Transdiaphragmatic transport of tracer albumin from peritoneal to pleural liquid measured in rats

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Lai-Fook, Stephen J., Pamela K. Houtz, and Philip D. Jones. Transdiaphragmatic transport of tracer albumin from peritoneal to pleural liquid measured in rats. J Appl Physiol 99: 2212–2221, 2005. First published August 11, 2005; doi:10.1152/japplphysiol.00731.2005.—In conscious Wistar-Kyoto rats, we studied the uptake of radioactive tracer 125I-albumin into the pleural space and circulation after intraperitoneal (IP) injections with 1 or 5 ml of Ringer solution (3 g/dl albumin). Postmortem, we sampled pleural liquid, peritoneal liquid, and blood plasma 2–48 h after IP injection and measured their radioactivity and protein concentration. Tracer concentration was greater in pleural liquid than in plasma ~3 h after injection with both IP injection volumes. This behavior indicated transport of tracer through the diaphragm into the pleural space. A dynamic analysis of the tracer uptake with 5-ml IP injections showed that at least 50% of the total pleural flow was via the diaphragm. A similar estimate was derived from an analysis of total protein concentrations. Both estimates were based on restricted pleural capillary filtration and unrestricted transdiaphragmatic transport. The 5-ml IP injections did not change plasma protein concentration but increased pleural and peritoneal protein concentrations from control values by 22 and 30%, respectively. These changes were consistent with a small (~8%) increase in capillary filtration and a small (~20%) reduction in transdiaphragmatic flow from control values, consistent with the small (3%) decrease in hydration measured in diaphragm muscle. Thus the pleural uptake of tracer via the diaphragm with the IP injections occurred by the near-normal transport of liquid and protein.

METHODS

This study was approved by University of Kentucky Animal Care Committee. We studied Wistar-Kyoto rats (Harlan, Indianapolis, IN) of body weight 150–270 g and 56–85 days old (n = 105). The uptake of radioactive tracer 125I-albumin (bovine serum albumin; Perkin Elmer, Boston, MA) from the peritoneal cavity into the pleural space and adjacent organs was studied with the following experiments. A 1- or 5-ml Ringer solution with 3 g/dl albumin (bovine serum albumin, Sigma, St. Louis, MO) and tracer was injected into the left lower quadrant of the abdomen of each conscious animal. We used both 1- and 5-ml IP injections to determine whether the tracer uptake into the pleural space and plasma depended on the size of the injection. Before use, the tracer was passed through a desalting column to remove unbound 125I (~5% of the total radioactivity) and molecular weight components below ~5,000 Da (PD-10 desalting column; Amersham Biosciences, Piscataway, NJ). All solutions were filtered (0.45 μm) before injection into the animals.

After a selected time (2, 4, 6, 12, 24, and 48 h for the 5-ml IP injections or 3 and 6 h for the 1-ml IP injections), the animal was anesthetized with an intramuscular injection of ketamine (50 mg/kg) and xylazine (4 mg/kg), a blood sample was withdrawn from a jugular vein, and the animal was euthanized with an intravenous injection of pentobarbital sodium solution (100 mg/kg) and exsanguination. Postmortem, we dissected the overlying tissue of the abdomen and sampled peritoneal liquid by suction with a syringe via a length of PE-10 tubing. Then, with the supine animal tilted to allow peritoneal liquid to drain into the costophrenic region, we punctured the ventral region of the diaphragm to produce a pneumothorax. Through two holes pierced in the diaphragm, we collected pleural liquid from the costophrenic region of both sides of the thorax by suction with a syringe via a length of PE-10 tubing. To avoid contamination by blood, no attempt was made to collect all of the peritoneal and pleural liquid. Plasma samples were separated from the whole blood by centrifugation. The lung, diaphragm, and a sample of left thigh muscle were removed by dissection. The diaphragm was separated into its central tendon (~0.4 g), central tendon (~0.2 g), lung (~0.8 g), and thigh muscle (~0.3 g) were weighed, and their radioactivity counted. Then the samples were dried in an oven at 70°C to a constant weight to determine their wet-to-dry weight ratios (W/D). In some experiments, we also dissected samples of intercostal muscles of the first and seventh intercostal space.

Specific radioactivity (counts per second per gram) of the collected peritoneal liquid, pleural liquid, and plasma was measured before and during postmortem collection. Liquid and protein concentrations were measured before and during postmortem collection. Protein concentration...
after separation of their albumin fraction by desalting columns. In initial experiments, we used IP injection volumes with unpurified tracer and measured the total specific radioactivity of peritoneal liquid, pleural liquid, plasma, diaphragm muscle, diaphragm central tendon, thigh muscle, and lung. In the latter experiments, we measured (total) protein concentration of pleural liquid, peritoneal liquid, and plasma using a protein assay (Coomassie Plus, Pierce Biotechnology, Rockford, IL).

In some studies, we measured pleural liquid volume (Vp) by the following procedure. Postmortem, after the pleural liquid was sampled, a 2-ml Ringer solution containing 1 g/dl albumin was injected into the chest cavity to lavage the visceral and parietal pleural surfaces. The lavage liquid was collected by suction and weighed, and its specific radioactivity measured. The weight and the radioactivity of the initial pleural liquid collected and of the lavage liquid were used to calculate the Vp using a mass balance analysis.

To determine whether the 5-ml IP injection volumes per se affected the normal transport of liquid and protein, we repeated the entire experiment in seven rats without IP injections.

In separate studies, we repeated the foregoing studies, but, instead of injecting tracer into the peritoneal cavity, we injected tracer into the circulation. In these studies, the rat was anesthetized, and purified tracer (2 × 10⁸ count/s in 0.3 ml of Ringer solution with 1 g/dl albumin) was injected into the tail artery. After 1 min, a sample of blood was withdrawn and its plasma radioactivity measured. The animal was allowed to recover from the anesthetic. After a selected time (0.5 and 1 h) following tracer injection, the animal was studied postmortem following the procedures identical to those used for the IP injection studies.

Statistics

Data are reported as means ± SD in Tables 1–4 and means ± SE in Figs. 2 and 3. We used a paired t-test or an unmatched t-test, where appropriate, to test for a significant difference between two groups. We accepted P < 0.05 to be significant.

RESULTS

Experiments

Tracer measurements in pleural liquid, peritoneal liquid, and plasma. Figure 1 shows an example of the relative fraction of bound ¹²⁵I-albumin in collected samples of peritoneal liquid (92%), pleural liquid (77%), and plasma (61%) separated by desalting columns. The IP injection volume consisted of 5 ml of 3 g/dl albumin in Ringer solution containing tracer with unbound ¹²⁵I removed. The time after injection was 2 h. The fraction of bound ¹²⁵I-albumin was the radioactivity of the first 6-ml eluent (3 min) relative to the total radioactivity eluted. In general, with the 5-ml IP injections, the fraction of bound ¹²⁵I-albumin in the three liquids was 70–90% after 2–24 h and fell to ~50% after 48 h. Tracer breakdown was faster with 1-ml IP injections: after 3 h, the bound tracer was 60%, and after 6 h it decreased to 40%. Accordingly, we reported no data of 1-ml IP experiments longer than 6 h. Specific activity (count per second per gram) was corrected by multiplying total tracer injection volume (counts/min) was measured in the volumes eluted every 30 s from desalting columns.

Fig. 1. Example of separation of unbound ¹²⁵I-albumin from tracer in collected peritoneal liquid (A), pleural liquid (B), and plasma (C). Radioactivity (counts/min) was measured in the volumes eluted every 30 s from desalting columns.

Table 1 summarizes pleural tracer concentration (Cp)-to-Ca (Cp/Ca), plasma tracer concentration (Cp)-to-Ca (Cp/Ca), and Cc-to-Ca (Cc/Ca) ratios at different times after 1- and 5-ml IP injections. We used subscripts p for pleural, a for peritoneal (abdominal), and c for plasma (capillary). Ratios were corrected for unbound tracer. Figure 2 is a plot of tracer ratios Cc/Ca and Cc/Ca (A), and Cc/Ca (B) vs. time (t in h) for 5-ml IP injections. Note that tracer concentration was always higher in pleural liquid (Cp/Ca) than in plasma (Cc/Ca) up to 12 h, consistent with a transdiaphragmatic transport of tracer into the pleural space from the peritoneal cavity, in addition to transport from the circulation. At 2 and 4 h, Cc/Ca values (0.14 and 0.54) were significantly greater than the Cc/Ca values (0.026 and 0.057). Smaller Cc/Ca than Cc/Ca values would be expected if the tracer were to enter the circulation from the peritoneal cavity before it entered the pleural space. Consistent with the greater Cc than Cc, Cc/Cc was considerably >1 and varied between 10 and 3 between 2 and 12 h. This also indicated a transdiaphragmatic source of tracer other than from...
injections is speculative (see DISCUSSION).

The reason for the smaller values with 1-ml IP injections were much smaller than the values with 5-ml IP injections (Table 1). At 6 h with 1-ml IP injections, values for Cp/Ca, Cc/Ca, and Cp/Cc measured with 1-ml IP injections were much smaller than the values with 5-ml IP injections. The reason for the smaller values with 1-ml IP injections is speculative (see DISCUSSION).

For 5-ml IP injections, tracer Cp/Ca and Cp/Cc tended to values of 0.67 and 0.55 at 48 h, respectively, slightly lower than the equilibrium values (0.77 and 0.59) measured for protein concentration (see Table 3). This might indicate that a steady state was not reached, not unexpected with the continual washout of the tracer (Fig. 2). However, tracer Cc/Ca tended to a value of 1.3 at 48 h, consistent with the measured protein Cc/Cc of 0.76 (see Table 3).

Tracer measurements in tissue. In the experiments in which we did not remove or correct for unbound $^{125}$I, the results of tracer uptake into the diaphragm (muscle and central tendon) provided further evidence of transport across the diaphragm. These results are shown for 5-ml IP injections in Table 2. Similar results (not shown) were obtained for 1-ml IP injections. Note that for 5-ml IP injections, diaphragm tracer concentration relative to Ca [diaphragm muscle (Cdm)/Ca] and central tendon (Cdt)/Ca] increased monotonically with time, with a lower equilibrium value (−0.5) for Cdm/Ca than for Cdt/Ca (−1) at 96 h. This behavior occurred in parallel with an increase in the Cc/Cc (Fig. 3). This indicated that pleural tracer was derived in part from the peritoneal liquid via the diaphragm. In contrast to the latter behavior, the response in lung was derived in part from the peritoneal liquid via the diaphragm. In contrast to the latter behavior, the response in lung was derived in part from the pulmonary liquid via the diaphragm.

The greater Cp/Ca than Cc/Ca values observed with 5-ml IP injections between 2 and 12 h was only observed at 3 h with 1-ml IP injections (Table 1). At 6 h with 1-ml IP injections, Cp/Ca and Cc/Ca values were not statistically different. The values for Cc/Ca, Cc/Ca, and Cp/Cc measured with 1-ml IP injections were much smaller than the values with 5-ml IP injections. The reason for the smaller values with 1-ml IP injections is speculative (see DISCUSSION).

Table 1. Pleural-to-peritoneal, plasma-to-peritoneal, and pleural-to-plasma tracer concentration ratios

<table>
<thead>
<tr>
<th>IP Volume, ml</th>
<th>Time After Injection, h</th>
<th>n</th>
<th>Cp/Ca</th>
<th>Cc/Ca</th>
<th>Cp/Cc</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>7</td>
<td>0.070±0.050*</td>
<td>0.032±0.015</td>
<td>2.1±1.6</td>
</tr>
<tr>
<td>1</td>
<td>6</td>
<td>3</td>
<td>0.078±0.053</td>
<td>0.057±0.029</td>
<td>1.3±0.36</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>3</td>
<td>0.14±0.058*</td>
<td>0.026±0.006</td>
<td>5.1±0.89</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>3</td>
<td>0.54±0.13*</td>
<td>0.057±0.009</td>
<td>9.5±2.5</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>5</td>
<td>0.74±0.32</td>
<td>0.15±0.066</td>
<td>5.5±3.2</td>
</tr>
<tr>
<td>5</td>
<td>12</td>
<td>5</td>
<td>1.1±0.96</td>
<td>0.44±0.22</td>
<td>3.1±2.8</td>
</tr>
<tr>
<td>12</td>
<td>24</td>
<td>4</td>
<td>0.58±0.11</td>
<td>0.78±0.032</td>
<td>0.74±0.17</td>
</tr>
<tr>
<td>48</td>
<td>48</td>
<td>4</td>
<td>0.67±0.11</td>
<td>1.3±0.17</td>
<td>0.55±0.15</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, no. of experiments. Tracer concentration was corrected for unbound $^{125}$I. IP, intraperitoneal; Cp, pleural tracer concentration; Cc, plasma tracer concentration; Cc/Cc, Cc/Cca, and Cp/Cc: ratios of Cp to Cc, Cc to Cca, and Cp to Cc. *Significantly greater than Cc/Cca values, P < 0.05.
of a contribution of a restricted microvascular filtration or an albumin-excluded volume in the intracellular diaphragm muscle (see Discussion).

Restricted tracer transport across the microvasculature was demonstrated by the ratios C/C and Cn/Cc for the lung and thigh muscle that attained steady-state values of ~0.3 and ~0.1, respectively, by 3 h with 5-ml IP injections (Table 2). The C/C values were overestimated by ~30% because of the trapped blood in the isolated lung, estimated to be 25% of the total protein concentrations in pleural liquid (Cp), peritoneal muscle, and lung wet weight in sheep (11). No correction was necessary for trapped blood in the isolated lung, estimated to be 25% of the total protein concentrations in pleural liquid (Cp), peritoneal muscle, and lung wet weight in sheep (11). No correction was necessary for the Cn/Cc values because residual blood in muscle has been estimated to be ~1% (29). Thigh muscle Cm/Cc values of ~0.1 were near the expected value for a reflection coefficient (0.9) for the vascular endothelial barrier (18) and somewhat lower than interstitial protein concentration of skeletal muscle (3). Intercostal muscle Cm/Cc values were similar to those of thigh muscle.

Total protein measurements. Table 3 summarizes data of (total) protein concentrations in pleural liquid (Cp), peritoneal liquid (Cp), and plasma (Cp), and of Cp/Cc, Cm/Cc, and Cm/Cc. These measurements were done on samples collected from the experiments of Table 2 for 5-ml IP injections and the similar experiments for 1-ml IP injections. The data from the 3- to 96-h IP injections were pooled because there was no significant trend with time. In the control animals without IP injections, protein concentration averaged 5.0 g/dl for plasma, 2.4 g/dl for pleural liquid, and 2.9 g/dl for peritoneal liquid. Compared with control, the 5-ml IP injections increased peritoneal and pleural liquid protein concentration by 30 and 22%, respectively, with no change in the plasma protein concentration. A similar behavior was observed for the 1-ml IP injections, except for the absence of any change in the pleural protein concentration compared with control.

The changes in pleural and peritoneal liquid protein concentrations with the IP injections provided further evidence in support of transport across the diaphragm. First, plasma protein concentration did not change with 5-ml IP injections compared with control. Thus microvascular filtration across pleural capillaries cannot alone be responsible for the 22% increase in pleural liquid protein concentration measured with the 5-ml IP injections. The increased protein concentration in pleural and peritoneal liquid was consistent with a small increase in pleural flow across pleural capillaries coupled with a small decrease in pleural flow across the diaphragm (see Discussion). Accordingly, the change in pleural protein concentration was attributed to the increase in protein concentration of peritoneal liquid that occurred with the 5-ml IP injections compared with control. Second, protein concentration was always less in Cp than in Cm. Cp/Cc averaged 0.82, 0.71, and 0.77 for control, 1-ml IP, and 5-ml IP injections, respectively. These values were consistent with transdiaphragmatic transport with a relatively small restriction to protein, with reflection coefficients (σd) of 0–0.3 based on a predominantly bulk flow (Qb) (see below and Eq. A2 of Appendix).

Previous measurements showed normal Cp/Cc values of 0.73 and 0.55 for albumin and protein, respectively, that were somewhat higher than values measured for tracer albumin (0.55) and protein (0.48) in the present study (17). These differences might reflect the different assays used to measure protein (Bromcresol Green) and albumin (Biuret) concentration in the previous study (17).

W/D. Table 4 summarizes the data of W/D values for the diaphragm tendon, diaphragm muscle, lung, and thigh muscle. W/D values did not vary with time (3–96 h) and were pooled. In general, there were only minor differences in W/D (~6%) among the control values and 1-ml IP and 5-ml IP injections. The diaphragm tendon had the lowest W/D values, followed by the diaphragm muscle, thigh muscle, and lung. The 1- and 5-ml

Table 2. Tissue tracer concentration relative to peritoneal tracer concentration after 5-ml IP injections

<table>
<thead>
<tr>
<th>Time After Injection, h</th>
<th>n</th>
<th>Cm/Cc</th>
<th>Cm/Cc</th>
<th>Cm/Cc</th>
<th>Cm/Cc</th>
<th>Cm/Cc</th>
<th>Cm/Cc</th>
</tr>
</thead>
<tbody>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>0.14±0.051</td>
<td>0.41±0.18</td>
<td>0.52±0.32</td>
<td>1.5±0.72</td>
<td>0.38±0.13</td>
<td>0.10±0.053</td>
</tr>
<tr>
<td>6</td>
<td>5</td>
<td>0.13±0.017</td>
<td>0.32±0.054</td>
<td>0.61±0.18</td>
<td>1.4±0.27</td>
<td>0.32±0.046</td>
<td>0.10±0.053</td>
</tr>
<tr>
<td>12</td>
<td>5</td>
<td>0.19±0.054</td>
<td>0.53±0.22</td>
<td>0.33±0.027</td>
<td>0.92±0.29</td>
<td>0.38±0.10</td>
<td>0.085±0.035</td>
</tr>
<tr>
<td>24</td>
<td>4</td>
<td>0.20±0.056</td>
<td>0.51±0.25</td>
<td>0.39±0.05</td>
<td>0.96±0.24</td>
<td>0.33±0.15</td>
<td>0.093±0.046</td>
</tr>
<tr>
<td>48</td>
<td>3</td>
<td>0.34±0.12</td>
<td>0.69±0.17</td>
<td>0.48±0.17</td>
<td>0.94±0.45</td>
<td>0.46±0.24</td>
<td>0.11±0.050</td>
</tr>
<tr>
<td>96</td>
<td>4</td>
<td>0.47±0.11</td>
<td>1.1±0.29</td>
<td>0.56±0.16</td>
<td>1.3±0.42</td>
<td>0.41±0.090</td>
<td>0.097±0.011</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, number of experiments. No correction was made for unbound 125I. Cm, diaphragm muscle tracer concentration; Cm, diaphragm tendon tracer concentration; Cc, lung tracer concentration; Cm, thigh muscle tracer concentration.

Table 3. Total protein concentrations in pleural liquid, peritoneal liquid, and plasma

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>1-ml IP</th>
<th>5-ml IP</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>7</td>
<td>29</td>
<td>22</td>
</tr>
<tr>
<td>Pleural liquid Cm, g/dl</td>
<td>2.37±0.22</td>
<td>2.57±0.28</td>
<td>2.89±0.71††</td>
</tr>
<tr>
<td>Peritoneal liquid Cm, g/dl</td>
<td>2.88±0.17††</td>
<td>3.61±0.50**</td>
<td>3.75±0.52††</td>
</tr>
<tr>
<td>Plasma Cm, g/dl</td>
<td>4.99±0.36</td>
<td>5.17±0.69</td>
<td>4.95±0.50</td>
</tr>
<tr>
<td>Pleural/plasma Cm/Cc</td>
<td>0.48±0.066</td>
<td>0.49±0.066</td>
<td>0.59±0.16††</td>
</tr>
<tr>
<td>Peritoneal/plasma Cm/Cc</td>
<td>0.58±0.068</td>
<td>0.70±0.069‡‡</td>
<td>0.76±0.13††‡‡</td>
</tr>
<tr>
<td>Pleural/peritoneal Cm/Cc</td>
<td>0.82±0.063</td>
<td>0.71±0.070</td>
<td>0.77±0.15‡‡</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, number of experiments. *Significantly different from control; †significantly different from 1-ml IP; ‡significantly greater than pleural values: P < 0.05.

Table 4. Wet-to-dry weight ratio of diaphragm, lung, and thigh muscle

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>1-ml IP</th>
<th>5-ml IP</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>7</td>
<td>31</td>
<td>32</td>
</tr>
<tr>
<td>Diaphragm tendon</td>
<td>3.34±0.44</td>
<td>3.15±0.62</td>
<td>3.24±0.47</td>
</tr>
<tr>
<td>Diaphragm muscle</td>
<td>3.75±0.15†</td>
<td>3.60±0.17†</td>
<td>3.66±0.18††</td>
</tr>
<tr>
<td>Lung</td>
<td>4.63±0.10</td>
<td>4.57±0.14</td>
<td>4.67±0.29</td>
</tr>
<tr>
<td>Thigh muscle</td>
<td>4.12±0.049</td>
<td>4.21±0.083‡‡</td>
<td>4.14±0.13</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, number of experiments. *Significantly greater than tendon; †significantly different from control; ‡significantly greater than 5-ml IP: P < 0.05.
IP injections produced a significantly small (2–4%) reduction in W/D values for diaphragm muscle compared with control. The reduced hydration might have resulted from a reduced flow and greater osmotic absorption of water from muscle tissue into the fluid that crossed the diaphragm with an inflow and greater osmotic absorption of water from muscle tissue into the fluid that crossed the diaphragm with an inflow and greater osmotic absorption of water from muscle tissue into the fluid that crossed the diaphragm with an increased protein concentration (20).

Pleural liquid volume. Pleural liquid volume ($V_p$) measured in the experiments after 3–48 h averaged 0.15 ± 0.082 ml with 5-ml IP injections ($n = 48$), 0.12 ± 0.053 ml with 1-ml IP injections ($n = 43$), and 0.14 ± 0.068 ml with intra-arterial tracer injections ($n = 18$). These values were not significantly different among the groups. The pooled values averaged 0.13 ± 0.070 ml ($n = 115$). $V_p$ normalized by dividing by body weight averaged 0.62 ± 0.33 ml/kg, comparable to published values of 0.6 ml/kg (19). The similar pleural volume measured with 1- and 5-ml IP injections and with control supported the estimated small changes in pleural flow due to the IP injections (see Transport Analysis and DISCUSSION).

Transport Analysis

Model of albumin and liquid transport into the pleural space. We developed a mathematical model of liquid and albumin transport from the circulation and peritoneal cavity into the pleural space. A brief outline is presented with details in the Appendix. Consider the pleural space as an input-output system (Fig. 4) with two input sources and one output source. One input source is the circulation, and the other is the peritoneal cavity via the diaphragm. The output is lymphatic outflow. $C_p/C_a$ is given by the following first-order linear ordinary differential equation:

$$\frac{d(C_p/C_a)}{dt} = K_a + K_c(C_p/C_a) - [(1/C_a)(dC_p/dt) + Q_f/V_p](C_p/C_a)$$

(1)

where $K_c$ and $K_a$ are two transport coefficients that describe the sieving properties of the pleural microvascular barrier and the diaphragm in terms of their reflection coefficients $\sigma_c$ and $\sigma_d$, respectively:

$$K_c = \left(\frac{Q_p}{V_p}\right)(1 - \sigma_c)$$

(2)

$$K_a = \left(\frac{Q_a}{V_p}\right)(1 - \sigma_d)$$

(3)

$Q_p$ and $Q_a$ are the steady-state flows across the pleural microvascular barrier and diaphragm, respectively, and are related to $Q_p$:

$$Q_p = Q_c + Q_a$$

(4)

In Eqs. 2 and 3, we assume only convective transport through cylindrical membrane pores and neglect diffusion (Ref. 25; Eq. A2 of Appendix). Numerical solutions to Eqs. 1–4 were matched to the experimental data with 5-ml IP injections using the following procedure. In Eq. 1, $C_a$ and $C_c/C_a$ were functions of time ($t$) chosen to fit the experimental data (see Figs. 2 and 3). By an iterative procedure, the solution for $C_p/C_a$ vs. time that matched the experimental data (Fig. 3) was found by numerical integration of Eq. 1. A good fit of the data was obtained with values of $K_c$ of 0.02 h$^{-1}$, $K_a$ of 0.23 h$^{-1}$, and $Q_p/V_p$ of 0.46 h$^{-1}$. With only these values, it was not possible to determine a unique set of values for the remaining four constants ($Q_p$, $Q_a$, $\sigma_c$, and $\sigma_d$) using the three equations (Eqs. 2–4). Accordingly, values for $Q_a/Q_p$ were obtained within the expected range for $\sigma_c$ (0.75–0.91) and $\sigma_d$ (0.03–0.39) that were consistent with restricted microvascular filtration (18) and a relatively unrestrictive diaphragm (20), respectively. Table 5 summarizes the calculated values. Here $Q_p$ was 0.06 ml/h based on the measured $V_p$ (0.13 ml) and the computed $Q_a/V_p$ value (0.46 h$^{-1}$). Note that $Q_a/Q_p$ was in the range 0.52–0.83, that is, 52–83% of the total flow into the pleural space was across the diaphragm. These estimates agreed with those based on a steady-state analysis of protein data (see below).

The tracer uptake data with 1-ml IP injections were limited to 6 h after injection and precluded any dynamic analysis to determine $Q_a/Q_p$.

The value of $Q_p$ of 0.06 ml/h, equivalent to 0.24 ml·h$^{-1}$·kg$^{-1}$, with 5-ml IP injections, was 12 times the value of 0.02 ml·h$^{-1}$·kg$^{-1}$ measured in sheep (28), dogs (22), and rabbits (9). Part of the greater pleural flow in the rat might be attributed to transdiaphragmatic flow that was reduced in the larger animals (see Discussion).

Estimates of $\sigma_c$, $\sigma_d$, and $Q_a/Q_p$ based on total protein concentrations. The steady-state values of protein concentrations $C_p$, $C_a$, and $C_c$ (Table 3) are related to $Q_a/Q_p$, $\sigma_c$, and $\sigma_d$ by the equilibrium solution of Eq. 1 at infinite time when $d(C_p/C_a)/dt = dC_p/dt = 0$:

$$\frac{C_p}{C_c} = \left[1 - \left(\frac{Q_a}{Q_p}\right)(1 - \sigma_d)\right] + \left(\frac{Q_a}{Q_p}\right)(1 - \sigma_d)\left(\frac{C_c}{C_a}\right)$$

(5)

Table 6 shows the solutions for $\sigma_c$, $\sigma_d$, and $Q_a/Q_p$ based on the average values for $C_p/C_a$ and $C_d/C_c$ measured in the control, 1-ml IP, and 5-ml IP experiments (Table 3). Note that, for $\sigma_c$ of 0.90–0.65 and $\sigma_d$ of 0–0.19, $Q_a/Q_p$ ranged from 0.5 to 0.8. Thus both the tracer uptake and equilibrium protein analyses showed that flow into the pleural space via the diaphragm was 50–80% of the total flow.

Table 5. Estimated values of $Q_a/Q_p$, $\sigma_c$, and $\sigma_d$ during tracer uptake after 5-ml IP injections

<table>
<thead>
<tr>
<th>$Q_a/Q_p$</th>
<th>$\sigma_c$</th>
<th>$\sigma_d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.52</td>
<td>0.91</td>
<td>0.03</td>
</tr>
<tr>
<td>0.57</td>
<td>0.90</td>
<td>0.12</td>
</tr>
<tr>
<td>0.71</td>
<td>0.85</td>
<td>0.30</td>
</tr>
<tr>
<td>0.78</td>
<td>0.80</td>
<td>0.36</td>
</tr>
<tr>
<td>0.83</td>
<td>0.75</td>
<td>0.39</td>
</tr>
</tbody>
</table>

$Q_a$, steady-state flow across diaphragm; $Q_p$, total flow into pleural space; $\sigma_c$, reflection coefficient of capillary membrane; $\sigma_d$, reflection coefficient of diaphragm.
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Table 6. Values of \( \frac{Q_a}{Q_p} \), \( \sigma_c \), and \( \sigma_d \) based on protein concentrations in pleural liquid, peritoneal liquid, and plasma

<table>
<thead>
<tr>
<th>( \frac{Q_a}{Q_p} )</th>
<th>Control</th>
<th>1-ml IP</th>
<th>5-ml IP</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \sigma_c )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.8</td>
<td>0.9</td>
<td>0.9</td>
<td>0.9</td>
</tr>
<tr>
<td>( \sigma_d )</td>
<td>0.02</td>
<td>0.16</td>
<td>0.07</td>
</tr>
<tr>
<td>0.8</td>
<td>0.7</td>
<td>0.85</td>
<td>0.85</td>
</tr>
<tr>
<td>( \sigma_c )</td>
<td>0.10</td>
<td>0.18</td>
<td>0.09</td>
</tr>
<tr>
<td>0.7</td>
<td>0.75</td>
<td>0.19</td>
<td>0.01</td>
</tr>
<tr>
<td>( \sigma_d )</td>
<td>0.01</td>
<td>0.06</td>
<td>0.07</td>
</tr>
<tr>
<td>0.7</td>
<td>0.7</td>
<td>0.05</td>
<td>0.02</td>
</tr>
<tr>
<td>( \sigma_c )</td>
<td>0.05</td>
<td>0.19</td>
<td>0.07</td>
</tr>
<tr>
<td>0.6</td>
<td>0.65</td>
<td>0.12</td>
<td>0.02</td>
</tr>
<tr>
<td>( \sigma_d )</td>
<td>0.03</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>0.7</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>( \sigma_c )</td>
<td>0.12</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>0.65</td>
<td>0.11</td>
<td></td>
</tr>
</tbody>
</table>

Although the foregoing analysis did not provide an estimate for \( Q_p \), the protein concentration data allowed the estimate of the fractional changes in \( Q_p \) and \( Q_a \) with the IP injections from control values by the application of the Starling and solute flux equations (see DISCUSSION).

DISCUSSION

The important results of this study are as follows. The transport of tracer albumin into pleural space after an IP injection in conscious rats occurred by two routes, a direct route across the diaphragm and an indirect route from the peritoneal cavity. This was evident from the greater \( C_p/C_a \) than \( C_c/C_a \) measured between 2 and 12 h after 5-ml IP injections and at 3 h after 1-ml IP injections. Furthermore, with 5-ml IP injections, \( C_p/C_a \) up to 12 h were much greater than 1. This indicated that microvascular filtration was not primarily responsible for the pleural tracer uptake. Consistent with the transdiaphragmatic tracer transport, tracer concentration relative to that of plasma was much greater in the diaphragm muscle (0.4) and central tendon (1.0) than in the thigh muscle (0.1) that was supplied only by microvascular filtration. Both dynamic modeling of pleural and plasma tracer uptake with 5-ml IP injections and steady-state modeling of protein concentrations in plasma, pleural, and peritoneal liquids showed that 50–80% of the \( Q_p \) occurred across the diaphragm.

Methods

To study albumin transport using tracer \( ^{125}\text{I} \)-albumin, we needed to correct for the unbound \( ^{125}\text{I} \)-albumin in the collected samples of the peritoneal liquid, pleural liquid, and plasma. This procedure for correcting for tracer breakdown was not applied to the radioactivity measured in tissue (lung, diaphragm, thigh skeletal muscle). Thus the tracer concentration ratios measured in the tissues involved the transport of both bound \( ^{125}\text{I} \)-albumin and unbound \( ^{125}\text{I} \). Accordingly, only qualitative estimates were possible with regard to the transport of albumin through these tissues.

Tracer breakdown also limited the time that could be studied after the IP injection. The fraction of unbound \( ^{125}\text{I} \) in the collected pleural liquid, peritoneal liquid, and plasma increased to 50% of the total tracer radioactivity after 48 h with 5-ml IP injections and after 6 h with the 1-ml IP injections. Thus our studies with 1-ml IP injections were limited to the initial stages of tracer uptake up to 6 h.

In general, tracer breakdown increased with the resident time in the body, tending to nearly the same fraction (50%) of total radioactivity in the peritoneal liquid, pleural liquid, and plasma at 48 h. This behavior might reflect recirculation that produced near steady-state values of bound and unbound tracer in the three compartments. The constant breakdown in the three compartments at 48 h also indicated that tracer concentration ratios based on the uncorrected total tracer radioactivity were subjected to little error. This was supported by the experimental data. In an in vitro experiment, the unbound \( ^{125}\text{I} \)-albumin measured in a 3 g/dl albumin solution with tracer at 37°C was 20% after 48 h, much less than the 50% measured in vivo. One reason for tracer breakdown in vivo might be an immunological reaction to the bovine serum albumin used in the experiments, a substance that was foreign to the rats studied. These effects warrant further study.

The continuous breakdown of tracer also put into question the interpretation of the tracer albumin concentration ratios measured at each time point. However, the correction for unbound \( ^{125}\text{I} \) did not substantially change the relative magnitudes of the tracer ratios measured in the pleural, peritoneal, and plasma liquids. Thus the data with no correction for unbound \( ^{125}\text{I} \) produced the same conclusion that pleural tracer uptake occurred across the diaphragm. That the bound albumin tracer reflected to a large degree the actual transport of endogenous albumin was supported by the values of tracer concentration ratios at 48 h that approached the equilibrium values of protein concentration ratios.

The control \( C_p/C_a \) for protein (0.48) were close to previous values (0.55) measured in Wistar-Kyoto rats (17). However, in the latter study (17), the \( C_p/C_a \) was greater for albumin (0.73) than for protein (0.55), a reflection of the higher molecular weights of some proteins. These differences between albumin and protein were not observed in the present study with tracer albumin and protein. This might be due in part to the separation of the lower molecular weight fraction (<5,000 Da) from the tracer before use and in part to the insensitivity of the assay used in the present study.

Comparison with Previous Studies

In addition to the present tracer studies, transdiaphragmatic transport of peritoneal liquid into the pleural space would be supported by the following studies. Zakaria and Rippe (31) reported a small pleural effusion with an increased IP pressure of 8 mmHg induced by abdominal cuff compression in anesthetized rats with a large dialysate. The present study showed this effect under conditions closer to normal in conscious rats with much smaller IP volumes. In vitro studies in the isolated rat diaphragm muscle (20) showed a relatively high hydraulic conductivity and low reflection coefficient, similar to those of the pleural membrane (16). The greater thickness of the diaphragm would be partly offset by the much lower hydraulic conductivity and high reflection coefficient associated with the capillary endothelium, the predominant resistance to microvascular filtration (18).

The present studies did not allow us to separate the relative contribution of the central tendon and muscle to total flow across the diaphragm. The faster tracer uptake into the diaphragm central tendon than into the diaphragm muscle (Table 2) might seem to indicate a predominant role for the central
tendon. However, the actual predicted transdiaphragmatic flow in conjunction with measured hydraulic conductivity of the isolated diaphragm muscle would suggest that most of the flow was across the diaphragm muscle. The flow across the diaphragm with 5-ml IP injections was estimated from the analysis to be 50–80% of the total pleural flow (0.06 ml/h); that is, 0.03–0.05 ml/h. Using a diaphragm muscle mass of 0.35 g and thickness of 0.07 cm (20), surface area was 5 cm². Hydraulic conductivity of the rat diaphragm measured in previous studies was 1.4 × 10⁻³ ml/cm²·h⁻¹·cmH₂O⁻¹ (20). This value is consistent with a flow of 0.05 ml/h for a diaphragm surface area of 5 cm² and transdiaphragmatic pressure of 7 cmH₂O (2, 20). For a transit time (t) of 1 h and flow (Q) of 0.05 ml/h, the tissue volume for flow (Q × t) was 0.05 ml. With the total diaphragm volume of 0.35 ml, the flow would occur through 14% of the total volume, close to the measured extracellular tissue volume (8, 15). Thus the estimated flow across the diaphragm was consistent with the estimated transit time for the tracer to cross the diaphragm via its extracellular volume. The flow of tracer albumin via 14% of the diaphragm volume would predict a Cc/Ca of 0.14 that was smaller than the values of 0.3–0.5 measured with the IP injections. However, the measured values included the effects of microvascular filtration, lymphatic clearance (32), and unbound¹²⁵I, which would increase tracer concentration in the muscle.

In the analysis, we assumed that transport across the diaphragm and pleural capillaries was due to Qₚ with a relatively high Péclet number, so that tracer concentration of the flow into the pleural space was flow independent and related only to the upstream tracer concentration and reflection coefficient of the membrane (Ref. 25; Eq. A2 of APPENDIX). Accordingly, the primary force for transdiaphragmatic transport was the difference between the IP pressure and pleural pressure. The effect of protein osmotic pressure differences was neglected because both the measured transdiaphragmatic protein concentration difference (0.5–1 g/dl, Table 3) and reflection coefficient (σ₆ of ~0.1, Ref. 20) was small (see Eq. 6 below). Transdiaphragmatic pressure has a mean value of ~8 cmH₂O based on pleural pressure (~9 to ~22 cmH₂O) measured in conscious rats by telemetry (21) and IP pressure (~2 cmH₂O) measured in anesthetized rats (33). In vitro studies in the diaphragm showed that this pressure difference was sufficient to produce flow-independent sieving coefficients of albumin and reliable estimates of reflection coefficient (20).

The Cc/Ca corrected for unbound¹²⁵I at 6 h after IP injections was threefold greater for 5-ml than 1-ml IP injections (Table 1). By contrast, the experiments with unpurified tracer and no correction for unbound¹²⁵I showed no significant difference in Cc/Ca between 1- and 5-ml IP injections. Thus the correction for unbound¹²⁵I was crucial to the determination of the true tracer uptake into the circulation from the peritoneal cavity.

We used a 3 g/dl albumin solution for the IP injections to approximate the normal peritoneal liquid protein concentration (23). Clearance of albumin from the peritoneal cavity has been reported to be independent of the albumin concentration in the dialysate (4). However, in the present study, the use of bovine serum albumin produced short-term and long-term (3–96 h) changes in both peritoneal and pleural protein concentrations, even for relatively small IP injections in the rat.

Differences Between the Rat and Other Species

There are major differences between the rat and other species with regard to pleural and peritoneal transport. Abdominal counterpressure resulted in improved fluid recovery with isotonic dialysis in rats but had no effect on recovery of fluid in humans (13). A reason for this interspecies difference might be that the rat has a significant transdiaphragmatic flow that is reduced in the larger human with a thicker diaphragm.

Pleural liquid protein concentration has been shown to be greater in smaller animals (19). In particular, the Cc/Ca of 0.5 measured in rats (17, 19) was greater than values reported for bigger species such as sheep (0.15; Ref. 28).

One explanation for this difference is an increased capillary-to-pleural pressure gradient in the larger animals (19). This was supported by studies in hypertensive rats compared with normotensive controls (17) and in sheep as vascular pressure increased during development from the fetus to adulthood (10). Another explanation is that pleural liquid protein concentration is increased in smaller animals because of absorption of protein-free pleural liquid by the visceral pleural capillaries with a relatively low pulmonary capillary pressure (16). In the present study, we propose the following explanation: a relatively unrestricted transport across the diaphragm.

Pleural liquid protein concentration relative to that in plasma measured in the larger animals, such as in sheep (0.15; Ref. 28), was near to that expected for a microvascular filtrate (0.10; Ref. 18) because transport across the diaphragm was relatively small. Microvascular filtration would produce a pleural liquid-to-plasma protein concentration ratio that was independent of body mass because the thickness of the microvascular barrier attributed to the endothelium is largely independent of species size (18). By contrast, transport across the diaphragm would decrease with body size because hydraulic conductance of the diaphragm is inversely proportional to its thickness, which increases with body size (thickness ∝ mass¹²).
plasma protein concentrations did not change significantly from control values with 1-ml IP injections, although peritoneal protein concentration increased by 25%. Thus the pleural flow was identical with 1-ml IP injections and control. Part of this flow must have been through the diaphragm to explain why tracer concentration measured 3 h after 1-ml IP injections was greater in pleural liquid than in plasma.

Second, the measured protein concentrations showed no change in plasma and small increases in pleural liquid (22%) and in peritoneal liquid (30%) with 5-ml IP injections. The following analysis using the Starling and solute flux equations showed these results to be consistent with relatively small changes in flow across the pleural microcirculation and diaphragm. According to the Starling equation, Q˙b is related to the microvascular-to-pleural hydrostatic pressure difference (ΔP), the plasma-to-pleural protein osmotic pressure difference (Δπ), and σc:

$$Q_b = K(ΔP - σ_cΔπ)$$  \[(6)\]

where K is the hydraulic conductance (filtration coefficient) of the pleural capillary endothelial membrane. ΔP was assumed constant with the IP injections, because previous results in anesthetized rats showed little change in vascular pressures, even with large amounts of dilaacsyate fluid (26). Studies in the rat showed a ΔP value of 37 cmH2O (7) and σcΔπ of 10 cmH2O (17) with σc of 0.9 (18). With 5-ml IP injections, Cp increased by 22%, resulting in a 22% decrease in σcΔπ. Thus, from Eq. 6 with ΔP constant, Qb increased by 8% with the 5-ml IP injections. The protein flux (Qs) associated with this increase in Qb is given by the solute flux equation (25):

$$Q_s = Q_b(1 - σ)C_m + D_A(ΔC)/L$$  \[(7)\]

where Cm, the mean protein concentration in the membrane, is the average of Cc and Ca values, σ is the solute drag reflection coefficient, D is the apparent diffusion coefficient, A is the surface area, L is the thickness, and ΔC is the concentration difference. The term on the right side of Eq. 7 is the Qs due to Qb, and the second term is the Qs due to diffusion. Neglecting diffusion, which was estimated to be small (see below), the fractional change in Qs (dQs/Qs) is related to the fractional changes in Qb, Cm, and σ by the following equation obtained by implicit differentiation of Eq. 7:

$$dQ_s/Q_s = (dQ_b/Q_b) + (dC_m/C_m) - [dσ/(1 - σ)]$$  \[(8)\]

Neglecting diffusion (18) and do (20) in Eq. 8, the 8% increase in Qs across the capillary membrane plus the 7% increase in Cm (Table 3) produced a 15% increase in Qs. However, the 22% increase in pleural protein concentration with the 5-ml IP injections was produced by an identical increase in Qs. Thus, of the total increase in Qs, 7% was associated with transport across the diaphragm. For transport across the diaphragm, with dC_m/C_m of 0.26 (Table 3) and dQ_b/Q_b of 0.07 in Eq. 8, dQ_s/Q_s was −0.19. That is, the flow across the diaphragm was reduced by 19%. For 1-ml IP injections with no change in Cp and dQ_b/Qb of 0, dQs/Qs was equal to −dC_m/C_m or −0.17 (Table 3), slightly smaller than the reduced flow across the diaphragm for 5-ml IP injections. The small reduction in flow across the diaphragm with the IP injections was consistent with the small (2–4%) reduction in tissue hydration (W/D) measured for the diaphragm muscle (Table 4).

In the present study, differences in protein concentration across the diaphragm averaged 0.5 g/dl under control conditions and increased by 70% after 5-ml IP injections. Thus the increase in Qc due to concentration-induced changes in diffusion per se could not explain why Cc relative to that of peritoneal liquid was ninefold greater with 5-ml IP than with 1-ml IP injections at 6 h after injection (Table 1). In in vitro studies of the rat diaphragm, albumin diffusion coefficient averaged 1 × 10⁻⁹ cm²/s (19). Thus the albumin diffusive flux was 2.5 × 10⁻⁹ g/h, based on ΔC of 1 g/dl, A of 5 cm², and L of 0.7 mm. The Qs due to Qb was 9 × 10⁻⁴ g/h, based on Qb of −0.04 ml/h and σd of −0.2 estimated in the analysis, and Cm of 3 g/dl (Table 3). Thus 80% of the albumin flux was attributed to Qb, which supported the assumption of high Peclet number used to model transport across the diaphragm (Eq. 2 of APPENDIX).

Several other factors might contribute to an increased Qs and diffusive Qc across the diaphragm with the IP injections compared with control. One factor is a tissue hydration-induced increase in hydraulic conductivity and diffusion that would result in an increased liquid and protein transport (12, 14). This effect was not consistent with the measured reduction (2–4%) in diaphragm muscle hydration (W/D) or the estimated reduction in transdiaphragmatic flow, with the IP injections. Another factor is an increased diaphragm surface area available for flow and diffusion that might increase with the size of the IP injections (14). This effect would not be supported by the tracer concentration measured in the diaphragm relative to that in the peritoneal liquid that was similar for both 1- and 5-ml IP injections. A third factor is an increase in IP hydrostatic pressure with the size of the IP injections. However, IP pressure measured in anesthetized rats was slightly subatmospheric (−2 cmH2O) and increased minimally by 0.5 cmH2O with 5-ml IP injections (33). Accordingly, the 1- and 5-ml IP injections had only minor effects on the transport characteristics of the diaphragm and forces driving flow into the pleural space.

In summary, tracer Cp/Ca were greater than Cc/Ca at ~3 h after both 1- and 5-ml IP injections. This behavior indicated a transdiaphragmatic tracer and liquid transport. The 5-ml IP injections produced no change in plasma protein concentration, with 22 and 30% increases in pleural and peritoneal protein concentrations, respectively, above control values. These increases in protein concentration were consistent with a small decrease in Qb across the diaphragm that produced a small (2–4%) reduction in diaphragm muscle hydration (W/D) with no change in Vp. We conclude that flow across the diaphragm changed only minimally with the IP injections and that the tracer transport into the pleural space with 1- and 5-ml IP injections occurred simultaneously with the near normal transport of albumin and liquid across the diaphragm.

In the analysis of tracer transport, we assumed that the transport processes were entirely passive (24), although active transport by the mesothelium has been proposed for both peritoneal and pleural liquid transport (6). The results of this study would support passive mechanisms to be dominant in the determination of pleural liquid transport.
Transport Model

The following analysis extends the single-source analysis (1) to two sources. Other more complex analyses of peritoneal transport related predominantly to peritoneal dialysis have appeared (13).

Consider the pleural space as an input-output system (Fig. 4). The mass flux of tracer (\(^{125}\)I-albumin) entering the pleural space across the capillary endothelial-interstitial membrane from the circulation (\(dM/dt\)) plus the mass flux entering from the peritoneal (abdominal) cavity across the diaphragm (\(dM_{di}/dt\)) minus the mass flux exiting the pleural space via lymphatics (\(dM_{lym}/dt\)) equal the rate of change of the total mass in the pleural space with respect to time \(t\) (\(dM/dt\)):

\[
dM/dt = Q_c C_{pc} + Q_d C_{ps} - Q_p C_p \quad (A1)
\]

Here, \(dM/dt = Q_c C_{pc}\), where \(Q_c\) is the flow from the circulation into the pleural space and \(C_{pc}\) is the tracer concentration in \(Q_c\); \(dM_{di}/dt = Q_{di} C_{di}\), where \(Q_{di}\) is the flow from the peritoneal cavity across the diaphragm into the pleural space and \(C_{ps}\) is the tracer concentration in \(Q_{di}\); \(dM_{lym}/dt = Q_{lym} C_{lym}\), where \(Q_{lym}\) is the flow out of the pleural space equal to \(Q_c + Q_d\), and \(C_{lym}\) is the tracer concentration in \(Q_{lym}\). We assume that the outflow occurs via the lymphatic vessels that do not restrict the passage of albumin (5). We consider only convective transport across the membranes so that, for a flow of relatively high Peclet number through cylindrical pores (25):

\[
C_{pc} = (1 - \sigma_p)C_c
\]

\[
C_{ps} = (1 - \sigma_d)C_d \quad (A2)
\]

This behavior was justified based on transport studies of single capillaries (18) and the isolated rat diaphragm (20). Here, \(\sigma_p\) is the reflection coefficient of the capillary membrane, and \(\sigma_d\) is the reflection coefficient of the diaphragm. With a constant \(V_p\) and \(V_d\), \(V_p dC/dt\) reduces to:

\[
V_p (dC/dt) = Q_c (1 - \sigma_p)C_c + Q_d (1 - \sigma_d)C_d - Q_p C_p \quad (A3)
\]

**IP injection experiments.** To study the transport of tracer after injection into the peritoneal cavity, we use the following equation that results from Eq. A3 after normalizing by dividing each term by \(C_a\) and \(V_p\):

\[
d(C/C_a)/dt = K_p + K_f (C/C_f) - [(1/C_f)(dC/dt) + Q_p/V_p] (C/C_f) \quad (A4)
\]

\[
K_p = (Q_p/V_p)(1 - \sigma_p) \quad (A5)
\]

\[
K_f = (Q_p/V_p)(1 - \sigma_d) \quad (A6)
\]

\[
Q_p = Q_c + Q_d \quad (A7)
\]

where \(K_p\) and \(K_f\) are treated as constants; that is, \(Q_c, Q_d, V_p, \sigma_p, \) and \(\sigma_d\) are constants. \(C_f(t)\) is the measured input function of the tracer from the peritoneal cavity (Fig. 2), and \(C_f/C_f(t)\) is the measured input function from the circulation (Fig. 3). Thus Eq. A4 is a first-order linear differential equation in the variable \(C_f/C_f\).

The input function representing the washout of tracer from the peritoneal cavity for 5-ml IP injections was obtained by a curve fit to the \(C_f/C_f(t)\) data (Fig. 2):

\[
C_f/C_{f0} = 0.97 \exp(-0.32t) + 0.03 \exp(-0.045t) \quad (A8)
\]

Note that tracer washout was given by an initial fast decay between 0 and 12 h described by a short time constant (3 h) followed by a slower decay described by a long time constant (22 h).

The input functions from the peritoneal cavity into the circulation for 5-ml IP injections were obtained by a polynomial curve fit to the \(C_f/C_f(t)\) data (Fig. 3):

\[
C_f/C_{f0} = -0.0228t + 0.0139t^2 - 0.0011t^3 + 4 \times 10^{-5}t^4 - 7 \times 10^{-7}t^5 + 5 \times 10^{-9}t^6 \quad (A9)
\]

**Vascular injection experiments.** An equation appropriate for intravascular (IV) tracer injection that is analogous to Eq. A4 for peritoneal injection results from Eq. A3 after normalizing by dividing each term by \(C_a\) and \(V_p\):

\[
d(C/C_a)/dt = K_p + K_f (C/C_f) - [(1/C_f)(dC/dt) + Q_p/V_p] (C/C_f) \quad (A10)
\]

where \(K_p\) and \(K_f\) are defined by Eqs. A4 and A5. This equation shows that, at initial time \(t = 0\), when \(C_a\) and \(C_p\) are zero, \(K_p\) is the slope of the \(C/C_f(t)\) curve and was measured experimentally (see below).

We used MATLAB (ode45) to solve numerically Eq. A4 for \(C_f/C_f(t)\). \(K_p\) value of 0.02 ml/h was specified from the slope of the \(C_f/C_f(t)\) curve near \(t = 0\) for IV tracer injection. Eq. A10 for IV injection shows that \(d(C/C_a)/dt = K_p + K_f \) at \(t = 0\). The IV injection studies produced \(C_f/C_f(t)\) values of 0.011 \pm 0.004 (SD; \(n = 3\)) and 0.018 \pm 0.003 \((n = 3\)) at times of 0.5 and 1 h after injection, respectively. A linear regression fit to the data was \(C_f/C_f = 0.019t, P = 10^{-5}\). The slope (0.019 h\(^{-1}\)) was near the value (0.02 h\(^{-1}\)) for \(K_p\) used in the computations. It was not possible to determine \(K_f\) from the \(C_f/C_f(t)\) curve using the IP injection data, because experiments at time periods of 0.5 and 1 h after injection showed an initial delay of \(\sim 1\) h for the tracer to cross the diaphragm (see below). With \(K_p\) specified and \(V_p\) measured, there are four unknown parameters (\(\sigma_p, \sigma_d, Q_p, \) and \(Q_d\)) in the three equations (A5–A7) that describe transport of tracer into the pleural space.

By a trial-and-error procedure, with \(K_p\) specified, \(K_f\), and \(Q_p/V_p\) were perturbed to fit the measured \(C_f/C_f(t)\) curve (Fig. 3). Simultaneously, values of \(\sigma_p, \sigma_d, Q_p,\) and \(Q_d\) that were consistent with \(Q_p/V_p, K_p,\) and \(K_f\) were calculated using the following procedure. \(Q_p\) was computed by the product of \(Q_p/V_p\) and measured \(V_p\). \(Q_p\) values corresponding to a range of \(\sigma_p\) values were calculated using the computed \(K_p\) value (Eq. A3). Then \(Q_p\) values equaled the difference between \(Q_p\) and \(Q_f\) values (Eq. A7). Next, values of \(\sigma_d\) were calculated by using the computed \(K_f\) value and values of \(Q_f\) (Eq. A6). We chose computed values of \(Q_p/V_p\) and \(K_f\) that fit the measured \(C_f/C_f(t)\) curve with values of \(\sigma_p\) and \(\sigma_d\) in the range 0.75–0.91 and 0.03–0.39, respectively (Table 5). The associated range for \(Q_f/Q_p\) was 0.52–0.83. To determine values of \(\sigma_p, \sigma_d,\) and \(Q_f/Q_p\) for 1-ml IP injection and control conditions, we used the equilibrium solution to Eq. A4 for infinite time given by Eq. 5 with total protein concentrations measured in pleural liquid, peritoneal liquid, and plasma (Table 3). The solutions are tabulated in Table 6.

The effect of the two time constants for the tracer decay in the peritoneal liquid (Eq. A8) was to produce a biphasic \(C_f/C_f(t)\) behavior, with \(C_f/C_f\) increasing to a peak value of 1.1 at \(\sim 12\) h and then decaying to a value of \(\sim 0.6\) after 24–48 h (Fig. 3). Note also that the initial part of the \(C_f/C_f(t)\) curve occurred earlier in time by 1–2 h than the measured data, a reflection of the delay time for the tracer to cross the diaphragm, a behavior that was ignored in the modeling. This biphasic behavior in \(C_f/C_f(t)\) was in contrast to the monotonic increase calculated if transport of tracer was entirely from the circulation (see below).

The long decay time constant (Eq. A8) determined the near equilibrium value for \(C_f/C_f\) at long time at \(t = 48\) h, when \(d(C_f/C_f)/dt = 0\) in Eq. A4:

\[
0 = K_p + K_f (C/C_f) - [(1/C_f)(dC/dt) + Q_p/V_p] (C/C_f) \quad (A11)
\]

Here, \((1/C_f)(dC/dt) = -0.045\) at \(t = \infty\) (Eq. A8), and with \(K_p = 0.23\) h\(^{-1}\), \(K_f = 0.02\) h\(^{-1}\), \(C_f/C_f = 1.3\), and \(Q_p/V_p = 0.46\) h\(^{-1}\), \(C_f/C_f = 0.66\). A lower equilibrium value \((C_f/C_f = 0.55)\) is obtained if microvascular filtration is neglected \((K_f = 0)\).
Single-source analysis. We considered the case of no transdiaphragmatic transport with only microvascular filtration so that \( K_a = Q_a = 0 \) and \( Q_p = Q_p/V_p \) in Eq. A4:

\[
\frac{d(C/C_p)}{dt} = K_a(C/C_p) - \left( \frac{1}{V_p} \frac{dV_p}{dt} \right) (C/C_p) \quad (A12)
\]

The numerical solution of this equation with the measured values of \( C/C_p \), \( C/C_a \), \( Q_p \), \( Q_p/V_p \) of 0.46 h\(^{-1}\) obtained from the two-source solution produced a monotonic increase in \( C/C_a \) to a value of 0.17 at 48 h. By contrast, with only transdiaphragmatic transport and no microvascular filtration so that \( K_a = Q_a = 0 \) and \( Q_p = Q_p/V_p \) in Eq. A4:

\[
\frac{d(C/C_p)}{dt} = K_a - \left( \frac{1}{V_p} \frac{dV_p}{dt} \right) (C/C_p) \quad (A13)
\]

The solution of this equation with the measured values of \( C/C_p \) \( \text{specifed by Eq. A8, and the use of } K_a \text{ of 0.23 h}^{-1}\) and \( Q_p/V_p \) of 0.46 h\(^{-1}\) obtained from the two-source solution produced a biphasic \( C_p/C_a(t) \) behavior with an equilibrium value of \( C_p/C_a \) of 0.55 at 48 h that closely approximated the solution of the two-source problem. Thus transport across the diaphragm was essential for modeling tracer uptake into the pleural space and was the predominant contributor to the measured behavior in tracer uptake.

GRANTS

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REFERENCES