A Method to Test Blood Flow Limitation of Peritoneal-Blood Solute Transport

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Abstract. Current transperitoneal transport models assume that effective blood flow to the microcirculation does not limit solute exchange with dialysate in the cavity. Despite evidence that gas transfer across the peritoneum (assumed to equal the effective blood flow) occurs at rates that exceed maximum urea transfer rates by a factor of two to three, the assumption has been strongly challenged. To address this problem at the tissue level, a technique to determine the effect of local blood flow on small-solute transport was developed in this study. Diffusion chambers were affixed to the serosal side of the anterior abdominal wall of rats, and solutions containing radiolabeled urea or mannitol were placed in the chambers. During each experiment, the local blood flow beneath the chamber was monitored with laser Doppler flowmetry and the disappearance of the tracer versus time was simultaneously measured under three conditions of blood flow: control, 30% of control, and zero blood flow. The results demonstrated no significant differences for either solute between control and the condition in which blood flow was reduced by 70%. However, there was a significant reduction in the rate of mass transfer with no blood flow. It was concluded that blood flow at $\geq 30\%$ of control values does not limit solute transfer across the abdominal wall peritoneum during dialysis. (J Am Soc Nephrol 8: 471–474, 1997)

All current theoretical models of peritoneal cavity blood transport depend on the assumption that, under normal physiologic conditions, blood flow does not limit the transfer of water or solutes (1). The assumption is derived from indirect evidence that shows that peritoneal gas clearance, which is equated to the functional or effective blood flow available for transport, exceeds rates of solute transfer. The effective blood flow available for transport will be only a fraction of the total blood flow through the tissues surrounding the peritoneal cavity, because most of the exchange capillaries are too far from the cavity to be active in the exchange process (1,2) or they are contained in tissues not in contact with the solution in the cavity (3). Clearances of various gases from the peritoneal cavity of rats (2,4), rabbits (5), pigs (6), and humans (7) have provided estimates of peritoneal blood flow of 2 to 7% of the cardiac output during dialysis. If a nominal cardiac output of a human is assumed to be 5000 mL/min, the minimal effective blood flow, derived from these studies, would be approximately 100 mL/min ($0.02 \times 5000$ mL/min). This is at least two to three times the estimated maximum peritoneal urea clearance of 30 to 40 mL/min (8). This excess capacity to deliver solute to the peritoneal exchange vessels is supported by dog studies in which profound hemorrhagic shock causes peritoneal urea clearances to decrease by only 10 to 25% (9,10). Based on this evidence, our hypothesis is that blood flow does not limit solute transport under normal physiologic conditions.

However, significant questions concerning blood flow limitations at the level of individual tissues (hereafter referred to as “local” blood flow) have recently arisen. Use of vasodilators during dialysis increases urea and creatinine transport rates by up to 50% above controls (11,12). In these studies, actual blood flow was not determined and changes in the capillary permeability or perfused capillary exchange area, which would change the peritoneal mass transfer-area coefficient (MTAC) (13), could not be ruled out. In contrast to in vivo, whole-animal experiments, studies of water and solute transport in an isolated, in vitro-perfused loop of human intestine demonstrated significant correlation between the rate of perfusion and the transport rates of water, urea, and creatinine (14). This group has recently argued the case further by qualitatively presenting a hypothesis of blood flow limitation in capillaries that are located adjacent to the peritoneum (15). Before major changes in current peritoneal transport theory or further human testing of vasodilators in an effort to improve dialysis, a fundamental examination of “local” or tissue-specific effects of blood flow on water and solute transport needs to be carried out in animals.

In this article, our goal is to propose a new method with which to determine both the level of blood flow in abdominal wall muscle and mass transfer rates of small solutes across the specific tissue surface simultaneously. To isolate transport to a specific tissue, we utilized a diffusion chamber, which we previously used to determine tissue-specific mass transfer coefficients (MTC) (3). By monitoring local blood perfusion with laser Doppler flowmetry and manipulating the supply of blood
flow to the tissue, we were able to correlate changes in the MTC with the relative local blood flow.

**Materials and Methods**

**Animals and Surgical Preparation**

Female Sprague-Dawley rats weighing between 200 and 350 g were procured from Charles River Laboratories (Wilmington, MA). All animals were anesthetized with an initial intramuscular injection of sodium pentobarbital (60 mg/kg) and maintained under anesthetized conditions with subsequent intravenous pentobarbital injections. Catheters were placed in the femoral artery and vein for blood pressure monitoring (maintained at > 100 mm Hg) and infusion of fluids and drugs, respectively. A tracheostomy was performed on each animal to ease the work of breathing in the supine position. Each animal's temperature was monitored continuously by rectal probe and kept at 37 ± 2°C with a servo-controlled heating blanket (Harvard Apparatus, South Natick, MA) and overhead heating lamps. Gauze pads that had been soaked with warm oil were used to cover all exposed tissues surrounding the chamber. All experiments were approved by the University of Rochester Committee for Animal Resources.

**Materials and Instrumentation**

The tracers used for the experiments were 14C-mannitol, purchased from Amersham Life Sciences (Arlington Heights, IL), and 14C-urea, purchased from Moravek Biochemicals (Brea, CA). These were certified to be 99% pure and were used directly from the original vial. Their activity (counts per minute) was determined by liquid scintillation counting (Beckmann LS6000IC, Fullerton, CA).

The chamber solution was Krebs-Ringer bicarbonate (KRB). Details concerning makeup of the solution and construction and testing of the diffusion chambers have previously been published (3).

Blood flow was monitored with the Perimed Periflux PF3 laser Doppler flow meter with a special probe (PF315-73; Perimed, Stockholm, Sweden). This device focuses a laser beam on the tissue and compares the backscatter light from the stationary tissue structures with the light reflected from moving red blood cells (RBC). The light reflected from the moving cells undergoes a frequency shift or Doppler effect. Laser Doppler flowmetry (16) produces a signal that correlates linearly with the product of the RBC velocity multiplied by the RBC volume fraction, where RBC volume fraction equals the volume of RBC divided by the total illuminated volume. This product is equivalent to the RBC perfusion through the tissue with typical units of flow rate through an unit area of tissue (mL/s per m²). This technique provides a measurement of total blood perfusion through the illuminated tissue, which includes the large vessels as well as the microvessels. It does not provide an absolute value for local blood perfusion, because its readings will vary from tissue to tissue. However, extensive testing (17) has demonstrated that within a single tissue preparation, its readings are linearly proportional to local blood flux or flow per unit area of tissue. The technique is sensitive to the hematocrit of the blood and the temperature, both of which were observed to be constant during a given experiment. Therefore, a decrease in the reading of the laser Doppler flowmeter correlates with a proportional decrease in total blood perfusion within the tissue volume illuminated by the laser. The laser Doppler flowmeter therefore does not differentiate between small-vessel flow and large-vessel flow. In manipulating blood flow, we assumed that the perfusion through the microvessels actively engaged with the peritoneal blood transport process was directly proportional to the total blood flow in the tissue. The instrument was kept in careful calibration, and we observed no large variation between animals in the readings of the abdominal wall blood perfusion under control conditions (160 to 186 PU, the arbitrary units of blood perfusion that are the output of the device).

The flow probe was positioned perpendicular to the peritoneum directly over the mesothelium by a micromanipulator. As shown in Figure 1, the flow probe is noninvasive, and the actual fiber optic tip

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**Figure 1.** In vivo cross-section of the abdominal wall with diffusion chamber glued to the serosal surface and the laser Doppler flow probe in place over the mesothelium at the base of the chamber. The free end of the muscle wall (laparotomy incision) is gently extended with a suture through the attached skin. The other end of the tissue is in continuity with the body.
sits in a layer of fluid above the mesothelial surface. Blood flow measurements were typically made within the chamber or directly adjacent to areas of chamber fixation to tissue; these showed blood perfusion very similar to that of areas 1 to 2 cm away. In addition to the testing of the chamber documented previously, we determined that local tissue blood perfusion was not affected by the application of the cyanoacrylate glue or by the presence of the chamber on the tissue.

**Experimental Protocol**

After surgical preparation, a medial laparotomy was performed and the animal was placed on its side. One side of the abdominal wall was carefully cleared of visceral tissue by retraction of the tissue. Care was taken not to stretch the abdominal wall or to touch the mesothelial surface that was to be studied. The chamber was fixed to the serosal side of the abdominal wall with cyanoacrylate glue (Figure 1), and a preweighed volume (approximately 1 mL, assuming a solution density of 1 g/mL) of KRB solution with approximately 1 µCi of tracer was carefully injected into the chamber, where it was mixed and sampled in 15-min intervals. At each 15-min interval, a 10-µL sample of chamber solution and a 20- to 30-µL sample of plasma was collected for determination of tracer concentration. After 1 h, the contents of the chamber were sampled, removed, and weighed. The inferior and superior epigastric arteries were subsequently tied off with 4.0 silk approximately 1 cm above and 1 cm below the outer perimeter of the chamber, to reduce blood flow to the experimental area. The blood perfusion was continuously monitored with the laser Doppler flowmeter. The chamber solution was returned to the experimental chamber when the blood perfusion was noted to be decreased and stable (typically 15 to 30 min after sutures were secured). The experimental procedure was then repeated under reduced blood perfusion conditions for an additional hour. In some animals (N = 3, urea), a third hour’s worth of measurements was made postmortem to check the effect of zero blood flow on the mass transport rates.

**Calculations**

The MTC was calculated for each period of observation by fitting the following equation, using the Scientist software program (MicroMath, Salt Lake City, UT), to the chamber data of tracer mass in chamber (M), which equals concentration in chamber (C_chamber) times the corrected volume of the chamber, and chamber concentration versus time:

\[
\frac{dM}{dt} = \frac{\text{mass transferred}}{\text{duration of experiment}} = \text{MTC}A_{\text{chamber}}(C_{\text{chamber}} - C_{\text{plasma}})
\]

\(C_{\text{plasma}}\) was < < \(C_{\text{chamber}}\) and approximately 0, because of the small surface area of the chamber (\(A_{\text{chamber}}\)). The first 15-min interval was considered a period of unsteady-state transport, and therefore the time beyond the first 15 min was used for the analysis. For each tracer, the values of MTC for each condition of altered blood flow were compared with the MTC from control blood flow conditions with a paired \(t\) test (\(\alpha = 0.05\) for significance) (detailed in Reference 3).

**Results**

The tracer mass versus time (normalized by dividing by the tracer mass at zero time) for each experimental condition is shown for urea in Figure 2. The plot for mannitol is analogous. Despite the reduction of blood perfusion during the second period of the testing by 70% (ratio of post-occlusion laser Doppler flow units (PU) to control PU = 0.28 ± 0.03 for urea and 0.24 ± 0.05 for mannitol), the slope of the disappearance curve does not change in Figure 2. The MTC in Table 1 reflect this and demonstrate no statistically significant differences between the mean MTC of control periods and of periods during 70% reduction of blood perfusion. However, the cessation of blood flow does cause a change of rate of tracer disappearance, as shown by the change in slope of the curve of Figure 1. In Table 1, the MTC for the condition of zero blood flow is significantly different from control conditions for urea. Mass transfer does not decrease to zero because the size of the chamber and the surface area of the base are small in comparison to the size of the animal’s body. It would likely take hours for the chamber to come into equilibrium with the volume of water in the carcass.

**Discussion**

Our study has demonstrated that a 70% reduction in local blood flow does not alter the clearance or MTC of urea or mannitol across the abdominal wall peritoneum. We chose these two solutes because their molecular sizes are at the low end of the range of typical solutes exchanged during dialysis and would more likely display some transport dependency on blood flow. Collins (6) studied the simultaneous clearance of several inert gases from the peritoneal cavity of pigs and found

![Figure 2](Image)

**Figure 2.** Transport of urea from abdominal wall chamber: effect of blood flow decrease. Time course of chamber urea mass (concentration \(\times\) volume) at time \(t\) normalized by dividing by the chamber urea mass at time, \(t = 0\). Nominal conditions of blood flow are listed under the corresponding portion of the curve. Open symbols are individual experiments. Filled circles are the mean values.

**Table 1.** Mass transfer coefficients: effect of blood flow

<table>
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<tr>
<th>Solute</th>
<th>Blood Flow Condition</th>
<th>(N)</th>
<th>Mean (cm/min)</th>
<th>SD</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mannitol</td>
<td>Control</td>
<td>10</td>
<td>0.0025</td>
<td>0.0007</td>
<td>0.0002</td>
</tr>
<tr>
<td></td>
<td>30% Control</td>
<td>10</td>
<td>0.0026</td>
<td>0.0011</td>
<td>0.0003</td>
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<tr>
<td>Urea</td>
<td>Control</td>
<td>6</td>
<td>0.0037</td>
<td>0.0010</td>
<td>0.0004</td>
</tr>
<tr>
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<td>0.0043</td>
<td>0.0013</td>
<td>0.0005</td>
</tr>
<tr>
<td></td>
<td>Postmortem</td>
<td>3</td>
<td>0.0014</td>
<td>0.0011</td>
<td>0.0005</td>
</tr>
</tbody>
</table>

* Significantly different from control (\(P < 0.02\)).
the clearance to be proportional to the gas diffusivity in water. Because it is generally assumed that transcapillary transport of gases is blood-flow limited, Collins’ findings point to the significance of the interstitial barrier, which separates blood capillaries in tissue underlying the peritoneum from the contents of the cavity. Dedrick and colleagues (18) developed a “distributed model approach” to peritoneal transport and have proposed that for substances limited in transport by blood flow: MTC = (Dq)^0.5, and for substances that are limited by the capillary barrier: MTC = (D(pa))^0.5. In these expressions, D = the effective tissue diffusivity of the substance, q = the blood flow per unit volume of tissue, and (pa) = the capillary permeability times the capillary surface area per unit volume of tissue. Thus gases or flow-limited small solutes with different diffusivities will have different values for MTC (or clearance, which can be estimated by MTC \times area), even if the value of q is unchanged. Others (19) have proposed combining blood flow and capillary permeability in the same equation but, in our opinion, have not justified their assumptions adequately to warrant use of their formulation. The dependency of the MTC on the square root of blood flow or of capillary permeability area also implies that, provided D is constant, a change to 30% of control value would lead to a reduction in MTC of approximately 50% (MTC \alpha (0.3)^0.5 = 0.54). This change would have been detected easily by our measurements.

When the blood perfusion is severely reduced, as in our experiment, there is the possibility that the tissue will become ischemic. Ischemia could lead to vasodilation, with a concomitant increase in the perfused capillary area in the tissue (20). As stated above, the rate of transfer of a solute across a capillary membrane-limited system is proportional to (pa)^0.5, whereas transfer across a flow-limited system is proportional to (q)^0.5. Thus the flow-limited system does not depend on the perfused capillary surface area. A substance becomes flow-limited when its coefficient of transport through a membrane (pa) equals or exceeds the local blood flow per unit tissue volume (q). Ischemia would likely produce an increase in (pa) because the perfused capillary surface area-density (a) would probably increase with the concomitant increase in (pa). If the transport at baseline or control conditions were limited by (q), an increase in (pa) with a decrease in (q) would enhance the possibility that a blood flow limitation would be detected. So despite the physiological changes that may occur in the tissue, the experiment provides a stringent test of our hypothesis.

In summary, for tissue blood perfusion > 30% of control levels, there is no limitation of small-solute transport across the abdominal wall peritoneum. We would therefore conclude that, under control conditions in the abdominal wall, it is unlikely that small-solute transfer is limited by blood flow. This conclusion does not necessarily extend to other peritoneal tissues, because organs such as the liver have unusually open circulations and high capillary permeability.

Acknowledgments
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References

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