Tissue Sources and Blood Flow Limitations of Osmotic Water Transport Across the Peritoneum

HAILU DEMISSACHEW, JOANNE LOFTHOUSE, and MICHAEL F. FLESSNER
Nephrology Unit, Department of Medicine, University of Rochester Medical Center, Rochester, New York.

Abstract. Despite the daily use of hypertonic solutions to remove fluid from patients throughout the world who are undergoing peritoneal dialysis, the tissue sources of this water flow are unknown. To study this phenomenon in specific tissues, small plastic chambers were affixed to parietal and visceral surfaces of the peritoneum and were filled with either an isotonic or hypertonic solution. The volume changes over 60 to 90 min were determined and divided by the chamber area to yield the volume flux. The hypertonic solution produced a positive flux into the chamber of 0.6 to 1.1 μl/min per cm² in all tissues tested. In contrast, the isotonic solution resulted in a net loss or an insignificant change in the chamber volume.

In the early days of peritoneal dialysis, clinical researchers noted that isotonic salt solutions were absorbed into the body (1). This absorption has been shown to be the result of hydrostatic pressure-driven flow into the tissue surrounding the cavity and lymphatic flow from the cavity (2,3). The intraperitoneal pressure depends on body size, the volume of dialysis fluid, and body position, with typical minimal pressures of 2 to 10 mmHg in adult patients (4,5). To counteract this fluid absorption in anephric patients, those pioneers in dialysis empirically added 2 to 5% glucose to make the solution hypertonic (1). Currently, nephrologists continue to adjust the glucose concentration in the dialysis solution to alter the osmotic pressure of the solution, in accordance with the need of the patient for fluid removal.

Conceptual models of peritoneal transport (6) place the source of the water at the blood capillary, but no one has adequately addressed the fact that the capillary exchange vessels are within the tissue surrounding the cavity and that the dialysis fluid does not make direct contact with the wall of the capillary. The mesothelium/peritoneum is highly permeable to small solutes and therefore is unlikely to provide a barrier to glucose that would allow the hypertonic solution in the cavity to exert a portion of its osmotic pressure on the underlying tissue (7). Therefore, we hypothesize that the parietal peritoneum does not act as a membranous barrier to small solutes in the cavity and that the tissue underlying this portion of the peritoneum is unlikely to be a source of osmotically driven water flow (often called ultrafiltration in dialysis studies).

Our first goal in this study was the measurement of water flux (flow rate/area) from different portions of the peritoneum, to determine the sources for osmotically induced fluid movement into the cavity. The second goal was the evaluation of the effect of blood flow on osmotic water transport in those tissues in which flow into the cavity occurs. To discover which tissues are the sources of water flow, we used small plastic chambers to isolate areas of the peritoneum, and we alternately exposed an area of the peritoneum to an isotonic solution or a hypertonic solution. During the hypertonic dwell, we observed a positive flow into the chamber from all tissues, including the abdominal wall; during the isotonic dwell, fluid was typically lost from the chamber. In the second series of experiments, we tested the effects of blood flow on solute transport. We found that reducing blood flow by 50 to 80% resulted in decreases in osmotic flow into the chamber, but these were not statistically significant, except in the case of the liver.

Materials and Methods

Animals and Surgical Preparation

Female Sprague Dawley rats (200 to 280 g) from Charles River Laboratories (Cambridge, MA) were used for these experiments. The animals were anesthetized with an initial intramuscular injection of sodium pentobarbital (60 mg/kg) in the hindleg, typically followed by 20% of the initial dose administered intravenously every 2 h. Catheters (PE50) were placed in the left carotid artery for BP monitoring, in the right internal jugular vein for intravenous fluid infusion, and in the tail artery for blood specimen collection. The mean BP was monitored continuously with a BP analyzer (MicroMed, Louisville,
KY) and was maintained at >100 mmHg. To facilitate breathing of the animals in the supine position, a tracheotomy was performed. The rectal temperature was monitored with a rectal probe and was maintained at 37 ± 2°C with a servo-controlled heating blanket (Harvard Apparatus, South Natick, MA) and overhead heating lamps.

After catheter placement, a midline laparotomy was performed and one of four tissues (anterior abdominal wall, cecum, stomach, or liver) was carefully exposed. A minimum of five animals for each tissue was used in each experimental protocol. For the experiments in which blood flow was manipulated, the blood supply of each of these tissues was dissected and isolated, as discussed in earlier publications. Briefly, in preparation to limit blood flow in the left lobe of the liver, to which the chamber was to be fixed, a loop of suture was placed around the proximal portion of the liver lobe. In the abdominal wall, the suture was placed around the inferior and superior epigastric arteries. In the cecum, the mesenteric vessels to the cecum were dissected and prepared for a vascular clamp. In the stomach, the suture was placed around the gastric vessels over the smaller curvature of the stomach. After isolation of the appropriate vessels, the chamber was fixed (with cyanoacrylate glue) to the serosal surface of the tissue to be studied. Gauze pads soaked with warmed (38°C) Krebs-Ringer solution were used to cover the exposed tissues around the chamber. All experiments were approved by the University of Rochester Committee for Animal Resources.

Materials and Instrumentation

[14C]Mannitol (Moravek Biochemicals, Brea, CA) was used as a tracer to determine the residual volume in the chamber. It was certified to be 99% pure and was used directly from the original vial. Its activity (cpm) was determined by liquid scintillation counting (LS6000IC; Beckman, Fullerton, CA).

Krebs-Ringer-bicarbonate solution (KRB) with 0.5% bovine serum albumin was the isotonic solution; KRB with 4% mannitol (M 9647; Sigma Chemical Co., St. Louis, MO) was used as the hypertonic solution. The composition of the KRB and the construction and testing of the diffusion chambers were reported previously.

Blood flow to the tissues was monitored with a Perimed Periflux PF3 laser Doppler flowmeter with a special probe (PF315-73; Perimed, Stockholm, Sweden). The device and its application to blood flow monitoring in the tissues underlying the peritoneum were discussed in a previous report. The technique does not discriminate between large vessels and microvessels. It also does not provide an absolute value for local blood perfusion, because the readings vary from tissue to tissue. However, it has been demonstrated that, within a single tissue, the readings are linearly proportional to local blood flux (flow/unit area of tissue). We assume that the perfusion through the microvessels that are actively involved in the peritoneal blood transport process is directly proportional to the total blood flow in the tissue. Because it depends on the backscatter of light from red blood cells moving through the tissue, the technique is sensitive to the hematocrit of the blood and the temperature of the animal, both of which were observed to be constant throughout the experiments presented in this report. The instrument was kept calibrated, and no large variations among animals in the readings for the various tissues under control conditions were observed.

The flow probe was positioned perpendicular to the peritoneum, directly over the mesothelium, using a micromanipulator as a support. The actual fiber-optic tip was in a layer of fluid above the mesothelial surface. Measurements were made to determine the relative tissue perfusion during experiments in which the solution was changed from the isotonic solution to the hypertonic solution, as well as experiments in which the blood flow was manipulated.

Osmolality was determined with a Wescor 5000 vapor pressure osmometer (Logan, UT). Weights were determined using a Mettler PM2500 digital balance (Mettler, Hightstown, NJ), which is calibrated annually and which was carefully used in each weight determination.

Calculations

The changes in chamber solution volume were calculated from the weight of the fluid before and after an experimental dwell and were corrected for the residual volume in the chamber. The average volume flux (flow/area) was calculated as:

\[ \text{Volume flux} = \frac{\text{Corrected volume change}}{\text{Chamber area} \times \text{dwell time}} \]

where the chamber area equals the area of the base measured at the end of the experiment, and the dwell time is the length of time the solution was in the chamber. A positive flux was defined as fluid transporting into the chamber. This was calculated for each solution with each experimental protocol and was used for comparison of isotonic versus hypertonic solutions and for evaluation of the effect of blood flow on the osmotically induced volume flow.

The rate of mass transfer of the mannitol tracer was calculated as:

\[ \text{Rate of mass transfer} = \frac{\Delta(\text{Chamber volume} \times \text{chamber concentration})}{\text{Dwell time}} \]

where the change in the chamber tracer mass (chamber volume times chamber concentration of [14C] tracer) can be divided by the initial mass of [14C] in the chamber to calculate the fraction of total tracer lost. The percent reduction in blood perfusion in tissues was calculated as:

\[ \% \text{ Reduction} = 100 \times \frac{\text{PU}_{\text{control}} - \text{PU}_{\text{reduced flow}}}{\text{PU}_{\text{control}} - \text{PU}_{\text{postmortem}}} \]

where perfusion units (PU) are the arbitrary units of output of the laser Doppler flowmeter. There is a small (usually ≤10 PU) signal present under conditions of no blood perfusion during the postmortem period, and this was used to correct all recorded PU values.

Experimental Protocols

Protocol 1: Sources of Transperitoneal Osmotic Water Flow.

The goal of the first protocol was to investigate the sources of osmotically induced water flow. In previous experiments with the chamber affixed to the peritoneum, the volume change with an isotonic solution in the chamber was always negative (volume loss) or near zero. We used the concept of the experiment was simple, i.e., to compare the flux into the chamber induced by an isotonic solution with that induced by a hypertonic solution.

In this experiment, animals were surgically prepared as detailed in the Animals and Surgical Preparation section, but the blood vessels supplying each tissue were left intact because this experiment did not involve manipulation of the blood supply. After the chamber was in place, a premeasured volume (determined by weight before and after injection from a syringe and needle) of isotonic KRB containing 0.5% bovine serum albumin and [14C]mannitol (0.5 µCi/3 ml) was placed in the chamber. The chamber solution height was measured every 15 min and was observed to be never more than 1 mm different from the initial height of 17 to 20 mm in the chamber. We showed previously that, at pressures of >2 cm H2O, significant flow into the tissue occurs (2); therefore, chamber levels of ≤2 cm H2O should result in
minimal hydrostatic pressure-driven flow. The solution volume was determined at 30 and 60 min by mixing the chamber contents by repeated withdrawal and reinjection into the chamber with the syringe needle used for the original injection, with final withdrawal of all of the fluid and weighing. The chamber solution height, mean BP, rectal temperature, and blood flow were recorded every 15 min. At the end of the 60-min dwell, the fluid was mixed, sampled, removed from the chamber, and weighed. The chamber was then washed twice with KRB containing no labeled mannitol. The volume of this wash fluid was also determined by weight. The $^{14}$C concentration of the chamber fluid at 60 min and the concentration of the wash fluid were determined by liquid scintillation counting. The total radioactivity in the wash fluid (concentration times volume) divided by the concentration in the 60-min sample provided an estimate of the residual volume of original solution left in the chamber. Residual volumes were typically in the range of 15 μl.

During the early development of these experiments in the abdominal wall, we found that the initial dwell with isotonic fluid resulted in a large flux from the chamber to the muscle of the abdominal wall. Serial dwell periods with isotonic fluid resulted in an almost negligible flux during 60- to 90-min dwell periods after the initial dwell (0.10 ± 0.07 μl/min per cm² versus −0.4 to −1.0 μl/min per cm² for the initial period). The larger negative flux may have resulted from drying of the abdominal wall during the surgical preparation or from compressive forces exerted on the wall during fixation of the chamber after laparotomy. Results reported reflect both periods, i.e., the initial period and those after an initial dwell with isotonic fluid to hydrate the tissue. In the other tissues, care was taken to use minimal force in placement of the chamber and to place KRB into the chamber as soon as it was fixed to the tissue (approximately 5 min after application of the glue).

During the second period, an equivalent amount (approximately 3 ml) of KRB with 0.5% bovine serum albumin, 4% mannitol, and $[^{14}$C]mannitol (0.5 μCi of tracer/3 ml of solution) was injected into the chamber. The experimental procedure described above was repeated for 90 min; the additional 30 min permitted increased accuracy in the observation of flow into the chamber. The chamber solution volume was mixed and weighed every 30 min as noted above. Samples for osmolality and tracer counts were obtained every 30 min. Blood samples were obtained at the beginning and the end of the 90-min experiment, for hematocrit, plasma osmolality, and tracer count determination. At the end of the 90-min experiment, the contents of the chamber were sampled, removed, and weighed. The residual volume was determined as described above. The animal was euthanized by pentobarbital overdose, the chamber was removed, and the surface area of peritoneum that was exposed to the fluid was measured.

The volume flux was calculated for each experimental solution dwell. Volume fluxes from both solutions and from all four tissues were compared by two-way ANOVA. Volume fluxes from each chamber solution were compared by one-way ANOVA. The fraction of tracer lost and the rate of loss were calculated as described above and were compared by two-way ANOVA.

**Protocol 2: Effect of Blood Flow on Transperitoneal Osmotic Water Flow.** A second set of experiments was carried out to determine whether significant decreases in blood flow in the tissue underlying the peritoneum would affect the rate of water flow into the chamber during exposure of the peritoneum to hypertonic solutions. Water flow was determined during three periods, i.e., control blood flow, decreased blood perfusion, and no blood flow.

The animals were surgically prepared as described above, with suture ties loosely in place around the vessel supplying the tissue and the chamber affixed to the appropriate tissue. Before and between hypertonic solution dwell periods, isotonic KRB was placed in the chamber for 15 to 20 min, to return the tissue to the same baseline conditions by hydration, to allow the tissue to stabilize between changes in blood flow, and to eliminate existing gradients of mannitol in the tissue underlying the chamber. After a period of KRB dwell, this solution was withdrawn from the chamber, and a preweighed amount (approximately 3 ml) of the hypertonic solution (KRB with 4% mannitol) containing $[^{14}$C]mannitol was added to the chamber for a 90-min period of control blood flow. As in the previous experimental protocol, solution height was monitored every 15 min (maintained at ±2 cm), as was BP, temperature, and blood flow. The chamber solution was mixed every 30 min and sampled for $^{14}$C and osmolality, and the chamber volume was determined. At the end of the 90-min dwell, the chamber contents were mixed and final samples were collected. The fluid was withdrawn and the final volume was determined. The chamber was washed twice, as noted above, to determine the residual volume. KRB was then placed in the chamber before the next period.

In general, the second period was one of reduced blood flow. In a series of experiments with the cecum and the stomach, the order of blood flow reduction was reversed, and the decreased blood perfusion period was carried out before the control blood flow. There was no significant effect of the order of control and reduced blood flow periods on the observed water flux, and all data were averaged together. The sutures that had been previously placed around the blood supply to the specific tissue were tightened, and the blood perfusion was observed to decrease markedly. Approximately 20 to 30 min was allowed for the blood perfusion readings to stabilize; during this period, KRB was placed in the chamber to keep the tissue under the chamber well hydrated. The KRB was then replaced with a fresh batch of the hypertonic solution containing labeled mannitol, and the experimental procedure described above was repeated. At the end of the second 90-min period, the chamber solution was mixed, sampled, and withdrawn for determination of volume. After the chamber had been washed twice for residual volume determination, the chamber fluid was replaced with KRB. The animal was given an overdose of sodium pentobarbital. After the BP was noted to be 0 for 15 to 20 min and the blood perfusion readings stabilized at a very low level (typically ≤10 to 20 PU), KRB was removed from the chamber and a third batch of hypertonic solution was injected. The experiment was repeated for another 60 min.

Calculations were carried out as in the previous experiments. The final volumes were corrected for the residual volume in the chamber. The fraction of tracer mass lost and the volume fluxes were analyzed by two-way ANOVA to study the effects of tissue type and blood perfusion. Paired t tests and one-way ANOVA were used to compare volume fluxes for different periods of blood perfusion.

**Statistical Analyses**

The flux into the chamber during isotonic dwell was compared with the flux resulting from hypertonic dwell by paired t test. Paired t tests were also used to compare calculated flux under control conditions with flux during reduced blood perfusion and with postmortem flow. A one-way ANOVA was used to compare the hypertonic fluxes from the four tissues. A two-way ANOVA was used to determine the effects of blood flow and tissue type on osmotic water flux into the chamber. A one-way ANOVA was carried out for each tissue type to determine the effects of blood flow on the osmotic water flux. Number Cruncher Statistical Systems software (version 6.0; Number Cruncher Statistical Systems, Kaysville, UT) was used for all statistical calcu-
lations. The probability of a type I error of <0.05 was used as the level of significance.

**Results**

**Effect of Solution Osmotic Pressure on Water Flow**

Figure 1 displays the volume flux (flow/area) into the chamber for the four tissues studied and for the isotonic and hypertonic solutions. The isotonic solution typically resulted in either fluid loss from the chamber or very little change. The hypertonic solution resulted in a positive flow from all tissues, and the flow from each tissue was significantly different from the flow induced by the isotonic solution \((P < 0.003)\). A one-way ANOVA of the hypertonic fluxes from the four tissues demonstrated no significant dependence on the tissue type. A one-way ANOVA of the isotonic fluxes obtained during the initial dwell did show a dependence on tissue type, chiefly because of the large negative flow from the chamber into the abdominal wall. When the value for the abdominal wall was replaced by the fluxes obtained in subsequent dwell periods, there was no significant trend in the values. The mean osmolality of the hypertonic solution in the chamber during the dwell period was 490 to 495 mosmol/kg, and the decrease in osmolality during the 90-min experiment was on the order of 5% (3 to 7%). In a two-way ANOVA, neither the tissue nor the tonicity of the dialysis solution significantly affected the fraction of mannitol lost from the chamber, which correlated with the decrease in osmolality. Plasma osmolality varied from approximately 290 to 310 mosmol/kg, with no discernible pattern of dependence on the chamber osmolality.

Blood flow perfusion (measured in PU) during the isotonic dwell periods was not significantly different from that during the hypertonic periods. For the cecum, the PU with isotonic solution (\(PU_{\text{isotonic}}\)) averaged 341 ± 23 (mean ± SEM), whereas the PU with hypertonic solution (\(PU_{\text{hypertonic}}\)) averaged 373 ± 53. For the stomach, \(PU_{\text{isotonic}}\) averaged 322 ± 19 (mean ± SEM), whereas \(PU_{\text{hypertonic}}\) averaged 394 ± 38. For the abdominal wall, \(PU_{\text{isotonic}}\) averaged 221 ± 24 (mean ± SEM), whereas \(PU_{\text{hypertonic}}\) averaged 210 ± 38. For the liver, \(PU_{\text{isotonic}}\) averaged 117 ± 10 (mean ± SEM), whereas \(PU_{\text{hypertonic}}\) averaged 136 ± 13.

**Effect of Blood Perfusion on Osmotic Flow**

The ratio of mean PU values (mean ± SEM) for each tissue and for each period of the second experimental protocol are shown in Figure 2. For each tissue, the PU values for reduced blood flow and for the postmortem periods were significantly different from those for the control period. The percent reductions in perfusion were as follows (mean ± SEM): cecum, 71 ± 3%; stomach, 76 ± 3%; abdominal wall, 53 ± 3%; liver, 77 ± 4%. Only the liver showed visible deterioration during periods of induced ischemia. Experiments with the liver were carried out during the three test periods but, because of deterioration of the tissue, data for the postmortem period are not reported. The liver data for the period of reduced flow must be evaluated by considering that, during the 77% reduction in blood flow, the tissue was apparently undergoing early necrosis; therefore, the results may not reflect the findings that would be observed with lesser degrees of ischemia. Unfortunately, our technique for decreasing hepatic blood flow to an isolated lobe of the liver could not be adjusted to cause a lesser reduction in perfusion.

Figure 3 displays the volume flux data for each tissue and for each period of the experimental protocol. A two-way ANOVA demonstrated dependence of the flux on the period of blood flow but not on the tissue type. Despite the apparent reduction in the volume flux in all of the tissues, paired \(t\) tests for comparisons between the flux during the control period and the

![Figure 1](image1.png)  
**Figure 1.** Effect of solution tonicity on peritoneal transport of water. Flux (flow/area; positive flux is into the chamber) was measured for each tissue (mean ± SEM, \(n = 5\) or 6/tissue). □, initial isotonic dwell period; △, isotonic dwell periods subsequent to the initial dwell period; ■, hypertonic dwell period. There were significant differences between the isotonic fluxes and the hypertonic fluxes for all tissues. The large fluid loss rate in the initial dwell period for the anterior abdominal wall (Ant Abd Wall) was attributable to effects of chamber placement in these experiments. Subsequent dwell periods did not show this large negative flow.

![Figure 2](image2.png)  
**Figure 2.** Arbitrary PU from laser Doppler flowmetry (mean ± SEM) during periods of control blood flow (□), reduced blood flow (■), and no blood flow (△). Numbers of animals were as follows: abdominal wall (Abd Wall), \(n = 10\); liver, \(n = 6\); cecum, \(n = 9\); stomach, \(n = 10\). \(^*P < 0.05\), significant difference from the control value.
flux during the period of reduced flow failed to demonstrate statistical significance, except in the case of the liver ($P = 0.04$). The postmortem period, on the other hand, demonstrated marked and statistically significant reductions in the volume flux, compared with the control period. There were no significant differences (by two-way ANOVA or $t$ test) among chamber osmolalities for the different tissues or periods of blood flow limitations. The average osmolality in the chamber varied between 487 and 500 mosmol/kg. The final plasma concentration at the end of two 90-min periods with hypertonic solution in the chamber averaged 300 mosmol/kg, with variation from 290 to 310 mosmol/kg. Each tissue during each test period thus experienced equivalent osmotic forces. Therefore, the chamber osmolalities could not account for the results in Figure 3.

Figure 4 displays the fraction of labeled mannitol lost for each tissue and for each test period. A two-way ANOVA demonstrated no effect of tissue type but a significant effect of the period of blood flow on the fraction of 14C lost during each 90-min period. Paired $t$ tests demonstrated a significant difference between the control period and the reduced-flow period in the liver only. Significant reductions in the fraction lost were measured when the postmortem periods were compared with the control periods. These results parallel our previous findings for urea transfer across the peritoneum; transfer across the liver surface is apparently limited by blood flow (8).

**Discussion**

**Tissue Sources for Osmotic Water Flow**

Our experiments have demonstrated significant water flow from all tissues into a chamber that contains a hypertonic solution. The hypertonic flux rates, as illustrated in Figure 1, averaged 0.8 μl/min per cm$^2$ for the abdominal wall, 1.1 μl/min per cm$^2$ for the cecum, 1.2 μl/min per cm$^2$ for the stomach, and 0.6 μl/min per cm$^2$ for the liver. The statistical analyses revealed no dependence on tissue type. If we assume that the remainder of the hollow viscera have rates of flow similar to those of the stomach and the cecum and that other parietal tissues (diaphragm and retroperitoneal muscle) have rates equivalent to that of the abdominal wall, we can estimate the overall average transperitoneal flux under these conditions as approximately 1 μl/min per cm$^2$. The following question arises: Is this a reasonable rate of flow for the degree of hypertonicity? Unfortunately, there are no data available for direct comparison.

To assess the value of hypertonic flux, it is necessary to estimate the total amount of flow that would be observed if the peritoneal cavity were filled with the same solution. To do this, the flux must be multiplied by the surface area in contact with the solution. This area is not equal to the area that is found by dissecting the tissues and measuring the total potential area. We and others (9,11) have estimated that approximately one-third of the total area is in contact with the solution. This area is not equal to the area that is found by dissecting the tissues and measuring the total potential area. The statistical analyses revealed no dependence on tissue type. If we assume that the remainder of the hollow viscera have rates of flow similar to those of the stomach and the cecum and that other parietal tissues (diaphragm and retroperitoneal muscle) have rates equivalent to that of the abdominal wall, we can estimate the overall average transperitoneal flux under these conditions as approximately 1 μl/min per cm$^2$. The following question arises: Is this a reasonable rate of flow for the degree of hypertonicity? Unfortunately, there are no data available for direct comparison.

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These results parallel data from an earlier whole-animal study by Rubin et al. (14). Those authors carried out hypertonic dialysis of intact or eviscerated rats with a solution with an initial osmolality of 1200 mosmol/kg. With the larger osmotic difference, they obtained flow rates of 14 ml/h and demonstrated osmotic flow from both parietal and visceral tissues. By design, the peritoneal transfer area in whole-animal dialysis is unknown. By using the chamber technique, we eliminated area as a variable and were able to maintain a more stable total osmolality in the chamber. Our results showed that, for the osmolality tested, nearly equivalent volume fluxes are produced from four different peritoneal tissues.

**Mechanism of Osmotic Water Flow**

In the first series of experiments, we demonstrated that all of the tissues tested, including the abdominal wall, are sources of osmotic water flow. We therefore disproved our hypothesis that the parietal tissues were not sources of osmotically induced water flow. In a previous study (7), we demonstrated that the abdominal wall peritoneum does not represent a significant barrier to the passage of mannitol (a sugar of the same molecular size as dextrose). If the hypertonic solution in the cavity did exert an osmotic effect across the anatomic peritoneum, theory would predict that the interstitial pressure would be <0 for 1 to 2 h after exposure of the tissue to the hypertonic fluid (15). Actual measurements of interstitial pressure in the abdominal wall muscle of rats during hypertonic dialysis have demonstrated that the interstitial pressures are all positive and virtually the same as those measured during isotonic dialysis (7). In another study, we showed that the magnitude of convective flow (solvent drag) of protein from the peritoneal cavity into the abdominal wall at a given intraperitoneally hydrostatic pressure is independent of the osmolality of the solution (2). Taken together, these data indicate a complex mechanism of bidirectional water flow during hypertonic dialysis. Although the theory of hydrostatic pressure-driven flow is well understood (Darcy’s law) (16), the mechanism of flow in the opposite direction has not been elucidated. Cellular dehydration of the mesothelial layer probably contributed to the osmotically induced flow; however, the very low fluxes under conditions of no blood flow demonstrate that the majority of the positive flow into the chamber is from the blood circulation. We can speculate that the peritoneum is a heteroporous structure (6), analogous to the capillary endothelium, with transcellular water channels (6) that respond to osmotic forces and very large intercellular gaps (which are reported to be on the order of 50 nm in radius) (17). Immunohistochemical studies have demonstrated aquaporins chiefly on the capillary endothelium, with a small amount of staining (18) or none (19) on mesothelial cells. Blockade of aquaporins with mercuric compounds caused a marked decrease in the osmotic flow in rats (18). However, the blocking agent was likely affecting the aquaporins of the blood capillary endothelia as well as those of the mesothelium, and the data could not distinguish the contributions of different cell layers. Further research will be required to fully delineate the mechanism involved.

**Osmotic Ultrafiltration: Is It Limited by Blood Flow?**

Several precautions were taken to minimize experimental variability in these experiments. To eliminate possible variations in tissue hydration, isotonic solution was allowed to dwell in the chamber for 15 to 20 min before each experimental dwell period. Reversal of the order of test periods in two of the tissues (stomach and cecum) demonstrated no specific dependence on this factor. Finally, the pattern of tracer solute loss was similar to our previous results with labeled urea and demonstrated blood flow limitations of mannitol treatment in the liver only. The finding that there was no reduction in the rate of mass transfer in the other organs provided further evidence that these experimental conditions were consistent with our previous results. The very significant reductions in blood perfusion in the tissues tested (Figure 2) resulted in statistically nonsignificant changes in the hypertonic volume flux in the abdominal wall, the cecum, and the stomach. Only the liver demonstrated a significant decrease in water flow as a result of the decrease in blood flow. Elimination of blood flow markedly reduced the volume flux in all tissues. These results demonstrate that relatively large decreases in blood flow cause decreases in osmotic ultrafiltration but do not result in elimination of the flux until the flow is quite small. Some osmotic flow occurs even in the postmortem period; this flow probably results from removal of water from the intracellular compartment.

Although there are no water flux data with which to directly compare the data obtained under conditions of control or reduced blood flow, there have been experiments in dogs subjected to hemorrhagic shock (20). When dogs were monitored during a control period (mean BP, 139 mmHg) and then subjected to bleeding to decrease the BP to a mean of 53 mmHg, there was no significant drop in the net ultrafiltration. However, in a third period, during which the BP was returned to control levels, the net fluid return was greater. In a second series of experiments, in which the period of reduced BP was first and the period of normal BP was second, the net ultrafiltration was increased significantly during the second period. The degree of blood flow reduction determined by those authors was similar to our results. The variable results in the dog study, depending on the order of the ischemic and control periods, illustrate the complexity of the phenomena we are observing. At a cellular level, ischemia may result in increases in the perfused capillary surface area resulting from vasodilation, despite the decline in total perfusion measured by laser Doppler flowmetry. Other changes may include increases in capillary hydraulic permeability or Starling forces, which would favor ultrafiltration despite a state of lower blood flow. This study focused only on changes in blood perfusion in the tissues, and therefore other factors may be quite variable.

The results of this study and our previous studies on solute flux suggest reconsideration of peritoneal dialysis in the acute intensive-care setting. In the past, high-flow peritoneal dialysis was regularly performed to treat acute renal dysfunction in hypotensive patients. Before the advent of continuous hemofiltration/hemodialysis, there were anecdotal reports of the use of 100 L of dialysate per day to save patients with severe renal
and hepatic failure. Studies with dogs placed in shock (20) have demonstrated only small (<20%) decreases in water or solute clearance. Our results support these earlier findings in animals, and it is hoped that they will lead to clinical trials of peritoneal dialysis in certain cases of acute renal failure in which continuous hemodialysis/hemofiltration is difficult.

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References