Fluorescein-labeled dextran concentration is increased in BAL fluid after ANTU-induced edema in rats

BENOIT P. GUERY,1 STEVE NELSON,2 NATHALIE VIGET,1 PATRICE FIALDES,3 WARREN R. SUMMER,2 ELIZABETH DOBARD,2 GILLES BEAUCAIRE,1 AND CAROL M. MASON2
1Laboratoire de Recherche en Pathologie Infectieuse and 2Laboratoire de Biophysique, Faculté de Médecine de Lille, 59045 Lille, France; and 3Department of Medicine, Louisiana State University Medical Center, New Orleans, Louisiana 70112

Guery, Benoit P., Steve Nelson, Nathalie Viget, Patrice Fialdes, Warren R. Summer, Elizabeth Dobard, Gilles Beaucaire, and Carol M. Mason. Fluorescein-labeled dextran concentration is increased in BAL fluid after ANTU-induced edema in rats. J. Appl. Physiol. 85(3): 842–848, 1998.—Several methodologies have been developed to assess alveolar capillary membrane permeability in acute lung injury. The purpose of this study was to determine the reliability of FITC-dextran compared with radioactive tracers to assess lung permeability alterations. After intraperitoneal administration of α-naphthylthiourea (ANTU, 50 mg/kg) or DMSO-ANTU vehicle, the animals were euthanized and their lungs were studied in an isolated-lung preparation. FITC-dextran or radiolabeled tracers were added to the perfusate. At 2 h the bronchoalveolar lavage (BAL) fluid from the ANTU group showed a significantly greater amount of fluorescence in the supernatant after centrifugation of BAL fluid compared with the DMSO group. Consistent results were observed with the radioactive tracers: there was an increase in extravascular albumin space and extravascular lung water compared with the control group. No cleavage of the FITC from the dextran molecule was evident by chromatography comparing samples recovered from the BAL fluid to the pure FITC-dextran molecule. In conclusion, measurement of FITC-dextran in the supernatant of BAL fluid after intravascular administration is a reliable method of assessing lung permeability changes in vivo and ex vivo.

bronchoalveolar lavage; α-naphthylthiourea; permeability; lung injury; dextran

DIFERENT METHODS HAVE BEEN USED to assess lung injury. These techniques can be classified according to whether they were performed in an ex vivo or in vivo experimental model. Each of these procedures is designed to study an aspect of the injury, including edema formation, changes in permeability-sieving properties of each component of the alveolar capillary membrane, or the histological modifications induced by the injury. Our study was designed to evaluate whether dextran accumulation in bronchoalveolar lavage (BAL) fluid was a reliable method of assessing α-naphthylthiourea (ANTU)-induced lung injury. In ex vivo techniques, the isolated perfused lung model allows an estimation of edema formation by measuring fluid accumulation in the lung by monitoring the absolute lung weight. Edema accumulation estimated by this method is confirmed by the lung wet weight-to-dry weight ratio (W/D) (11, 15). Initially, in vivo studies assessed vascular parameters to explain the mechanisms leading to edema formation (28). Subsequently, normal circulating proteins or tracer molecules were introduced to characterize and quantify experimentally induced lung injury. Different-size molecules (immunoglobulin, vasopressin, and serum albumin) were used as permeability markers after tracheal instillation (10). These markers were then quantified in the blood of the animal. Radioactive tracers are now the most common molecules used to estimate lung injury and permeability changes. The quantification of two radioactive tracers, one injected into the alveolar space and the other injected into the circulation, allowed the characterization of lung endothelial and epithelial permeability (16, 24). Other authors have used the ratio of 125I-labeled albumin in whole lung tissue to that in plasma after the administration of the tracer intravenously (6, 14, 29); blood-based methods using lymph and indicator dilutions have also been used (13, 26). In addition to these dynamic studies, permeability alteration can be visualized by histological examination (31, 32). However, radioactive molecules provide a very accurate and sensitive method of detecting an impairment of the lung endothelial and epithelial barriers, but they require a laboratory authorized to use radioactive material and special precautions in handling the samples and all disposable material waste. A method using FITC-dextran (molecular wt 73,100) is described, which results in an easy and radioactive-free technique for defining lung fluid accumulation.

We decided to evaluate whether dextran accumulation in BAL fluid was a reliable method for assessing ANTU-induced lung injury. To do so 1) lung FITC-dextran egress from the vascular side into the alveolar space was assessed in an isolated perfused lung model; 2) results were compared with the classic method by using albumin space (AS) and the measurement of extravascular lung water (EVLW) with radiolabeled tracers (6, 7); 3) additionally, cleavage of FITC from dextran was assessed by chromatography; 4) the use of FITC-dextran as a tracer was evaluated in two additional groups in vivo; and 5) finally, this method was tested during hydrostatic stress. The results indicate that FITC-dextran can be used in vivo or ex vivo to assess lung injury and that it is a consistent and reproducible method of measuring injury in both of these situations.

MATERIALS AND METHODS

Animals

Specific-pathogen-free rats (250–274 g) were obtained from Hilltop Laboratories (Scottdale, PA) and housed in the Louisi-
ana State University Medical Center Animal Care Facility. Animals were allowed food and water ad libitum. All experiments were performed with approval of the Louisiana State University Medical Center Institutional Animal Care and Use Committee.

**Intraperitoneal (ip) and/or Intravenous (iv) Injections**

For ip injection, ANTU (50 mg/kg) diluted in DMSO (Mallinckrodt, Paris, KY) was given 90 min before euthanization of the animals and harvesting of the lungs for the isolated perfused lung preparation, or 4 h before for the in vivo study. Iv injections of ANTU or DMSO were performed with a sterile 25-gauge needle without anesthesia by injecting the total volume (0.5 ml) after ip placement of the needle was confirmed. For iv injection, animals were anesthetized with ether (Mallinckrodt), and the injection was performed in the dorsal penile vein.

**Isolated Perfused Lung Preparation**

The isolated perfused lung model has been previously described (12, 20). Briefly, animals were anesthetized with ketamine and xylazine intramuscularly (50 and 5 mg/kg, respectively; Fort Dodge Laboratories, Fort Dodge, IA), and the trachea was cannulated and connected to a small-animal ventilator (Harvard Apparatus, South Natick, MA). The lungs were ventilated with a constant tidal volume of 10 ml/kg of 95% O₂-5% CO₂ at 60 breaths/min, with 2 cmH₂O positive end-expiratory pressure. A catheter was inserted into the left carotid artery and, after the administration of 1.0 ml sodium heparin solution (5,000 U/ml), the animal was exsanguinated. The chest was then opened, and the main pulmonary artery was exposed. The pulmonary artery was cannulated through a right ventriculotomy, and the lungs were perfused with Krebs-Henseleit buffer. Next, the left atrial appendage and the lower one-third of the left ventricle were excised to ensure zero left atrial pressure (LAP). The lungs and heart were removed en bloc from the chest and placed in a heated water jacket. The lungs were then perfused with a mixture of one part Krebs-Henseleit buffer to three parts heparinized whole blood (taken from rats that had not been injected with ANTU) at a rate of 4.5 ml/min with a Masterflex pump (Cole-Parmer Instrument, Barrington, IL). The perfusate was circulated through a 37°C heat exchanger. The perfuse reservoir was positioned below the isolated lung so that all drainage from the preparation returned to the reservoir, which was constantly stirred magnetically to provide adequate mixing. Airway pressure, pulmonary arterial pressure (PAP), and the pressure in the blood-buffer reservoir were recorded continuously with an eight-channel recorder (Grass Instruments, Quincy, MA). The isolated lungs were ventilated and perfused ex vivo for a total period of 180 min. To quantify lung fluid accumulation, perfusate reservoir pressure was recorded continuously during the experiment and change was correlated with volume in the perfusate reservoir. Changes in perfusate volume are reported as milliliters of volume lost from the reservoir. The lung fluid accumulation was confirmed by W/D for each study group; the correlation can be made because the measurements were performed in the same animal.

Fluorescence and radiolabel measurements were performed at 120 min instead of 180 min so as to avoid the occasional alveolar flooding.

Fluorescence Measurements

In another subset of animals, 1 ml of a solution containing 0.5 mg/ml of FITC-dextran (Sigma Chemical, St. Louis, MO) was added in the perfusate for the ex vivo experiments or was iv injected into the animal for the in vivo experiments. Lungs from each experimental group, 2 h after either injection (iv) or addition of FITC-dextran to the perfusate, were lavaged with a total of 30 ml of PBS with 3 mM EDTA in 5-ml aliquots. The fluid was centrifuged (200 g × 10 min), and the fluorescence was measured in the lavage fluid supernatant with a fluorescence spectrophotometer (Perkin-Elmer 650–40) for which the excitation wavelength was 495 nm and the emission wavelength was 517 nm. A standard curve was prepared with known amounts of FITC-dextran to calculate the values recovered in the BAL fluid. The results were then expressed as milligrams of FITC-dextran per milliliter of BAL fluid.

**Radiolabeled Red Blood Cells and Albumin**

To evaluate the change in permeability observed with FITC-dextran with another method, in a different subset of animals, edema was estimated with radiolabeled markers in the isolated perfused lung model. Radiolabeled albumin and red blood cells were added to the perfusate immediately after hanging of the lung. We estimated EVLW content and bloodless dry lung weight (DLW) by using red blood cells labeled with 51Cr to determine lung blood content. Red blood cells from a donor rat were incubated for 20 min with 370 kBq sodium chromate (51Cr, CIS BioInternational) at room temperature. Labeled cells were washed twice with 5 ml of isotonic saline, and 51Cr activity was measured in the supernatant of the last wash. 51Cr activity of ~5% of free activity was considered satisfactory. Microvascular permeability was assessed by 125I-human albumin (CIS BioInternational) uptake by the lung at the end of the 120 min of the experiment (0.1 ml of a solution at 185 kBq/ml). The mixture of radiolabeled albumin and red blood cells was added to the perfusate of the preparation 15 min after hanging of the lung, the time necessary to reach a steady state. After the end of the experiment, lungs and blood sample obtained were weighed, the lungs were homogenized, and their tracer activities were determined by gamma counting (LKB 1282, Wallach). Corrections were made for the overlap between channels.

The fraction of blood (Fblood) in the lung was

\[
F\text{blood} = \frac{(51\text{Cr counts} \cdot \text{min}^{-1} \cdot \text{g lung}^{-1})}{(51\text{Cr counts} \cdot \text{min}^{-1} \cdot \text{g blood}^{-1})}
\]

After this first measurement, lungs and blood samples were desiccated for 7 days at 37°C. Bloodless wet lung weight (WLW), DLW, and EVLW were calculated according to the following equations

- \[
WLW = \text{wet weight} \times (1 - F\text{blood})
\]
- \[
DLW = \text{dry weight} - \text{dry weight of blood in lungs}
\]
- \[
\text{WLW} = \text{wet weight} \times F\text{blood}
\]
- \[
\text{EVLW} = \text{WLW} - \text{DLW}
\]

The extravascular AS was defined as the space in which albumin would be distributed in the blood-free lungs, assuming the tissue concentration of albumin was identical to its...
plasma concentration (we therefore assumed that 1 ml of blood weighs 1 g)

\[
AS = (1 - \text{hematocrit}) \\
\times \left(\frac{125\text{-albumin cpm in lungs}}{125\text{-albumin counts} \cdot \text{min}^{-1} \cdot \text{ml blood}^{-1}}\right) \\
\times \left(\frac{\text{Cr cpm in lungs}}{\text{Cr counts} \cdot \text{min}^{-1} \cdot \text{ml blood}^{-1}}\right),
\]

where cpm is counts per minute.

All these radioactive data were standardized by body weight and expressed as means ± SE.

**Lung W/D**

To confirm that lung fluid accumulation correlated with the volume change in the perfusate reservoir, each group of animals had lung W/D performed. For these experiments, lungs from the four groups were prepared as described above and set up in the isolated perfused lung model for the ex vivo groups. At the end of the experiment (180 min), the lungs were removed and the wet weight was recorded. For the in vivo groups, lung wet weight was recorded after euthanization of the animal, 120 min after injection of FITC-dextran. The lungs were then placed in a 37°C incubator for 7 days, at which time the dry weight was recorded. For each pair of lungs, W/D was calculated.

**Estimation of the Capillary Filtration Coefficient**

This method has been previously used to estimate, in this setting of isolated perfused lung model, the capillary filtration coefficient (20). The flux during each 30-min time interval was divided by the mean PAP to estimate the capillary filtration coefficient of each group. This variable relates the increase in flux to the change in PAP.

**Chromatography**

To evaluate the possible degradation of FITC-dextran in the isolated perfused lung preparation, 700 µl of Biogel P-6 (Bio-Rad, Hercules CA) were added atop acid-washed glass beads (212–300 µm, Sigma Chemical) in an 800-µl tube. The column was then packed for 3 min by centrifugation at 1,200 g. Known amounts of pure FITC-dextran or BAL fluid samples obtained ex vivo (50 ml) were layered on the top of the column. After a second centrifugation of 5 min at 1,200 g, the fluorescence was measured in the collected effluent. The molecular weight cutoff of Biogel is between 1,000 and 6,000, allowing exclusion of larger molecules and thus more rapid efflux of these large molecules through the column. The molecular weight of FITC-dextran is 73,100; the FITC component alone has a molecular weight of 390. The fluorescence of the effluent in the column was measured, and the result was divided by the total fluorescence measured in the solution before chromatography. This ratio evaluates the percentage of FITC linked to dextran.

**Experimental Protocol**

Four experimental groups were studied, two ex vivo and two in vivo.

**Ex vivo:** ip ANTU (ANTUin) and ip DMSO (DMSOin). Ninety minutes after the DMSO or ANTU injection, the animals were euthanized and their lungs were removed and studied in the isolated perfused lung preparation. In each group, n = 5 animals unless otherwise noted.

**In vivo:** ipANTU (ANTUin) and ip DMSO (DMSOin). The ip injections in vivo were followed by iv injection of 1 ml of a solution containing 0.5 mg/ml of FITC-dextran (Sigma Chemical) 120 min later. The animals were euthanized after an additional 120 min, the lungs were removed, and BAL was performed.

An additional group with hydrostatic edema was studied ex vivo. In the isolated perfused lung setup, the left atrial appendage was cannulated through the left ventricle, and the effluent was directed to the perfusate reservoir so that all drainage from the preparation returned to the reservoir. The LAP was set with a mechanical tourniquet on the tubing draining this cannula. All the other parameters remained unchanged.

After a 15-min equilibration period, 1 ml of a solution containing 0.5 mg/ml of FITC-dextran (Sigma Chemical) was added in the perfusate and the LAP was increased to 12 mmHg for a 120-min period. BAL was performed after this time to measure the alveolar accumulation of FITC-dextran.

**Statistical Analysis**

Group mean data were compared with ANOVA to detect differences among the groups. When only two groups were studied, a t-test was performed. Where appropriate, a Bonferroni and/or Dunn multiple-comparison test was performed. A P value < 0.05 was accepted as statistically significant (4). Filtration coefficient data were analyzed by using a two-way ANOVA, allowing assessment of the independent effects of time and injury, respectively.

**RESULTS**

**Lung Fluid Accumulation and Lung W/D**

Figure 1 shows that there was a greater leak in the ANTUex group than in the DMSOex group. After 120 min this difference between the two groups reached statistical significance. At 180 min, the final leak is 11 ml in the ANTUex group and 1.7 ml in the DMSOex group. The lung W/D demonstrate the same pattern ex vivo, with significantly higher values in the ANTUex group: 16.2 ± 1.4 vs. 6.0 ± 0.2 for the DMSOex group. In vivo the lung W/D are also significantly different between the two groups (6.59 ± 0.29 vs. 4.69 ± 0.04) (P < 0.05).

**PAP and Airway Pressure**

PAP and airway pressure in the ANTUex group increased with time, but in the DMSOex group these parameters remained stable throughout the observation period. By using a constant flow, the increase in

![Fig. 1. Lung fluid accumulation (ml) for DMSO and α-naphthylthio-urea (ANTU) ex vivo groups (DMSOex and ANTUex groups, respectively) over 180-min ex vivo period, during which isolated perfused lungs were monitored. Values are means ± SE. *ANTUex group statistically different from DMSOex group (P < 0.05).](http://jap.physiology.org/)
these two variables at the end of the experiment most likely reflects an increase in EVLW, decreasing the system’s compliance on the vascular side as well as on the alveolar side (Fig. 2).

Fluorescence Measurements

In both in vivo and ex vivo experimental models, the total amount of FITC-dextran recovered in the BAL fluid was significantly greater in the ANTu groups compared with the DMSO groups. Consistent with these findings, lung wet weights were higher after ANTu administration than in the DMSO groups in vivo or ex vivo. The induction of hydrostatic edema did not increase the FITC-dextran level in the BAL compared with the DMSO groups. All results are shown in Table 1.

Radiolabeled Red Blood Cells and Albumin

The AS was significantly increased in the ANTu group of animals compared with the DMSO group. Similarly, EVLW was higher in the injured group. The results are shown in Table 2.

Estimation of the Capillary Filtration Coefficient

The two-way ANOVA did not evidence any difference between the ANTu group and the DMSO group ex vivo. In the ANTu group, the flux-to-PAP ratio (flux/ PAP) does not statistically change throughout the experiment. On the other hand, a slight but significant increase is observed for the DMSO group as shown in Table 2.

DISCUSSION

One of the first reports on ANTu in a rat model was produced by Latta (17) in 1947. This microscopic study of the lungs after injection of 33 mg/kg of ANTu revealed a “great increase in the permeability of the capillaries of the lung.” The fluid collected in the pleural space and the lungs had a similar protein content to the blood serum. Later, by histological examination, the main features of ANTu-induced pulmonary edema were described (5, 21). Maximum edema occurs between 2 and 4 h after ip administration. Much of the leak is from the capillaries into the pericapillary interstitium (22). These histological findings were confirmed by the assessment of pulmonary vascular permeability in the ANTu-injured lung. ANTu increases the filtration coefficient and decreases capillary pressure, indicating a seriously damaged endothelium (2, 27). Although the consequences of ANTu administration have been precisely studied, the etiology remains unclear. Oxygen radicals are probably important in this injury (19), but other mediators have been postulated, including serotonin, eicosanoids, and prostaglandins.

Table 1. Fluorescence recovered in BAL fluid of each group and lung wet weight

<table>
<thead>
<tr>
<th>Group</th>
<th>FITC-Dextran, mg/ml</th>
<th>Lung Wet Wt, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSOex</td>
<td>4.1 ± 0.5 × 10⁻⁵</td>
<td>1.54 ± 0.05</td>
</tr>
<tr>
<td>ANTuex</td>
<td>4.1 ± 0.7 × 10⁻⁴*</td>
<td>2.81 ± 0.22*</td>
</tr>
<tr>
<td>DMSOex</td>
<td>3.3 ± 0.8 × 10⁻⁵</td>
<td>1.87 ± 0.13</td>
</tr>
<tr>
<td>ANTuex</td>
<td>1.9 ± 0.5 × 10⁻⁵*</td>
<td>3.27 ± 0.85*</td>
</tr>
<tr>
<td>Posthydrostatic edema</td>
<td>3.17 ± 1.2 × 10⁻⁵</td>
<td>1.92 ± 0.5</td>
</tr>
</tbody>
</table>

Values are means ± SE. BAL, bronchoalveolar lavage; DMSOex and DMSOin, DMSO in vivo and ex vivo groups, respectively; ANTuex and ANTuin, o-naphthylthiourea in vivo and ex vivo groups, respectively. *P < 0.05, significantly different from DMSO group.

Fig. 3. However, the values for the DMSO group remain low, below 0.27 ml · 30 min⁻¹ · mmHg⁻¹ (Fig. 3).

Chromatography

When the pure FITC-dextran sample was layered on top of the column, 67.3 ± 5.2% (n = 4) of the total fluorescence was recovered in the chromatography column effluent. With BAL fluid samples, 58.6 ± 3.9% (n = 5) of the total fluorescence was recovered in the effluent. There is no difference among the groups. This confirms that FITC remains largely bound to dextran in our experiments.

Table 2. Estimates of albumin space and extravascular lung water measured ex vivo by using radiolabeled red blood cells and albumin

<table>
<thead>
<tr>
<th>Group</th>
<th>Albumin Space, ml/kg BW</th>
<th>EVLW, ml/kg BW</th>
</tr>
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<tbody>
<tr>
<td>DMSOex</td>
<td>1.4 ± 0.6</td>
<td>2.45 ± 0.6</td>
</tr>
<tr>
<td>ANTuex</td>
<td>2.3 ± 0.7*</td>
<td>3.64 ± 0.5*</td>
</tr>
</tbody>
</table>

Values are means ± SE. EVLW, extravascular lung water; BW, body wt. *P < 0.05, significantly different from DMSOex.
increased PAP is a consequence of the permeability lung decreases by accumulation of EVLW. In this case, could be that, as edema increases, compliance of the zone 3 condition was estimated by inducing an elevation of 10 mmHg in LAP. We found that there was no results probably reflect the leak of a smaller-molecular-(as opposed to the globular aspect of albumin), the collection of endothelial and epithelial permeability. However, there is a limitation to measurement of the combination of endothelial and epithelial permeability edema, allowing us to study, in and ex vivo, the validity of FITC-dextran as a marker of alveolar capillary membrane injury compared with a more conventional method using radiolabeled markers. In the isolated perfused lung model, the development of ANTu injury can be closely monitored and is a good model in which to assess the utility of FITC-dextran as a marker for alveolar capillary leak. Compared with the DMSO group, a leak from the perfusate to the lung can be seen 90 min after hanging of the preparation, as evidenced by the decrease in volume in the reservoir chamber and pressure recorded from it. The leak rapidly increases after 150 min, associated with a rise in PAP and airway pressure. These observations are consistent with previous studies performed under similar conditions with the same dose of ANTu (15, 30). Several factors could be responsible for reservoir weight loss. Possible explanations are change in extravascular water, protein, and blood; change in intravascular volume; extrapulmonary perfusate leak; edema clearance; and evaporative water loss. Both extrapulmonary perfusate leak and edema clearance should be returned to the reservoir, but these measurements do not allow differentiation of increased vascular permeability from changes in extravascular volumes. However, we performed the measurement of EVLW and AS, which both indicate an increase in permeability and rule out the occurrence of an increase in intravascular volume alone as an explanation of differences in lung fluid accumulation in this model. We also checked that there were no differences in the fraction of the lung in West’s zone 3 condition. This was estimated by inducing a small change in LAP and recording the resulting change in PAP. The percentage of lung in the zone 3 condition was estimated by inducing an elevation of 10 mmHg in LAP. We found that there was no difference among the groups. 

We also observed an increase in PAP; one explanation could be that, as edema increases, compliance of the lung decreases by accumulation of EVLW. In this case, increased PAP is a consequence of the permeability edema and should occur concomitantly with alveolar flooding. The second hypothesis is that there is a worsening of the injury related primarily to an increase in PAP. This situation would falsely induce an increase in FITC-dextran recovered in the BAL by a hydrostatic mechanism, compounding the permeability injury. To clarify this issue, because we did not measure microvascular pressure, the capillary filtration coefficient was estimated by dividing the flux by the mean PAP for each 30-min period of the ex vivo experiment (Fig. 3) (20). In the ANTu group, flux/PAP ratio is high throughout the experiment. The injury remains stable during the study, and the permeability edema is reflected in the higher flux. The increase in the leak during the final phase of the study is only related to the augmentation of PAP, as shown by the unchanging flux/PAP ratio. However, we must underscore that this method only approximates and does not measure microvascular permeability and particularly neglects osmotic driving forces in Starling’s equation. Nevertheless, similar results were found by Rutili et al. (27) in dogs. He demonstrated that capillary hydrostatic pressure in the ANTu lung injury was the major determinant of the amount of EVLW. In this study, constant LAP was maintained by bleeding the animal after either colloid or crystalloid infusions, and EVLW was unchanged compared with preinfusion values in ANTu-treated dogs. From these results, we can conclude that our model is an epithelial permeability-type edema. After 120 min, a hydrostatic component is superimposed on the permeability injury, as shown by the increase in PAP with a constant flux/PAP. In the DMSO group, a slight but significant increase in the flux/PAP ratio can be seen. PAP is stable with no significant increase during the experiment (Fig. 2A), so changes in flux/PAP can be attributed to an increase in flux. The absolute values of the flux are small compared with the ANTu group (1.73 vs. 11 ml at 180 min). This minimal leak in the DMSO group is most likely related to a mild deterioration of the isolated lung preparation over the 3 h of the experiment. Thus microvascular pressures may be similar and therefore imply that the filtration coefficient is higher for the ANTu group. Ex vivo, the total amount of fluorescence recovered in the BAL of the ANTu group is statistically greater than that from the DMSO group. Because FITC-dextran is a high-molecular-weight compound, its extravasation into the alveolar compartment indicates a permeability-type edema. Because dextran is a snakelike structure (as opposed to the globular aspect of albumin), the results probably reflect the leak of a smaller-molecular-weight structure.

These results obtained with FITC-dextran are in agreement with the increase in AS and EVLW measured with radiolabeled tracers. Dextran extravasation, as well as AS and EVLW, only reflects a global measure of the combination of endothelial and epithelial permeability. However, there is a limitation to dextran used according to our protocol. In fact, we measured the quantity of dextran leaking from the vascular compartment into the alveoli and only into the alveoli. We cannot assess the leak of dextran into the extravascular water, protein, and blood; change in intravascular volume; extrapulmonary perfusate leak; edema clearance; and evaporative water loss. Possible explanations are change in extravascular water, protein, and blood; change in intravascular volume; extrapulmonary perfusate leak; edema clearance; and evaporative water loss. Both extrapulmonary perfusate leak and edema clearance should be returned to the reservoir, but these measurements do not allow differentiation of increased vascular permeability from changes in extravascular volumes.
interstitium, which is the first step of the injury; we therefore underestimate lung injury. On the contrary, AS and EVLW are measured in the global lung and, even if interstitial and alveolar leaks cannot be differentiated, this measurement remains more sensitive but less specific than that of dextran leak.

FITC-dextran has been previously used to assess lung permeability changes (22, 25), but mostly to localize, morphologically, protein leakage after different kinds of injuries. Abernathy et al. (1) have used radiolabeled dextran compared with albumin to evaluate macromolecule transport across the alveolar capillary membrane; however, their goal was not to measure a leak. Zheng et al. (33) investigated effects of acute hyperoxia on solute transport in isolated rat lung by filling air spaces with solutions containing FITC-dextran and other markers. However, to our knowledge, FITC-dextran has not yet been measured in the BAL to evaluate lung epithelial injury. Two other methods have been used to describe ANTU-induced lung injury: Minty et al. (23) studied the clearance of 99m-labeled technetium-diethylene-triamine pentaac- etate from the lung into the blood and found an increased permeability of the alveolar capillary barrier after ANTU administration. These authors also studied the clearance from one compartment to the other and could not appreciate a difference between epithelial or endothelial permeability, therefore raising the same difficulties as in our study. One of the most accurate studies on ANTU-induced lung injury was performed by Rutili et al. (27) in a dog model. By assessing lymph flow, they managed to calculate changes in lymph protein clearance for given changes in pulmonary capillary pressure. This study represents the gold standard of assessing lung permeability alterations; however, our model does not allow us to measure all of the same parameters, particularly lymph flow. Nevertheless, dextran leak provides reliable data on alveolar epithelial permeability in a rat model.

In vivo, the results are consistent with a statistically greater amount of FITC-dextran recovered in the ANTU group (Table 1). The chromatographic data confirm that the measured fluorescence is not free fluorescein but label that is still linked to high-molecular-weight dextran. Thus there is no evidence that a false-positive result due to an in vivo deterioration of the compound occurred. Serum osmolality values before and after FITC-dextran injection were also obtained and were not significantly different (data not shown). Therefore, measurement of FITC-dextran in BAL fluid is a reliable means of detecting lung permeability changes in vivo and ex vivo.

The quantity of FITC-dextran recovered in vivo is greater than that recovered ex vivo. Ex vivo, to perfuse the lung, the blood of a noninjured animal is used to obtain a stable and reproducible injury. In vivo, native blood is used, with ongoing injury likely occurring over the course of the experiment. For the lung W/D, we observed the opposite phenomenon, higher values for the ex vivo lungs. King et al. (15) observed the same differences between in vivo and ex vivo with higher lung W/D in the isolated-organ chamber. A difference in perfusion pressure could be an explanation as well as a change in lymph clearance, which would allow a better clearance of fluid from the lung in vivo compared with an ex vivo preparation.

Finally, to validate the reliability of our method, we added a group with noninjured lungs, in which the microvascular pressure was elevated to create a hydrostatic stress. We observed no leak of FITC-dextran; this control group underscores the need for an increase in permeability to observe a leak of dextran within the alveoli and eliminates a hydrostatic component as the primary cause of the leak in the ANTU group.

In conclusion, measurement of FITC-dextran in BAL fluid is a reliable method of assessing lung injury in vivo or ex vivo. Compared with radiolabeled molecules ex vivo, labeled dextran measures a global permeability alteration, but this measurement, in its neglect of the interstitial compartment, could underestimate the injury. However, fluorolabeled dextran is easier to use and does not require the special safety precautions required when radioisotopic markers are used. A future application may be the use of different-size dextran molecules to assess the sieving properties of the alveolar capillary membrane, therefore describing more accurately the characteristics of the injury.

The authors are grateful to Dr. Claude Foucher for help and Xavier Marechal for input in the fluorescence data management.

This work was supported in part by the research group Réponse à l’agression septique et mécanismes d’adaptation (JE 2084, Univ. of Lille) and the Centre Hospitalier Régional Universitaire of Lille.

Address for reprint requests: B. Guery, Service de Réanimation Médicale et Maladies Infectieuses, Soins intensifs de Cardiologie, Centre Hospitalier de Tourcoing, 135 Rue du Pdt Coty, 59208 Tourcoing, France (E-mail: bguery@compuserve.com).

Received 6 August 1997; accepted in final form 21 April 1998.

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