In vivo determination of diffusive transport parameters in a superfused tissue

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Flessner, Michael F., Ravi Deverkadra, Jeremy Smitherman, Xiarong Li, and Kimberly Credit. In vivo determination of diffusive transport parameters in a superfused tissue. Am J Physiol Renal Physiol 291: F1096–F1103, 2006.—To address the hypothesis that functional changes in tissue transport can be related to structural alterations, we combined mathematical modeling with in vivo experimentation. The model concept includes interstitial diffusion and removal by a distributed microvasculature. Transport of solute and water across the peritoneum is measured via a plastic chamber affixed to the abdominal wall of anesthetized Sprague-Dawley rats. Solutions containing [14C]mannitol, with or without vasotoxic compounds [control (C; n = 10), C + nitroprusside (NP; n = 10), C + norepinephrine (NE; n = 10)], were infused into the chamber, and the volume and tracer concentrations were determined over 60 min to calculate the mass transfer coefficient (MTC) and the water flux. At 60 min, FITC-dextran (500 kDa) was given to mark the peritoneal vascular density. After euthanasia, the tissue under the chamber was frozen, dried, sliced with a cryomicrotome, and examined with fluorescent autoradiography. The microvessel density (×10⁷/cm²: NE, 50 ± 10; C, 180 ± 7.0; NP, 225 ± 15) resulted in marked differences (P < 0.05) in water flux (μl·min⁻¹·cm⁻²: NE, 0.1 ± 0.1; C, 1.6 ± 0.4; NP, 1.0 ± 0.2) and in mannitol MTC (×10⁸ cm³/min: NE, 0.9 ± 0.3; C, 3.8 ± 0.3; NP, 3.6 ± 0.6). Concentration profiles and calculated capillary permeability and tissue diffusivity were significantly different among the groups. These results demonstrate a direct correlation of mass transfer, diffusion, capillary permeability, and water flux with peritoneal vascular density and validate a method by which mechanistic changes in transport may be measured.

In the study of biological transport phenomena, the investigator attempts to quantify altered mechanisms that result in a change of the measured rate of transfer. With a simple membrane, transport may be defined by measuring the rate of change in concentration and volume in each compartment on either side of the membrane (Fig. 1A; membrane model as illustrated for the peritoneal cavity) and fitting the data to a mathematical model, which typically consists of first-order ordinary differential equations (see Eq. 1). However, the problem becomes more challenging in the case of a superfused tissue, in which solute and water transport occurs between plasma within microvessels distributed within a cell-interstitial matrix (Fig. 1B; distributed model) and a fluid bathing the surface of the tissue. Such transport occurs in the pleural or peritoneal cavity when fluid exists as a result of a pathological or therapeutic process. Clinical applications of this transport phenomenon are peritoneal dialysis and intracavitary regional chemotherapy. As seen in Fig. 1B, the potential transport barriers are typically 1) peritoneum, 2) tissue interstitium, and 3) blood capillary endothelium. Intrapерitoneal chemotherapy is a clinical technique in which sterile salt solutions containing antineoplastic agents are infused into the potential space of the peritoneal cavity to treat metastatic disease on the peritoneum. Solutions transfer chiefly via diffusion from the peritoneal solution into the tissues in contact with the solution. In the typical animal or human clinical study, the concentrations of the plasma (Cplasma) and solution in the peritoneal cavity (PC; Cpc) are sampled, and the peritoneal volume (Vpc) are measured. The mass transfer area coefficient (MTAC), which combines all of the diffusive transport processes into a single “lumped” parameter, characterizes the solute transport in the membrane model. The following equation illustrates the use of the MTAC to relate the rate of mass transfer from the peritoneal solution (8)

\[
\frac{d(C_{PC}V_{PC})}{dt} = -\text{MTAC} \cdot (C_{PC} - C_{plasma})
\]


Equation 1 can be fitted to Cpc, Cplasma, and Vpc vs. time to obtain the MTAC. While the MTAC can be used to compare the effects of various treatments on the relative rates of mass transfer across a biological membrane structure in Fig. 1A, it does not provide any information on the physiological processes within the system portrayed in Fig. 1B. We hypothesize that a relatively simple mathematical model combined with specific experimental methods will permit the characterization of transport parameters that describe transport phenomena at the level of the tissue.

The purpose of this paper is to document a method to determine and quantify alterations in tissue structure and transport function. The method includes the coupling of a simplified mathematical model with in vivo measurement of the rate of mass transfer across the tissue surface via a diffusion chamber fixed to the tissue. The chamber defines the contact area, eliminates the transfer area as an unknown, and facilitates measurement of solutes above the tissue and within the tissue at the end of the experiment. These procedures permit the calculation of intrinsic parameters that characterize diffusive transport with microvascular exchange within the tissue space.

Theory

Mathematical Model

The details of the mathematical model and the experimental calculations are contained in the Appendix and in previous publications (2, 7). In the experiments, the concentration in the transport chamber (Cchamber) is equivalent to that of the solution bathing the surface of the tissue (see Fig. 2). We previ-
ously showed that, under normal conditions, there are no blood flow limitations in the abdominal wall muscle (18, 19) and that the stagnant layer adjacent to the base of the chamber is an insignificant barrier to transport (5). We assume that the small solutes are not bound to protein or tissue, that lymph flow is an insignificant transport mechanism for small solutes, that small solutes transport exclusively via diffusion between the peritoneal cavity and blood capillaries, which are uniformly distributed in tissue interstitium, and that the process is at a steady state. Further justification and discussion of these assumptions are located in the APPENDIX and DISCUSSION. We have previously shown that transport of small solutes (molecular mass <6,000 Da) is equivalent in either direction of transport (7) and that the concentration profile approaches a pseudo-steady state within 30 min (10).

The MTAC in Eq. 1 is actually the product of an area term (A), equivalent to the area in contact with the dialysis fluid, and a mass transfer coefficient or permeability term (MTC), which combines all of the resistances in the barrier. Because the bottom of the chamber (Fig. 2) defines a specific transport area and $C_{\text{plasma}} = 0$ (the transfer rate is limited by the small area and does not alter $C_{\text{plasma}}$ significantly), we calculate the tissue MTC by fitting the following equation to data of the disappearance of the tracer mass from the chamber ($M_{\text{chamber}}$) fixed to tissue (3)

$$\frac{dM_{\text{chamber}}}{dt} = \frac{\text{mass transferred}}{\text{duration of experiment}} = -\frac{MTC \cdot A_{\text{chamber}} (C_{\text{chamber}} - C_{\text{plasma}})}{MTC \cdot A_{\text{chamber}} (C_{\text{chamber}} - C_{\text{plasma}})}$$

where $M_{\text{chamber}} = C_{\text{chamber}} \cdot V_{\text{chamber}}$.

The following equation links the microcirculatory transport parameters of the effective tissue diffusivity ($D_{\text{eff}}$) and capillary permeability-area density ($P_a$) with the MTC of the two major barriers to peritoneal transport, namely, the blood capillary wall and the interstitium which surrounds the capillary (2, 7, 18, 19)

$$MTC = \sqrt{D_{\text{eff}}(P_a)}$$

where $D_{\text{eff}}$ is the effective tissue diffusivity of the solute, and $P_a$ is the capillary permeability-area density parameter. Therefore, variation in the rate of transport of small-molecular-weight substances such as urea, creatinine, or glucose may be due to three possible mechanisms: 1) a change in the transcapillary transport ($P_a$), which is made up of capillary permeability ($P$) and the capillary area density ($a$); 2) an alteration in the rate of diffusion through the tissue interstitium that surrounds the capillaries ($D_{\text{eff}}$); or 3) a change in contact area ($A_{\text{chamber}}$) between the peritoneum and the chamber solution.

When a blood flow limitation exists, then the following equation applies (2)

$$MTC = \sqrt{D_{\text{eff}} \cdot q}$$

where $q$ is local blood flow per unit volume of tissue.

The model also predicts that there should be an exponentially decreasing concentration in the tissue interstitium ($C_s$) adjacent to the peritoneal surface vs. the distance from the peritoneum ($x$) according to (2) (see the APPENDIX)
of the concentration in the chamber and in the plasma can be measured vs. time, and the MTC can be found from the fitting of Eqs. 2 to the data. Using Eqs. 3 or 4, the value for MTC may be equated to \( [(P_a) D_{\text{eff}}]^{0.5} \) or \( [(q) D_{\text{eff}}]^{0.5} \), respectively. If, at the end of the experiment, the concentration profile is measured in the tissue below the chamber, this data can be fitted to Eq. 4 to find a value for \( [(P_a) D_{\text{eff}}]^{0.5} \) or \( [(q) D_{\text{eff}}]^{0.5} \). The resulting Eqs. 3 and 5 or Eqs. 4 and 6 can be solved simultaneously for values of \( P_a \) and \( D_{\text{eff}} \) or \( q \) and \( D_{\text{eff}} \). The capability to calculate \( D_{\text{eff}} \) and \( P_a \) or blood flow \( q \) permits the separation of effects on the vascular system from those in the interstitium. Further measurement of “\( a \)” permits the separation of \( P_a \) into its components.

### EXPERIMENTAL METHODS

#### Materials

The tracer molecule used for these experiments was \([^{14}\text{C}]\text{mannitol (182 Da)}\) purchased from Moravek Biochemicals (Brea, CA). The tracer was stated to be at least 97% pure by the manufacturer and was used as received. Thin-layer chromatography of this tracer demonstrated no spread of the tracer to lower molecular weights.

The chamber solution consisted of 3 \( \mu\text{g} \) of the labeled tracer to \( \sim 3.0 \text{ ml of an isotonic Krebs-Ringer-bicarbonate solution with 5\% mannitol (see Ref. 7 for details of this solution; 538 \pm 9 \text{ mosmol/k} \text{gH}_2\text{O}). (Note that the hypertonic solution was used to demonstrate the effect of the changes in perfused vascular area on osmotic ultrafiltration. Use of the theory and technique is more straightforward if an isotonic solution is used. See the APPENDIX.)} \)

For experiments involving vasodilation, the chamber solution was prepared by adding the tracer molecule to a Krebs-Ringer solution containing 10 \( \mu\text{mol} \) nitroprusside (NP) to cause vasodilation (based on observations of Nolph (25) and an increase in mass transfer, or 10 \( \mu\text{g/ml} \) norepinephrine (NE; based on observations of Hirszel et al.) (16, 17) to cause vasoconstriction and a decrease in mass transfer. While the former solution resulted in no change in blood pressure, the latter concentration resulted in an increase in the mean arterial pressure range from control values of 95–120 to 110–128 mmHg.

Diffusion chambers were constructed of 15-ml polystyrene centrifuge tubes (Dow Corning, Corning, NY). In brief, the lower portion of the tube was sawed off, and the rough edge was melted smooth with a hot plate to form a flange. A hole was then drilled into the conical top of the diffusion chamber to allow access to the contents once it is affixed to the tissue (see Ref. 7 and Fig. 2). The base of the melted tube was ground out to the original diameter of the tube; tubes filled with Evans blue dye bound to serum albumin consistently stain an area of the surface equivalent to the base of the tube. Therefore, the tube base defines the area of fluid contact.

#### Animals and Surgical Preparation

Female Sprague-Dawley rats (minimum \( n = 5 \) for each experimental maneuver) weighing between 200 and 300 g were obtained from Charles River Laboratories (Wilmington, MA). Animals were initially anesthetized with an intramuscular injection of pentobarbital sodium (50 mg/kg). Subsequent doses of anesthesia were given intravenously. On loss of the blink reflex, a tracheostomy was performed to ease breathing, and catheters were inserted into the carotid artery and jugular vein for blood sampling and infusion of fluids and drugs, respectively. A midline laparotomy was then performed to gain access to the peritoneal cavity. After the laparotomy, the animal was infused intravenously with 2 \text{ ml/h} of normal saline. Blood pressure was monitored continuously with an arterial catheter connected to a P10-EZ Statham transducer connected to a Gould Ponemah Physiology Platform (Gould, Valley View, OH); the mean arterial pressure was always >90 mmHg. The animal was placed on its side and, with care taken not to stretch or otherwise deform the tissue, a polystyrene diffusion chamber was affixed to the serosal side of the anterior abdominal wall with cyanoacrylate glue by applying a thin film of the glue around the underside of the chamber and lightly pressing the chamber to the tissue. The chamber was held in this position for 4–5 min to ensure proper adhesion. Once the chamber was affixed to the anterior abdominal wall, 1.5 ml of an isotonic Krebs-Ringer solution were added to the chamber and allowed to sit for 30 min. This hydration period allows the tissue to recover from the process of chamber placement and to come to a uniform state of hydration similar to that present during the mass transfer experiment. Previous experiments (5, 12) demonstrated that only the first two cell layers of tissue which adhered to the glue were disrupted and that the histology through the base of the chamber was unchanged from adjacent normal tissue. Other experiments (12) showed that the product of the MTC derived from chamber experiments and the measured peritoneal surface contact area was equivalent to the MTAC in whole-cavity experiments; this validates the use of the transport chamber in the determination of MTC.

Gauze pads soaked in an isotonic Krebs-Ringer solution were placed on all exposed tissues to prevent drying. Throughout the experiment, the animal’s rectal temperature was monitored and maintained at \( 37 \pm 2 \)°C through the use of an overhead heating lamp and a servo-controlled heating blanket (Harvard Apparatus, South Natick, MA). All animal procedures were reviewed and approved by the University of Mississippi Institutional Animal Care and Use Committee.

#### Experimental Protocol

Following the 30-min tissue hydration period, the chamber solution was removed and replaced with \( \sim 1.5 \text{ ml of solution containing the tracer. This volume was chosen because it results in a hydrostatic pressure of } \pm 1 \text{ cmH}_2\text{O}, a pressure that has been shown to cause a negligible convective flux into interstitial tissue, thereby reducing the analysis to a diffusion problem (13). Initially, and at 15-min intervals, the chamber solution was mixed, and 50-\( \mu\text{l} \) samples of fluid were taken for activity determination by liquid scintillation counting (LS 6000IC or LS Tri-Carb, Beckman Instrument, Fullerton, CA). The concentration of tracer molecule in the diffusion chamber was determined as the counts per minute (cpm) value divided by the sample volume. The volume of the chamber solution was determined by weight before the experiment and at 30-min intervals. To measure the volume, the entire fluid volume was withdrawn from the chamber into a 5-\( \text{ml syringe and weighed; the mass of the empty syringe before sampling was subtracted from the total mass of the filled syringe to obtain the net fluid volume, with corrections for sampling and residual fluid on the chamber walls. M}_{\text{chamber}} \) was calculated from the product of \( C_{\text{chamber}} \) and \( V_{\text{chamber}} \). Blood samples were taken both initially and after the experiment for determination of tracer present in the plasma. Blood was collected in 50-\( \mu\text{l} \) heparinized capillary tubes and centri-
fuged for 3 min to separate the blood cells from the plasma. The volumes of plasma in the capillary tubes were measured, and the activity was determined via liquid scintillation counting. Tracer concentration was calculated from the ratio of the tracer activity to the sample volume. One minute before termination, a 300-μl injection of 15% FITC-dextran 500 (500 kDa, Sigma, St. Louis, MO) was injected intravenously (iv) to mark the perfused vessels in the tissue. After 60 min, iv administration of a KCl solution or decapitation arrested the circulation, and the chamber solution was immediately withdrawn into a syringe. The chamber was removed, and the abdominal wall tissue was flash frozen to block any additional diffusion of the tracer by immersion in an isopentane bath cooled to −70°C. The total elapsed time from induction of circulatory arrest to tissue freezing was ~40 s. However, it was found that despite these rapid maneuvers, careful removal of the chamber volume and simultaneous preservation of the tissue concentration profile could not be accomplished within this period, so identical experiments with freezing times of <10 s were carried out to carefully preserve the concentration profiles in the tissue for each different type of treatment (n = 5 each).

**Quantitative Autoradiography**

To determine the tissue concentration, quantitative autoradiography was performed on the frozen tissue from under the chamber. Tissue samples were sliced to a thickness of 20 μm with a cryomicrotome (Leica CM3050S, Leica Microsystems, Nussloch, Germany) operating at −25°C. These frozen sections were placed on glass slides and nearly instantaneously dehydrated by placing the slides on a hot plate. These slides, along with slides containing standards for 14C (Amersham Radiochemicals, Arlington, IL) were then placed against X-ray film (Kodak BioMax, Eastman Kodak, Rochester, NY). After 5–7 days, the films were developed and the slides were stained with hematoxylin/eosin. By superimposing the film images on the stained tissue sample, it was possible to exclude from analysis those regions in which the peritoneum or the underlying tissue had been damaged. The films were analyzed using image-analysis software (MCID, Imaging Research, St. Catherine, CA), which, in conjunction with the 14C standards, permits determination of the concentration profile of tracer molecule as a function of position within the tissue. Care was taken to ensure that only undamaged tissue with an intact peritoneum was analyzed (refer to Ref. 11 for more details).

**Measurement of Local Vascular Density**

Frozen tissue below the chamber was sliced to 4–10 μm and examined with a fluorescence microscope and a calibrated graticule. Two independent examiners performed counting measurements at ×400 in 5 animals/treatment over 10 areas/animal, each area 250 × 250 μm. The depth of measurement was 500 μm but is plotted to 300 μm, at which the vascular density appeared similar in all treatments.

**Calculations and Statistics**

Statistical calculations were carried out using NCSS97 (Number Cruncher Statistical System, Kaysville, UT). Calculated parameters were compared with one-way ANOVA, and the NE and NP parameters were compared to the control values with a t-test. The probability of a type I error was set at P ≤ 0.05 for significance.

**Equations 2 and 4** were fitted to data with the program Scientist (MicroMath, Salt Lake City, UT). The resulting values for the product $D_{eq}(Pa)$ and for the $Pa/D_{eq}$ ratio (or in conditions of blood flow limitation: $D_{eq}/q$ and $q(D_{eq})$) were then solved simultaneously with Microsoft Excel.

The osmotically induced fluid flux from the tissue to the chamber was calculated as follows

$$\text{Fluid flux} = \frac{\Delta V_{\text{chamber}}}{\Delta t \cdot A_{\text{chamber}}}$$

where $\Delta t$ is the duration of the experiment, and $\Delta V_{\text{chamber}}$ is corrected for sampling and residual fluid left in the chamber after removal.

**RESULTS**

**Effect of Vasoactive Drugs on Submesothelial Perfusion**

Figure 3 shows the perfused vascular density vs. distance from the mesothelium and demonstrates the variation in perfusion for each case in the first 150 μm of tissue and the marked difference between the NE- and the control or NP-treated tissue. The effectiveness of these vasoactive drugs apparently dissipates after ~150–200 μm and presumably indicates that the interstitial concentration of the drugs has decreased to ineffective levels.

**Effect of Vasoactive Drugs on Transperitoneal Solute and Water Transport**

Figure 4A is a plot of the tracer mass in the diffusion chamber vs. time for mannitol under the different experimental conditions. NE clearly retards the disappearance of mannitol, whereas NP had minimal effects on the process. Figure 4B demonstrates the effect of the drugs on the mannitol concentration profile in the tissue. NP results in higher tissue concentration adjacent to the peritoneum, but it has a more rapid decrease in concentration than the controls. That the NE mannitol concentration deep in the tissue is significantly greater than either of the two profiles presumably demonstrates the effect of retarded transfer to the plasma because of the lower blood capillary perfused area (see Fig. 3). Figure 5 demonstrates the effects of the drugs on ultrafiltration ($P < 0.05$ control vs. NP; $P < 0.005$ control vs. NE). While the loss of ultrafiltration in the case of NE was anticipated, that the measured flux for NP was less than that of controls was a surprise. One-way ANOVA demonstrated significant differences in all measured parameters ($P < 0.05$).

**Calculated Transport Parameters**

The values for MTC [$(Pa/D_{eq})^{0.5}$, Pa, and $D_{eq}$] are shown in Figs. 5 and 6 for each dialysate condition. The results indicate the influence of vasodilation and vasoconstriction on Pa but only minimal effects on the effective tissue diffusivity (not
significant for control-NP comparison; $P < 0.05$ for control-NE comparison). If the vascular number densities in Fig. 3 are distance averaged for each treatment and multiplied by an assumed capillary diameter of 8 $\mu$m and an average capillary length of 650 $\mu$m (20), the surface area of these vessels is 0.000163 cm$^2$/capillary. By multiplying the number of perfused capillaries/cm$^3$ of tissue (Fig. 3) times the assumed surface area of each capillary, we arrive at an estimate of the parameter $a$, the "distance-averaged," capillary surface area density (cm$^2$ of perfused capillary area/cm$^3$ tissue). Figure 7 shows the estimates of $a$ and the values for $P$. The value of $P$ for the NE experiments is markedly reduced ($P < 0.005$).

There is the possibility that NE may have resulted in a significant reduction in local blood flow; the alternative solution contained in Eqs. 4 and 6 yields a value for $q = 0.00025$ ml/min/cm$^2$ tissue$^{-1}$ (see Fig. 6, in which $q$ is equated to $Pa$).

**DISCUSSION**

**Technique Assumptions and Limitations**

In this study, we have combined in vivo experimentation and mathematical modeling to estimate fundamental parameters, which determine the rate of transport of small solutes across the peritoneum. In a single experiment, we collected data on the solute’s mass transfer to or from a chamber that is affixed to the tissue of interest. The chamber restricts solute transfer to the area of its base and removes the factor of contact area of the solution to the subject tissue (peritoneum in our model) as an unknown in the transport equation. The experiment yields two

![Fig. 4](image-url)

Fig. 4: A: chamber mass of mannitol tracer normalized to the initial chamber mass (concentration × volume) vs. time for each treatment. Values are means ± SE of the chamber mass for each treatment. ● Control; ■ norepinephrine; ▲ nitroprusside. Solid lines are linear fits to the data with the intercept set to 1. B: concentration profile of mannitol in abdominal wall, normalized to the concentration in the chamber, vs. distance from the peritoneum for each treatment. Values are means ± SE of tissue concentration. Symbols are the same as in A. Dashed lines are fits of Eq. 5 or Eq. 6 to the data using the derived parameters.
sets of data: mass transfer vs. time to or from the chamber and the concentration profile of the solute in the tissue underlying the chamber. With the use of a simple mathematical model, two equations with two unknowns are derived from the data, and these are easily solved for the unknown parameters. While our approach is novel, it obviously represents a simplification of a very complex system. However, it is an initial step toward a much more detailed mathematical and experimental analysis of the problem. The solution of Eq. AI with variable coefficients, time dependence, and a more sophisticated formulation of transcapillary transport (14) will likely require finite element techniques for an adequate solution. Much more experimental data are needed to justify this complicated and laborious approach. In the following, we discuss some of the limitations in our relatively simple approach.

**Blood flow limitation of transport.** The model assumes that, under control conditions, individual capillary blood flow does not limit the transport. This is based on previous experiments carried out with similar techniques to those in this study in which mean systemic blood pressure was maintained in the normal range, whereas the local blood flow was locally reduced 50–80% from its control value (19). One other group (27) demonstrated a very modest 25% reduction in the MTC after decreasing the mean blood pressure from 133 to 61 mmHg. Because the blood pressure in our experiments was maintained in the normal range, the condition of shock was not present and therefore the findings of these other investigators do not apply. However, a decrease in perfused microvascular area does limit the mass transfer in the NE case, and there may or may not be a lack of blood flow in each exchange microvessel. Estimates of control values of the rat abdominal wall perfusion vary from 0.07 (29) to 0.28 (30) ml·min⁻¹·g⁻¹. Assuming the lower value of 0.07 ml·min⁻¹·g⁻¹ for q in the control and NP cases, we calculate the following ratios of Pa/q: C, 0.029 and NP, 0.036; both of these justify the assumption of no blood flow limitation. However, a measurement of blood flow for the case of the vasoconstrictor does not exist, and therefore a more conservative assumption is that the transport may be blood flow limited and that the Pa in Fig. 6 represents the effective perfusion of the tissue. Measurement of the local blood flow is required under the specific physiological condition to make a conclusive determination.

**Effect of osmotically induced convection.** Convective transport in the tissue space requires a more sophisticated approach than our simple diffusion model. Certainly in the case of the convection of proteins, we have found very significant effects than our simple diffusion model. Certainly in the case of the vasoconstrictor does not exist, assumption of negligible effects of convection appears valid.

**Assumption of uniform parameters within the tissue.** The most obvious parameter, which we have demonstrated in Fig. 3 to vary within the tissue, is the capillary area density parameter (a). In each group, there is considerable change in the number of perfused capillaries over the initial 150 μm of tissue. To simplify calculations, we have chosen to average the capillary counts throughout the tissue. Although changes in D_eff would indicate alterations in the interstitium vs. the microvasculature, the calculation of Pa does not demonstrate the mechanism of microvascular alteration. Pa can be separated from the perfused capillary area a in a given volume of tissue with a separate measurement coupled with certain assumptions and calculations. While a few investigators (15, 26) have used India Ink to mark open vessels, we used perfusion with a high-molecular-weight FITC-dextran and fluorescent microscopy to estimate the capillary density, and from this measurement, we estimated parameter a. A more sophisticated approach would utilize a variable a as a function of distance, but, in our opinion, this would only be worthwhile if we had detailed mapping of the actual vessel area in a two- or three-dimensional plane.

Other assumptions also include a constant D_eff and θ_o over time and space. Because the concentration at the surface of the tissue should be equilibrated with chamber concentration, its value divided by the chamber concentration provides an estimate of θ_o, at x = 0; however, θ_o(x) may vary within the tissue, depending on the local alterations in the microvasculature. The relative concentration of mannitol at the tissue surface (x = 0, Fig. 4B) indicates that the interstitial space was likely increased with NP: this would increase the effective diffusivity (28) and the rate of interstitial diffusion. In the case of NE, the decrease in D_eff (Fig. 6) may have been due to the decreased perfusion of the tissue, which might result in decreased capillary pressure, an increased absorption of extracellular fluid, and a relative dehydration of the affected tissue. To simplify the calculations, we have assumed constant values of θ_o or D_eff throughout the tissue. To include a variable θ_o and D_eff to measure θ_o, a separate set of experiments would be required (see Ref. 31).

**Parameter comparison with values in the literature.** The values of the parameters are dependent not only on the data but also on the model used to calculate the parameters. Therefore, our determinations for D_eff and Pa are likely unique to our technique and may be different from others that have been derived from different data sets, using different mathematical models. In the paper by Dedrick and colleagues (2), literature from a
variety of sources were pulled together into correlations that yielded results similar to those of this paper: $D_{\text{eff}} = 4 \times 10^{-7}$ cm$^2$/s and $Pa = 10 \times 10^{-6}$ cm/s. Although these parameter values were obtained from disparate sources, our results are very close to those from the literature.

As shown in Figs. 6 and 7, there were small but relatively insignificant differences in the estimated parameter values between the control and NP cases. The lack of change in the case of the vasodilator could have been due to two factors: 1) insufficient drug and/or 2) the induction of near-maximum vasodilation by the hypertonic solution (21, 23). Other researchers have noted no effect of NP on the osmotic ultrafiltration, due to the more rapid transfer of glucose from the cavity (3, 24); this may in part explain the decrease or lack of increase in the osmotic flux.

More significant changes were noted between the control and NE groups. The significant change in osmotic flux was expected with the vasoconstrictor. The decrease in the $D_{\text{eff}}$ was likely due to the decreased perfusion induced by the vasoconstriction and a decrease in the extracellular space, which would, in turn, decrease $D_{\text{eff}}$ (28). The value for $Pa$ or $q$ changed dramatically, due to reduction in $a$ and $P$ and possibly $q$; the alteration in $P$ has been noted in recent animal studies that demonstrated significant effect of NE on capillary permeability to sodium fluorescein (22).

The use of vasoactive drugs has resulted in changes of perfusion in the tissue, transperitoneal transport, and tissue level parameters that characterize the transport of this system. Our major purpose was the development of a simple technique to differentiate between changes in tissue diffusion and the rate of transcapillary transport. We have carried out experiments with a vasodilator and a vasoconstrictor to perturb the value of $Pa$ or $q$ separately from any alterations in interstitial diffusion. This study presents a simple, algebraic model as a first attempt to quantitatively describe a complex system under altered physiological conditions or after pathophysiological changes in a superfused tissue. More complex models with variable parameters will need to be applied to fully simulate the local tissue phenomena. Our simple model will help design the future experiments and mathematical approaches that will address the complexities of such a system.

**APPENDIX**

**Detailed Theory and Justification of Assumptions**

The mass balance on the tissue space is derived from previous work (4)

$$\frac{\partial(\theta_1 C_1)}{\partial t} = \frac{\partial}{\partial x} \left[ D_{\text{eff}} \frac{\partial C_1}{\partial x} + C_1 \cdot f \cdot j_v \cdot \theta_1 \right] + R_{\text{cap}} \quad (A1)$$

where $\theta_1$ is the fraction of tissue available to the solute; $C_1$ is the concentration of the solute in the interstitial space; $D_{\text{eff}}$ is the effective tissue diffusivity of the solute; $x$ is the distance into the tissue from the peritoneum; $f$ is the solute retardation factor (solute velocity divided by the solvent velocity); $j_v$ is the fluid flux through the tissue; and $R_{\text{cap}}$ is the rate of solute exchange with the microvasculature $[-Pa(C_1 - C_{\text{plasma}})]$ in the case of no blood flow limitation, where $P$ is capillary permeability, and $a$ is the capillary area per unit tissue volume or mass. In the case of blood flow limitation, $R_{\text{cap}} = -q(C_1 - C_{\text{plasma}})$, where $q$ is blood flow per unit mass or volume of tissue. Eq. A1 assumes that unidirectional transport is dominant in this system and that there is no generation, metabolism, binding, or lymphatic uptake of the solute. All of these quantities may be functions of time and distance, which makes the solution of Eq. A1 complex, typically requiring finite element techniques to solve.

**Steady-State Simplification**

To simplify the solution of Eq. A1 and apply it to experimental data, a steady state is assumed along with constant transport coefficients. Equation A1 becomes

$$\frac{\partial (\theta_1 C_1)}{\partial t} = 0 = D_{\text{eff}} \frac{\partial^2 C_1}{\partial x^2} + f \cdot \theta_1 \left[ \frac{d}{dx} (C_1 \cdot j_v) \right] - Pa(C_1 - C_{\text{plasma}}) \quad (A2)$$

The fluid flux ($j_v$) is dependent on the osmotic and hydrostatic pressure differences between the interstitium and the microvascular lumen and will vary within the tissue due to the decreasing concentration of the osmotic solute in the tissue and the declining hydrostatic pressure from the peritoneum inward $-0.5$ to $2$ mm (11, 13). At this time, no one has solid data on convection of small solutes through tissue. To derive the simple equations of Eqs. 3–5, $j_v$ needs to be zero or the middle term must be negligible in its effect on mass transport.

**Negligible Effect of Osmotic Convection on Small-Solute Transport**

To test whether the observed osmotic flux from the tissue influences small-solute diffusion in the opposite direction, a series of chamber experiments was carried on 12 Sprague-Dawley rats in the same fashion as in the experiment. In these experiments, there were two 90-min periods of testing in which [H]$\text{H}^4$ mannitol transport from the chamber to the tissue was measured under isotonic and hypertonic conditions. If convection from the tissue to the chamber had a significant effect, the mass transfer would be significantly affected. Using identical techniques to those outlined above, our results for the isotonic case demonstrated a mean $\pm$ SE (cm/min) for $j_v$ of $-0.000053 \pm 0.000027$ and MTC of $0.00039 \pm 0.00040$. The hypertonic case demonstrated a markedly positive flux into the chamber of $0.00233 \pm 0.00033$ ($P < 0.05$ vs. isotonic) and no significant change in the MTC ($0.00280 \pm 0.00035$). The isotonic solution produced a $j_v \approx 0$; however, our experiments were designed to check the effects of vasoactive drugs on both solute diffusion and osmotic filtration. That the apparent mass transfer of mannitol was not affected by this convection justifies our assumption of neglecting the middle term in Eq. A3 in this animal model.

**Further Simplification**

From the above data, Eq. A2 becomes

$$D_{\text{eff}} \frac{d^2 C_1}{dx^2} = Pa(C_1 - C_{\text{plasma}}) \quad (A3)$$

or for blood flow limitation

$$D_{\text{eff}} \frac{d^2 C_1}{dx^2} = q(C_1 - C_{\text{plasma}}) \quad (A4)$$

The solutions for these equations are, respectively

$$C_1 - C_{\text{plasma}} \bigg|_{C_{\text{PC}}} = e^{-\frac{D_{\text{eff}}}{Pa} x} \quad \text{or} \quad C_1 - C_{\text{plasma}} \bigg|_{C_{\text{PC}}} = e^{-\frac{D_{\text{eff}}}{q} x} \quad (A5)$$

Through calculation of the diffusive flux at $x = 0$, the following relationships can be calculated (2)

$$\text{MTC} = \sqrt{D_{\text{eff}} \cdot Pa} \quad \text{or} \quad \text{MTC} = \sqrt{D_{\text{eff}} \cdot q} \quad (A6)$$

for the permeability-limited case or the blood-flow limited cases, respectively.
REFERENCES


