Optical measurement of isolated canine lung filtration coefficients after alloxan infusion

JOSEPH W. KLAESNER,1 N. ADRIENNE POU,2 RICHARD E. PARKER,2 CHARLENE FINNEY,2 AND ROBERT J. ROSELLI1

1Department of Biomedical Engineering and 2Center for Pulmonary Research, Vanderbilt University, Nashville, Tennessee 37235

Klaesner, Joseph W., N. Adrienne Pou, Richard E. Parker, Charlene Finney, and Robert J. Roselli. Optical measurement of isolated canine lung filtration coefficients after alloxan infusion. J. Appl. Physiol. 84(4): 1381-1387, 1998.—In this study, lung filtration coefficient (Kfc) was measured in eight isolated canine lung preparations by using three methods: standard gravimetric (Std), blood-corrected gravimetric (BC), and optical. The lungs were held in zone III conditions and were subjected to an average venous pressure increase of 8.79 ± 0.93 (mean ± SD) cmH2O. The permeability of the lungs was increased with an infusion of alloxan (75 mg/kg). The resulting Kfc values (in milliliters·min⁻¹·cmH2O⁻¹·100 g dry lung weight⁻¹) measured by using Std and BC gravimetric techniques before vs. after alloxan infusion were statistically different: Std, 0.527 ± 0.290 vs. 1.966 ± 0.283; BC, 0.313 ± 0.290 vs. 1.384 ± 0.290. However, the optical technique did not show any statistical difference between pre- and postinjury with alloxan, 0.280 ± 0.305 vs. 0.483 ± 0.297, respectively. The alloxan injury, quantified by using multiple-indicator techniques, showed an increase in permeability and a corresponding decrease in reflection coefficient for albumin (σf). Because the optical method measures the product of Kfc and σf, this study shows that albumin should not be used as an intravascular optical filtration marker when permeability is elevated. However, the optical technique, along with another means of measuring Kfc (such as BC), can be used to calculate Kfc of a tracer (in this study, σf of 0.894 at baseline and 0.348 after injury). Another important finding of this study was that the ratio of baseline-to-injury Kfc values was not statistically different for Std and BC techniques, indicating that the percent contribution of slow blood-volume increases does not change because of injury.

Evans blue; permeability; gravimetric techniques

The ability to measure lung filtration coefficient (Kfc) in humans would aid the clinician in the diagnosis and treatment of several pulmonary diseases, including acute respiratory distress syndrome. Until recently, isolated lung preparations were used to measure Kfc. An estimate of Kfc could be made by using animal preparations in which lung lymph was available, but these values could be influenced by problems such as the unknown fraction of lung lymph collected and microvascular pressure (Pmv) (4). Both of these methods are highly invasive, thus preventing their being extended to use in humans.

The gravimetric method performed on an isolated lung preparation has been considered the "gold standard" for measurement of Kfc. The technique entails monitoring the weight of an isolated lung preparation that is subjected to step increases of pulmonary venous pressure (Ppv). The lung gains weight rapidly during the first 1–3 min because of vascular filling. The slow weight gain that follows is normally attributed to fluid filtration across the microvascular barrier (5). The constant-slope method of calculating Kfc assumes that the slow weight gain is entirely caused by filtration and is linear with respect to time. Then Kfc can be calculated by dividing the slope of the weight gain by the Pmv increase and the dry lung weight (DLW) (5).

Harris et al. (8) have shown with the use of 51Cr-labeled red blood cells (RBC) that vascular volumes can increase for up to 30 min after the pressure elevation. Maron and Lane (17) have shown, with the use of indicator dilution methods, that blood volume (BV) may continue to increase for longer than the 1–3 min after Ppv increases if the isolated lung was not perfused for an extended period of time before the pressure elevation (17). Thus, slow vascular BV changes can adversely affect Kfc calculation based on gravimetric techniques.

Oppenheimer et al. (19) introduced an optical technique for measuring Kfc. Changes in optical-tracer concentrations in lung venous blood are measured after a step change in Ppv. The tracer concentrations increase after a pressure elevation, because fluid flows across the pulmonary capillary barrier more readily than proteins or large macromolecules can move across. Kfc can be calculated by determining the rate of change of optical-tracer concentrations. However, RBC strongly absorb and scatter light at the wavelengths used to measure the optical-tracer concentrations. Multiple wavelengths (19) have been used to correct for oxygen saturation levels or small hematocrit changes caused by the Fahraeus-Lindqvist effect (22) or by respiration (16). With the use of this optical system, Hancock et al. (7) found Kfc values measured optically were ~25% of those calculated by using weight changes, possibly because of slow vascular volume changes that were misinterpreted as filtration when using weight analysis.

Harris et al. (8) have optically measured Kfc in isolated canine lungs at low flows and small hematocrits by using a spectrophotometer. Lung venous blood was monitored with two wavelengths, so that optical-tracer concentration changes could be corrected for fluctuations in hematocrit. The Kfc values obtained by using optical techniques were significantly smaller than those obtained via the gravimetric method until the gravimetric values were corrected for BV changes. Unfortunately, RBC artifacts masked small changes in plasma optical-tracer concentrations, thus restricting this method to low hematocrits and low flow rates.
Earlier, we (15) showed that on-line separation of RBC from plasma before optical measurements allows for measurements of \( K_{fc} \) at physiological hematocrits and flow rates. A polysulfone filter cartridge provides on-line separation of plasma and RBC so that small concentration changes of an optical tracer can be measured by using a spectrophotometer. The \( K_{fc} \) values measured with this technique were within physiological ranges (15), but the values were not compared with simultaneous gravimetric measurements on the same isolated canine lung preparation. In another study, we used a commercially available filter from Cellco (model 4007-10, Germantown, MD) to measure the \( K_{fc} \) of an isolated canine lung by using normal hematocrits and flow rates. We showed that values obtained by using the optical technique compared favorably with those obtained via the blood-corrected (BC) gravimetric method (14).

Alloxan has been used in many studies to alter permeability in isolated canine lungs because it increases permeability while minimally affecting surface area (2, 11, 25). Harris et al. (10) and Zelter et al. (26) used alloxan injury (62.5 mg/kg) to increase lung microvascular permeability which was measured by using \([^{14}C]\)urea permeability-surface (PSU) area product in canine preparations. Olson et al. (18) used alloxan to induce injury in canine lungs to show that the PSU to 1,4-[\(^{14}C\)]butanediol permeability-surface (PSB) area product ratio (PSR) was sensitive to changes in permeability that were independent of surface area changes (18). To our knowledge, the optical method of measuring \( K_{fc} \) has not been applied to lungs injured with alloxan or with any other agent that causes lung injury.

In this study, \( K_{fc} \) was measured in isolated canine lung preparations by using gravimetric, BC gravimetric, and optical methods. The permeability of the lungs was altered with the use of alloxan to determine the sensitivity of the optical technique to changes in the permeability of the capillary barrier. Changes in permeability, independent of surface area, were determined with multiple-indicator dilution (MID) studies by using two barrier-limited tracers, \([^{14}C]\)urea and 1,4-[\(^{14}C\)]butanediol.

**METHODS**

Optical \( K_{fc} \) measurement theory. The basic principle employed by the optical method for calculating \( K_{fc} \) is that, when \( Ppv \) is increased, fluid crosses the microvascular barrier, leaving large solutes in the plasma to become slightly more concentrated. This small transient concentration change can then be used to calculate the product of the reflection coefficient, for the optical tracer (\( \sigma_r \)) and \( K_{fc} \) (8)

\[
\sigma_r K_{fc} = \frac{Q_p \Delta C_{pv}}{\Delta P_{mv} C_{pv} m_{dvw}}
\]

where \( Q_p \) is arterial plasma flow (in ml/min), \( \Delta C_{pv} \) is change in plasma concentration of nondiffusing tracer (in mg/dl), \( C_{pv} \) is initial plasma concentration of nondiffusing tracer (in mg/dl), \( m_{dvw} \) is blood-free dry lung weight (BFDLW in g), and \( \Delta P_{mv} \) is change in microvascular pressure (in cm\(H_2O\)).

In baseline studies, \( \sigma_r \) was assumed to be 1.0 for the optical tracer (14) \([\text{albumin labeled with Evans Blue (EBA)}]\). Thus the actual value of \( \sigma_r \) is probably \(<1.0\), which implies that the optical values of \( K_{fc} \) estimated with Eq. 1 were slightly underestimated.

The capillary pressure was estimated by using the formula developed by Gaar et al. (5)

\[
P_{mv} = P_{pv} + R_{av}(P_{pa} - P_{pv})
\]

where \( P_{mv} \) is capillary pressure and \( R_{av} \) is ratio of postcapillary resistance to total resistance of 0.4 (23).

Gravimetric \( K_{fc} \) measurement theory. The gravimetric technique depends on measuring the weight gain of the lung caused by fluid filtration. The Ppv is increased in a stepwise fashion, and the weight gain is monitored. The initial rapid weight gain is normally attributed to BV increases, but the slower secondary weight gain is assumed to be caused by filtration. This rate of weight gain is then divided by the change in pressure and DLW to determine the \( K_{fc} \).

\[
K_{fc} = \frac{dw/dt}{\Delta P_{mv} \cdot m_{dvw} \cdot \rho}
\]

where \( \rho \) is density of fluid (in g/ml) and \( dw/dt \) is rate of weight gain (in g/min).

MID theory. The MID measurements of permeability-surface area product (PS) involved the bolus injection of several radioactive tracers with different diffusing characteristics near the inlet of the lung and the collection of samples downstream of the lung. Two separate sets of injections were prepared, with different barrier-limited diffusing markers. One was \([^{14}C]\)urea, which is sensitive to PS (10, 26), and the other was 1,4-[\(^{14}C\)]butanediol, which is sensitive to perfused surface area (18). The vascular, or reference, tracers used in this study were \( ^{99} \)Tc-labeled RBC and \( ^{125} \)I-labeled albumin. Forty samples were collected downstream of the lung by using a revolving collection wheel which turned at a rate of one well/s. The gamma isotope activity of each sample and injectate was measured in a Packard Auto-Gamma Scintillation Spectrometer (model 5921), and the beta activity was measured in a liquid-scintillation counter (Bedman LS 3150T). The radioactive counts of the isotopes in each sample were then converted to tracer concentrations and were plotted with respect to time. These tracer concentration-time profiles were then used in the equations described by Harris and Roselli (9) and Haselton et al. (12) to calculate the PS. The integral extraction (\( E_i \)) for urea (U) was computed by using the equation

\[
E_i = \frac{\int_{0}^{t_{peak}} (C_R - C_D) dt}{\int_{0}^{t_{peak}} C_R dt} (4)
\]

where \( t_{peak} \) is time of peak of reference curve, \( C_R \) is concentration of reference tracer normalized to injectate concentration, and \( C_D \) is concentration of diffusing tracer normalized to injectate concentration.

\( E_i \) was then used to calculate the urea extraction PSU, neglecting the back diffusion from the extravascular space

\[
PS = -F_w \log_{10}(1 - E_i)
\]
F\textsubscript{w} is the water flow rate through lungs. Although PSU is known to be flow dependent (24), we kept flow constant in these studies. Thus any changes in PS were caused by a change in permeability or PS area. As is known to be flow dependent (24), we kept flow constant in these studies. Thus any changes in PS were caused by a change in permeability or PS area.

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The lungs were ventilated with a 95% O\textsubscript{2}-5% CO\textsubscript{2} gas mixture which was kept at a pressure of 2 cmH\textsubscript{2}O by adjusting the resistance of the air outflow. The lungs were held in zone III by setting Ppv > 3 cmH\textsubscript{2}O relative to the top of the lung. RBC were radiolabeled with 20–30 µCi of 51Cr. The NaI detectors were positioned over the suspended lung and the perfusate to monitor radioactivity. Baseline radioactivity was monitored for 5 min at a rate of two samples/min before addition of 51Cr-labeled RBC to the perfusate to obtain baseline radioactivity. The labeled RBC were distributed throughout the lung by raising and lowering Ppv several times. The increase in radioactivity caused by the 51Cr-labeled RBC was converted to a weight increase. This rate of weight gain caused by RBC was then subtracted from the total rate of lung weight gain to correct the gravimetric K\textsubscript{fc} values for vascular volume increases (8).

EBA was prepared by centrifuging enough blood (normally 100–120 ml) to obtain 65 ml of plasma and then adding 13 mg of Evans Blue to the resultant supernatant. This was mixed on a rocker for ~10 min so that the Evans Blue became bound to the albumin. Plasma (~20 ml) and EBA (~5 ml) were set aside for making a set of calibration standards. Once the lung-perfusion and filtrate-sampling systems were operating properly, the remaining 60 ml of the EBA was added to the reservoir. This provided a step increase that approached a baseline plasma absorbance of ~0.2 absorbance units.

Once the optical signal reached a stable state, the lungs were subjected to two to three pressure elevations, consisting of a Ppv increase of 8–15 cmH\textsubscript{2}O for 12 min, then lowering the Ppv back to the original value. Samples from both the cerebral and femoral arteries were collected and hemolysis was determined by measuring the absorbance of EBA in the supernatant following centrifugation.

The lungs were ventilated with a 95% O\textsubscript{2}-5% CO\textsubscript{2} gas mixture which was kept at a pressure of 2 cmH\textsubscript{2}O by adjusting the resistance of the air outflow. The lungs were held in zone III by setting Ppv > 3 cmH\textsubscript{2}O relative to the top of the lung. RBC were radiolabeled with 20–30 µCi of 51Cr. The NaI detectors were positioned over the suspended lung and the perfusate to monitor radioactivity. Baseline radioactivity was monitored for 5 min at a rate of two samples/min before addition of 51Cr-labeled RBC to the perfusate to obtain baseline radioactivity. The labeled RBC were distributed throughout the lung by raising and lowering Ppv several times. The increase in radioactivity caused by the 51Cr-labeled RBC was converted to a weight increase. This rate of weight gain caused by RBC was then subtracted from the total rate of lung weight gain to correct the gravimetric K\textsubscript{fc} values for vascular volume increases (8).

Table 1. Average pulmonary venous and arterial pressures before and after alloxan infusions

<table>
<thead>
<tr>
<th></th>
<th>Baseline Ppv</th>
<th>Baseline Ppa</th>
<th>Elevated Ppv</th>
<th>Elevated Ppa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prealloxan average</td>
<td>3.94 ± 0.38</td>
<td>14.45 ± 2.71</td>
<td>13.93 ± 0.37</td>
<td>21.17 ± 1.94</td>
</tr>
<tr>
<td>Postalloxan average</td>
<td>4.05 ± 0.45</td>
<td>17.43 ± 4.71</td>
<td>14.32 ± 0.72</td>
<td>23.86 ± 4.70</td>
</tr>
<tr>
<td>Total</td>
<td>4.00 ± 0.42</td>
<td>16.01 ± 4.13</td>
<td>14.13 ± 0.61</td>
<td>22.58 ± 3.86</td>
</tr>
</tbody>
</table>

Values are means ± SE in mmHg; n = 9 dogs. Ppv, pulmonary venous pressure; Ppa, pulmonary arterial pressure.
The solution was added at a rate of 1 ml/min if the lungs did not recover after the initial dose of alloxan. The remainder of the alloxan solution were added to the reservoir at 1 ml/min while monitoring the Ppa. The Ppa normally increased with the addition of the alloxan. The rate was decreased if the Ppa exceeded 30 cmH2O. To determine the extent of the injury, we observed the weight gain and pressures for 30 min and the pressure changes (model 1290A, Hewlett-Packard) were recorded at 1 Hz by using a Keithley-Metrabyte DAS-20 analog-to-digital card. The Kfc was then calculated by using Eq. 2.

At the end of the final baseline measurement, urea and 1,4-butanediol PS values were measured by using the MID techniques outlined above. An alloxan solution was prepared by mixing 75 mg/kg into 20 ml saline (1, 18). Then 10 ml of the alloxan solution were added to the reservoir at 1 ml/min while monitoring the Ppa of the lung. The Ppa normally increased with the addition of the alloxan. The rate was decreased if the Ppa exceeded 30 cmH2O. To determine the extent of the injury, we observed the weight gain and pressures for 30 min after the initial dose of alloxan. The remainder of the alloxan solution was added at a rate of 1 ml/min if the lungs did not appear to be adequately injured. Two to three more Kfc measurements were performed 30 min after the last injection of alloxan. PS measurements were made again at the end of the second pressure elevation.

When the experiment was completed, the lungs were weighed, homogenized, dried in a microwave oven at low temperature to determine the initial concentration of EBA before each run. The optical responses were observed by using the spectrophotometer (model 1706 UV/Vis monitor, Bio-Rad), and the pressure changes (model 1290A, Hewlett-Packard) were recorded at 1 Hz by using a Keithley-Metrabyte DAS-20 analog-to-digital card. The Kfc was then calculated by using Eq. 2.

Table 2. Average Kfc values before and after alloxan injury

<table>
<thead>
<tr>
<th>Dog</th>
<th>Gravimetric Baseline</th>
<th>Alloxan</th>
<th>Blood-Corrected Gravimetric Baseline</th>
<th>Alloxan</th>
<th>Optical Baseline</th>
<th>Alloxan</th>
</tr>
</thead>
<tbody>
<tr>
<td>FA1</td>
<td>0.68</td>
<td>2.77</td>
<td>0.43</td>
<td>1.32</td>
<td>0.28</td>
<td>0.34</td>
</tr>
<tr>
<td>FA2</td>
<td>0.42</td>
<td>0.60</td>
<td>0.31</td>
<td>0.47</td>
<td>0.42</td>
<td>0.46</td>
</tr>
<tr>
<td>FA3</td>
<td>0.29</td>
<td>0.77</td>
<td>0.19</td>
<td>0.59</td>
<td>0.23</td>
<td>0.22</td>
</tr>
<tr>
<td>FA4</td>
<td>0.60</td>
<td>5.96</td>
<td>0.26</td>
<td>4.71</td>
<td>0.16</td>
<td>0.31</td>
</tr>
<tr>
<td>FA5</td>
<td>0.57</td>
<td>1.51</td>
<td>0.40</td>
<td>1.23</td>
<td>0.42</td>
<td>0.95</td>
</tr>
<tr>
<td>FA6</td>
<td>0.83</td>
<td>1.57</td>
<td>0.47</td>
<td>0.77</td>
<td>0.41</td>
<td>0.34</td>
</tr>
<tr>
<td>FA7</td>
<td>0.56</td>
<td>3.65</td>
<td>0.24</td>
<td>2.44</td>
<td>0.28</td>
<td>0.48</td>
</tr>
<tr>
<td>FA8</td>
<td>0.81</td>
<td>0.70</td>
<td>0.27</td>
<td>0.50</td>
<td>0.28</td>
<td>0.35</td>
</tr>
<tr>
<td>FA9</td>
<td>0.65</td>
<td>1.79</td>
<td>0.31</td>
<td>0.29†</td>
<td>0.31†</td>
<td>0.48†</td>
</tr>
<tr>
<td>Average</td>
<td>0.53 ± 0.29*</td>
<td>1.97 ± 0.28*</td>
<td>0.31 ± 0.29†</td>
<td>1.38 ± 0.29†</td>
<td>0.28 ± 0.31</td>
<td>0.48 ± 0.30</td>
</tr>
</tbody>
</table>

Values are in ml·min⁻¹·cmH₂O⁻¹·100 g dry lung weight⁻¹ (DLW). Kfc, capillary filtration coefficient. *Statistically different from each other, P < 0.05. †Statistically different from each other, P < 0.05.

RESULTS

Eight isolated canine lung preparations were used in this study. Each lung was subjected to three to seven step increases in Ppv, averaging 8.79 ± 0.93 (mean ± SD) cmH₂O. The baseline and elevated Ppv and Ppa were listed in Table 1 for pre- and posttreatment with alloxan. The optical, gravimetric, and RBC-corrected gravimetric Kfc values (in ml·min⁻¹·cmH₂O⁻¹·100 g DLW⁻¹) were measured for each run, and the average values are listed in Table 2. Although the average value of Kfc increased after alloxan infusion, the difference from baseline was not significant as shown by two-way ANOVA, with the Student-Newman-Keuls test for multiple comparisons. The gravimetric and BC values measured before and after alloxan infusion were statistically different. Time-course analysis was not done on these experiments, because this analysis was performed in an earlier paper, and it was shown that any changes in Kfc caused by time were small compared with those measured before and after alloxan infusion in this study (14).

In this study, the BC gravimetric and the optical technique both gave results that were not statistically different in the uninjured lung, similar to the results we presented earlier (14). However, after injury, the optically measured Kfc values were significantly differ-

Table 3. Values of αf calculated by optical and blood-corrected Kfc

<table>
<thead>
<tr>
<th>Dog</th>
<th>αf Baseline</th>
<th>αf Alloxan</th>
<th>αf Alloxan/αf Baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td>FA1</td>
<td>0.65</td>
<td>0.26</td>
<td>0.39</td>
</tr>
<tr>
<td>FA2</td>
<td>1.35</td>
<td>0.97</td>
<td>0.72</td>
</tr>
<tr>
<td>FA3</td>
<td>1.21</td>
<td>0.38</td>
<td>0.31</td>
</tr>
<tr>
<td>FA4</td>
<td>0.63</td>
<td>0.07</td>
<td>0.11</td>
</tr>
<tr>
<td>FA5</td>
<td>1.05</td>
<td>0.77</td>
<td>0.74</td>
</tr>
<tr>
<td>FA6</td>
<td>0.87</td>
<td>0.45</td>
<td>0.52</td>
</tr>
<tr>
<td>FA7</td>
<td>1.17</td>
<td>0.20</td>
<td>0.17</td>
</tr>
<tr>
<td>FA9</td>
<td>0.48</td>
<td>0.70</td>
<td>1.43</td>
</tr>
<tr>
<td>Average ± Std</td>
<td>0.92 ± 0.31</td>
<td>0.28 ± 0.31</td>
<td>0.55 ± 0.43</td>
</tr>
</tbody>
</table>

Values are as measured, except for average ± Std (standard gravimetric), αf, reflection coefficient for albumin; αf Alloxan/αf Baseline, αf after alloxan/αf at baseline.

Table 4. Ratio of postinjury Kfc to preinjury Kfc

<table>
<thead>
<tr>
<th>Dog</th>
<th>Gravimetric</th>
<th>Blood-Corrected Gravimetric</th>
<th>Optical</th>
</tr>
</thead>
<tbody>
<tr>
<td>FA1</td>
<td>4.17</td>
<td>3.03</td>
<td>1.20</td>
</tr>
<tr>
<td>FA2</td>
<td>1.43</td>
<td>1.49</td>
<td>1.09</td>
</tr>
<tr>
<td>FA3</td>
<td>2.63</td>
<td>3.22</td>
<td>0.98</td>
</tr>
<tr>
<td>FA4</td>
<td>10.0</td>
<td>20.0</td>
<td>1.96</td>
</tr>
<tr>
<td>FA5</td>
<td>2.63</td>
<td>3.03</td>
<td>2.22</td>
</tr>
<tr>
<td>FA7</td>
<td>1.89</td>
<td>1.61</td>
<td>0.85</td>
</tr>
<tr>
<td>FA8</td>
<td>6.67</td>
<td>10.0</td>
<td>1.69</td>
</tr>
<tr>
<td>FA9</td>
<td>0.51</td>
<td>0.83</td>
<td>2.63</td>
</tr>
<tr>
<td>Average ± Std</td>
<td>3.89 ± 3.00</td>
<td>5.33 ± 6.41</td>
<td>1.58 ± 0.65</td>
</tr>
</tbody>
</table>
ent than the BC gravimetric $K_{fc}$ values. Assuming that the difference between these two values was caused by a change in $\alpha_{f}$, we computed $\alpha_{f}$ by taking the ratio of the optical $\alpha_{f}K_{fc}$ product to the BC $K_{fc}$. These values are listed in Table 3. With the exception of results for animal FA9, the results suggest that $\alpha_{f}$ decreases when $K_{fc}$ increases, and the product of $\alpha_{f}K_{fc}$ may not change significantly with injury.

The ratios of post- to preinjury $K_{fc}$ values (optical is $\alpha_{f}K_{fc}$) were calculated for the eight studies and are also listed in Table 4. The average ratios for the gravimetric and the BC gravimetric techniques were not statistically different from each other, whereas the ratio for the optical technique was significantly different from that for the other two techniques. BV increases account for ~40% of the preinjury gravimetric value of $K_{fc}$ and for ~30% of the postinjury gravimetric value.

The extent of injury to the lung caused by alloxan was measured by using M1 techniques, $P_{SU}$, $P_{SB}$, and PSR for the eight dogs used in this study are listed in Table 5. After alloxan infusion, the decreases in $P_{SB}$ and the increases in $P_{SU}$ were not statistically significant, but the PSR increased significantly, indicating an increase in permeability.

Table 6 lists the pulmonary vascular resistance (PVR) values calculated for the eight studies. The average baseline PVR value, 0.60 ± 0.15 cmH$_2$O·s·ml$^{-1}$, was statistically smaller than the average postinjury PVR value, 0.75 ± 0.29 cmH$_2$O·s·ml$^{-1}$, indicating that the alloxan injury caused an increase in vascular resistance.

Vascular compliance values were calculated for the eight studies by dividing the initial weight change by the pressure step and the BFDLW. These data are shown in Table 7. The preinjury compliance value of 15.57 ± 5.48 g·cmH$_2$O$^{-1}$·100 g DLW$^{-1}$ is statistically greater than the value calculated after alloxan injury, 13.45 ± 5.49 g·cmH$_2$O$^{-1}$·100 g DLW$^{-1}$. This indicates that the lung vasculature became stiffer after the alloxan injury. This small change is consistent with the BV increase being smaller after alloxan injury.

**DISCUSSION**

Several studies have been devoted to the comparison of different methods for measuring lung $K_{fc}$. Gravimetric methods are limited to isolated lung preparations and have been shown to be influenced significantly by vascular stress relaxation that accompanies vascular pressure elevations (8, 14). The optical technique can be used in vivo (15) and is unaffected by BV changes that can occur during a measurement (6–8, 13–15, 19, 20, 22). One drawback to the optical method is that it requires precise plasma-concentration measurements, because only small changes occur during a filtration measurement. Significant artifacts can be introduced by RBC, which absorb and scatter light. In addition, absorption can be influenced by the extent of oxyhemoglobin saturation (19). These artifacts can be elimi-
nated by removing RBC with a filter or by centrifugation before making the optical measurements (14, 15).

The optical method has only been used to estimate $K_{fc}$ in normal lungs. In the present study, simultaneous gravitational and optical measurements were used to estimate lung $K_{fc}$ and albumin $\sigma_f$ after inducing lung injury. Alloxan was used to damage the lungs, and damage was confirmed by a significant increase in the ratio of $P_{Sh}$ to $P_{Sw}$. A significant increase in gravimetric and BC gravimetric $K_{fc}$, a decrease in albumin $\sigma_f$, an increase in PVR, and a decrease in pulmonary vascular compliance.

In the use of the optical method, we encountered a potential difficulty which occurs when comparing filtration before and after lung injury. The optical method measures the product of $K_{fc}$ and $\sigma_f$ for the molecular tracer used in the measurement. Although the $K_{fc}$ increased by a factor of three in this study, the $\sigma_f$ for albumin decreased, so the product did not increase significantly. This is a serious limitation, because the detection of lung injury is the principal reason for making filtration measurements.

This limitation can be overcome by making an independent measure of either $K_{fc}$ (as was done in this study) or $\sigma_f$. Such methods will normally apply to isolated organ preparations. A much better approach would be to select a tracer that is truly intravascular and thus always has a $\sigma_f$ of unity. Albumin, with a $\sigma_f$ below unity in normal lungs, is not a good choice. Labeled RBC would seem to be an ideal choice, but the Fahraeus-Lindqvist effect severely limits the interpretation of filtration experiments with RBC (16, 22). Very large macromolecules, such as blue dextran (mol wt 2,000,000) would be expected to remain in the plasma during a postinjury measurement. This would allow the optical method to be used to accurately estimate $K_{fc}$ during lung injury without a separate measure of $\sigma_f$.

The values of $\sigma_f$ listed in Table 3 were calculated by dividing the optical $\sigma_f K_{fc}$ values by the BC $K_{fc}$ values. Both of these values have some error associated with them, so that when the two values were used to calculate $\sigma_f$, the error could be compounded. Thus, some of the calculated $\sigma_f$ baseline values were larger than unity.

Results from this study confirm earlier reports (8, 14) that suggest that vascular relaxation occurs during gravimetric filtration measurements. The resulting BV accumulation causes lung $K_{fc}$ to be overestimated by $\sim 40\%$. In the present study, however, we found that the rate of intravascular volume change is similar after alloxan injury (30%).

Thus, although the absolute value for $K_{fc}$ is overestimated both before and after injury, the postinjury-to-baseline ratio is nearly identical to the actual BC ratio. This would imply that percent changes in gravimetric measurements already reported in the literature after alloxan injury are valid, without the need to correct for stress relaxation. It remains to be seen whether this is valid for injurious substances other than alloxan. Lungs treated with mediators that cause simultaneous changes in permeability and vascular tone may exhibit different rates of vascular filling postinjury than preinjury.

In summary, care should be taken in performing and interpreting both optical and gravimetric lung filtration studies. Intravascular stress relaxation during a measurement can significantly contribute to gravimetric $K_{fc}$. However, the percent change after lung injury measured with this method will be valid if vascular accumulation is similar before and after lung injury. The optical method is independent of vascular stress relaxation but measures the product of $\sigma_f K_{fc}$, $\sigma_f$. Therefore, if a measure of $K_{fc}$ is desired in an injured lung, without an independent measure of $\sigma_f$, macromolecules much larger than albumin should be used as the intravascular tracer.

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