underestimate the volume of total lung water present. The lungs with a wet-to-dry weight ratio in excess of 7 appeared grossly edematous and, on microscopic section, had alveolar flooding as has been noted at wet: dry ratio above 6.5 by other authors (Muir et al., 1972). In these lungs, prolonging the equilibration time by another 2 minutes failed to reduce the discrepancy substantially. Therefore, the remainder of this paper considers only those lungs with wet: dry ratios of 7 or less, for which accuracy of measurement with the isotopic technique was very high compared to the gravimetric method.

Below a wet-to-bloodless dry weight ratio of 7, the correlation of the wet-to-dry with isotopic measurements of extravascular lung water was good ($r = 0.8, P < 0.001$) (Fig. 4) with the average value of 70 ml obtained by the gravimetric technique compared to 64 ml obtained with the isotopic technique. The tendency to underestimate EVLW isotopically was random in these lungs and did not correlate significantly with increasing wet-to-dry weight ratios ($P > 0.3$).

**Figure 2** Intravascular blood volume in six isolated perfused lungs as determined by the $^{15}$O-tagged red cell technique showed a correlation coefficient of 0.93 with the gravimetric technique. Solid line represents identity.

**Figure 3** Total lung water as measured by the $H_2^{18}O$ method in single isolated perfused dog lungs revealed a correlation coefficient of 0.92 with total lung water determined by the gravimetric technique. Wet-to-dry ratios varied from 3.3 to 7.0. Solid line represents identity.

**Figure 4** Measurement of extravascular lung water in single isolated perfused dog lungs by the radioisotopic technique had a correlation coefficient of 0.8 with the gravimetric technique. Solid line represents identity.
To determine the location and distribution of extravascular water in the 17 lungs, shown in Figure 4, each lung was divided into four zones of equal height, with zone 1 being the most dependent zone. The zones covered the entire lung but, in so doing, necessarily included varying widths and volumes of lung not only in each dog but from dog to dog. Therefore, the absolute volume of extravascular lung water in any zone had little meaning because it reflected not only the wet-to-bloodless dry weight ratio of lung in that zone, but also the actual volume of lung tissue contained in the zone. Since the isotopic measurement of total lung water correlated so closely with the measurement of total lung water by the wet-to-bloodless dry weight technique, we elected to normalize the zones by the fraction of scintigraphically determined total lung water in each zone. When the fraction of extravascular lung water in each zone was compared to the fraction of total lung water in that zone, the ratios were found as shown in Figure 5. There was a small increase in the fraction of extravascular lung water as compared to the fraction of total lung water from the apex of the lung to the more dependent zones. Apical zones 4 and 3 were not significantly different from each other but were significantly (p < 0.01) less than zones 2 or 1. The same distribution was true for lungs of wet-to-dry ratio of under four as well as those of wet-to-dry ratios between five and seven. The distribution of extravascular lung water in the isolated lung then assumed a slight gravity-related accumulation.

The uniformity of the increase from apex to base in increasingly edematous states was surprising though. We worried that this uniformity may have arisen from our use of regional total lung water to normalize lung zones, since the volume of lung tissue in a given zone may not be the same as the total lung water content in that zone. Therefore, we performed a serial analysis of the accumulation of extravascular lung water in four lungs as they progressed into mild edema to determine again where extravascular lung water increased. The results on these four lungs (Fig. 6) reveal that the distribution of blood volume remained constant and that there was a uniform increment in extravascular lung water from apex to base, each zone now containing the same relative quantity of lung tissue from one study to the next, i.e., from non-edematous to edematous state. The fractional increase in extravascular lung water was 39% in zone 1 (base), 26% in zone 2, 41% in zone 3, and 34% in zone 4 (apex). Thus, although the increments in extravascular lung water were variable in different zones, there was no tendency for edema to accumulate preferentially in the dependent parts of the lung.
Discussion

Total lung water (TLW) and EVLW, as estimated by the camera and steady state double indicator positron-emitting technique (Figs. 3 and 4), were 96% and 90%, respectively, of that determined by the gravimetric technique in normal and moderately edematous lungs (wet-to-bloodless dry weight ratios of 3.3–7.0). However, the accuracy of the positron isotopic measurement estimation of extravascular lung water worsened in severe edema with wet-to-dry weight ratios above 7.0 so that only 72% of EVLW was detected compared to gravimetric measurements. Increasing the equilibration time of the isotopes in the grossly edematous lungs by 2 minutes failed to improve the error. Thus, the diffusible tracer ($^3$H$_2$O) fails to equilibrate fully with extravascular lung water in advanced stages of pulmonary edema. This may be due to the inability of the positron tracer to label intraalveolar edema rapidly, or it may be due to the continued accumulation of interstitial edema beyond that with which the positron tracer could equilibrate readily. However, lungs below a wet-to-dry weight ratio of 7 showed grossly no intra-airway edema at the end of the experiment and also demonstrated no evidence of alveolar edema on histological section, even from dependent lung zones.

Because the values for total lung EVLW determined isotonically approximated closely that found by the gravimetric technique in lungs with mild to moderate edema, we felt justified in subdividing these lungs into zones and thereby quantifying EVLW on a regional basis. Analysis of extravascular fluid accumulation in the 17 lungs which were normal to moderately edematous (wet: dry < 7.0) revealed more EVLW compared to intravascular lung water (IVLW) in the dependent zones and less at the apices (Fig. 7). This relative distribution did not change from normal to edematous lungs. The failure of Jones et al. (1976) to note this gradient may reflect the inability of their detection equipment to correct accurately for regional differential $\gamma$-ray absorption but more likely reflects the fact that a large proportion of their lungs was perfused in a zone II condition in which venous pressures were low and less than alveolar pressure. Inherent in zone II perfusion is an inequality of the distribution of perfusion, and this could influence the equilibration time of poorly perfused or less dependent zones. They would see an increase in EVLW in lung zones in which the capillaries are recruited more fully and the diffusion distance for the diffusible indicator is less. Since they could not quantify total EVLW, they may well have been underestimating total EVLW and thus were not assessing an index of intravascular lung water in advanced stages of pulmonary edema. This may be due to the inability of their detection equipment to correct accurately for regional differential $\gamma$-ray absorption but more likely reflects the fact that a large proportion of their lungs was perfused in a zone II condition in which venous pressures were low and less than alveolar pressure. Inherent in zone II perfusion is an inequality of the distribution of perfusion, and this could influence the equilibration time of poorly perfused or less dependent zones. They would see an increase in EVLW in lung zones in which the capillaries are recruited more fully and the diffusion distance for the diffusible indicator is less. Since they could not quantify total EVLW, they may well have been underestimating total EVLW and thus were not assessing an index of EVLW reflecting regional blood volume and perfusion and not true regional EVLW.

The lungs in the present series were perfused in zone III conditions (pulmonary venous pressure > alveolar pressure) below the hilum and in zone II conditions (alveolar pressure > pulmonary venous pressure) above the hilum. It has been suggested (Glazier et al., 1969) that the majority of capillary recruitment in zone II occurs in the first 10 cm below the point of no flow. In our lungs, the point of no flow can be calculated to have occurred at least 5 cm above the lung apex. Thus, only the very apex of the lung would be expected to have a substantial decrease in capillary recruitment. In these conditions of relatively good capillary recruitment, the regional detection of extravascular lung water was independent of regional blood volume.

The distribution of regional isotopic extravascular lung water compared to regional total lung water showed a small, but significant, tendency for more extravascular lung water to occur in dependent lung. The distribution of EVLW, however, was remarkably constant in increasingly edematous lungs and the diffusion distance for the diffusible indicator persisted even when we arbitrarily added to the upper two zones the excess 6 ml of water determined by the gravimetric technique for the whole lung. This produced a more uniform distribution of EVLW with a ratio of the percent regional EVLW to the percent regional TLW of 0.94 in the upper two zones compared to 1.06 in the lower two zones. This difference,
although small, was still significant ($P < 0.01$).

The small gravity-related increase in EVLW down the non-edematous isolated lung contrasts to a uniform presence of EVLW independent of gravity in the in vivo studies using the freezing technique (Naimark et al., 1971; Baile et al., 1979; Flick et al., 1979). However, one of these studies (Flick et al., 1979) did note a trend, though not significant, for 16 to 30% more EVLW to be present in the dependent lung than in the non-dependent. The isolated lung differs from that of the intact animals in having had the lymphatics cut. Although the lymphatics drain freely, their function may be diminished compared to the in vivo setting due to trauma and/or the high dose of barbiturate (Staub, 1974). Less effective lymphatic drainage could influence the basal distribution of EVLW in the non-edematous isolated lung compared to the in vivo lung. Regardless of the difference in baseline distribution of EVLW, the important common feature between the lungs studied, either with the radioisotopic technique or by the freezing method, is that formation of EVLW in normal to moderately edematous lungs seems to be largely independent of the influence of gravity. Thus, although microvascular hydrostatic pressure is increasing down the lung, an offsetting change in perimicrovascular pressure or in osmotic pressure is occurring which prevents an excess formation of EVLW in the dependent lung. This observation is still consistent with the clinical picture of pulmonary edema appearing first in the dependent part of the lung, since, although apex and base of the lung both were accumulating more extravascular water, the greatest volume of lung, and therefore of extravascular lung water which would be best detected by x-ray, is at the bases.

It is also possible, in latter stages of edema when alveolar flooding is occurring, that edema formation is different and becomes more highly gravity dependent. The positron isotopic technique, although more sensitive to EVLW than any other non-destructive technique, is still not sufficiently accurate to allow topographic mapping of the distribution of EVLW in advanced pulmonary edema. Thus, the positron camera and the steady state radioisotopic technique using C$^{15}$O-labeled red cells as the non-diffusible indicator and O$^{17}$-labeled water as the diffusible indicator are accurate in states of mild to moderate edema in quantifying and localizing extravascular lung water in lungs perfused in approximately normal physiological states. Disadvantages of the system are that the labeled substances cannot be infused together for simultaneous measurement of total lung water and intravascular volumes because the $\gamma$ energy of both labels is the same. An interval of 10–15 minutes must separate each isotope infusion and, therefore, a rapidly changing state cannot be studied. In studies of intact animals or humans, the chest wall contributes substantially to the background counts for measurements of intravascular and extravascular compartments and may mask subtle changes in lung EVLW. With the positron emission transverse section tomograms (Chesler et al., 1975), it is possible to separate the chest wall from the lung so that with the use of this technique it may be possible that the intra- and extravascular compartment lung water volumes can be calculated in intact subjects.

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