Transient transcapillary exchange of water driven by osmotic forces in the heart

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Kellen, Michael R., and James B. Bassingthwaighte. Transient transcapillary exchange of water driven by osmotic forces in the heart. Am J Physiol Heart Circ Physiol 285: H1317–H1331, 2003. First published May 8, 2003; 10.1152/ajpheart.00587.2002.—Osmotic transient responses in organ weight after changes in perfusate osmolarity have implied steric hindrance to small-molecule transcapillary exchange, but tracer methods do not. We obtained osmotic weight transient data in isolated, Ringer-perfused rabbit hearts with NaCl, urea, glucose, sucrose, raffinose, inulin, and albumin and analyzed the data with a new physicochemically based model accounting for 1) transendothelial water flux, 2) two sizes of porous passages across the capillary wall, 3) axial intercapillary concentration gradients, and 4) water fluxes between myocytes and interstitium. During steady-state conditions ~28% of the transcapillary water flux going to form lymph was through the endothelial cell membranes [capillary hydraulic conductivity (Lp) = 1.8 ± 0.6 × 10⁻⁸ cm·s⁻¹·mmHg⁻¹], presumably mainly through aquaporin channels. The interendothelial clefts (with Lp = 4.4 ± 1.3 × 10⁻⁸ cm·s⁻¹·mmHg⁻¹) account for 67% of the water flux; clefts are so wide (equivalent pore radius was 7 ± 0.2 nm, covering ~0.02% of the capillary surface area) that there is no apparent hindrance for molecules as large as raffinose. Infrequent large pores account for the remaining 5% of the flux. During osmotic transients due to 30 mM increases in concentrations of small solutes, the transendothelial water flux was in the opposite direction and almost 800 times as large and was entirely transendothelial because no solute gradient forms across the pores. During albumin transients, gradients persisted for long times because albumin does not permeate small pores; the water fluxes per milliosmolar osmolarity change were 200 times larger than steady-state water flux. The analysis completely reconciles data from osmotic transient, tracer dilution, and lymph sampling techniques.

capillary permeability; reflection coefficient; transport modeling; microcirculation; isolated rabbit heart; porous transport

The osmotic weight transient method is one of the few techniques available to measure the reflection coefficient (σ) of small hydrophilic solutes in the vascular beds of whole organs. Using a single-membrane model of events during this experiment, Vargas and Johnson (49) developed a relationship, σ = Jv(LpΔII), where Lp is capillary hydraulic conductivity, ΔII is osmotic perturbation, which relates the volume flux across the capillary wall, Jv, to the solute reflection coefficient, σ, immediately following a step change in perfusate osmolarity. In a whole organ, the total fluid movement across the capillary wall, JS, is nearly equal to the rate of weight change of the organ; knowledge of the magnitude of ΔII and Lp is all that is needed to estimate σ. Estimates of σ using this analysis method and osmotic weight transient measurements have been obtained in many mammalian tissues including heart (19, 47, 49, 50), skeletal muscle (15, 39), and lung (16, 37). For small solutes like sucrose, estimates of σ in skeletal muscle range from 0.08 (39) to 0.41 (54) and in heart are 0.30 (49) and 0.14 (19).

A few investigators have attempted to extract additional information from the complete time course of the weight transient by using two-compartment (50), three-compartment (7), or three-region axially distributed models (19). The intended use of these models was to provide estimates of the solute permeability (P), but it is not clear that any of these models have captured the underlying physiology in enough detail to provide meaningful parameter estimates. The standard methods for analyzing osmotic weight transient data have several shortcomings. First, although the necessity of viewing transport across the capillary wall as a process occurring simultaneously through several disparate pathways is now recognized (40), this concept has not been built into any of the models used to analyze osmotic weight transient data. Therefore, the σ obtained from all previously developed methods of analysis is only a functional description of the behavior of the capillary wall as a whole, not information on specific pathways. Second, estimates of σ obtained by compartmental models tend to increase with increasing flow through an organ, even though there is no reason to suppose that the capillary wall morphology is actually changing. Finally, previously described analytical models depend on the prior knowledge of Lp to obtain estimates of σ and/or P.

In the companion paper to this article (29), we developed an anatomically and physiologically realistic model of the transcapillary exchange process and showed that the model was consistent with data from three different sources: multiple-indicator dilution, osmo-
motic weight transient, and lymph sampling experiments. Although previous models had integrated two of the three types of data sets (23, 41), this was the first model to be applied to all three of the major methods of investigating transcapillary exchange rates. The objective of the present paper is to determine key parameter values in our model (29), particularly those that govern water and solute exchange, with osmotic weight transient data obtained from isolated, Ringer-perfused rabbit hearts. This approach has allowed us to extract more complete and more accurate information from the weight transient record than previous methods of analysis. The parameters obtained from this analysis do not require prior measurement of total \( L_p \) and are independent of the flow. Thus our approach marks a significant improvement over previous attempts to analyze osmotic weight transient experiments.

**METHODS**

An osmotic weight transient experiment consists of the gravimetric measurement of the net transcapillary flow \( (J_v) \) induced by a step change in perfusate osmolarity. We based our experimental protocols on the basic experimental methodology developed by Vargas and Johnson (49) but used a new model (29) for the analysis of the record of weight changes. We used sensitivity analysis on preliminary data and parameters obtained from one heart to determine the model parameters identifiable from the data. We then optimized the model parameters governing transcapillary exchange with a larger data set of 111 transients on 12 different hearts.

**Experimental Methods**

Our standard protocol was to induce weight transients in the isolated, Ringer-perfused rabbit heart by using as the osmotic agent one of a series of hydrophilic solutes with molecular weights ranging between 58 (NaCl) and 68,000 (albumin). Steps were made in osmolarity from control to a hyperosmotic solution. The step durations were 4–30 min, with longer transients for larger solutes. The perfusate was then switched back to control for a reequilibration period that lasted at least as long as the original transient. Typically, four to five test solutes were used in one experiment. For each solute, one to three transients were recorded; the total time for one experiment was 3–4 h (Fig. 1). In a second protocol, we conducted a set of transients using sucrose as the osmotic agent while varying the flow through a range from 1.75 to 3.9 ml·min\(^{-1}\)·g\(^{-1}\). Details of the experiment are given in this section regarding 1) the standard and osmotically active Ringer solutions, 2) the perfusion system, 3) the surgical procedures, and 4) the data recording devices.

**Solutions.** Physiological Krebs-Ringer perfusates were prepared fresh each morning before the experiments. The control perfusate contained (in mM) 118 NaCl, 3.8 KCl, 1.2 KH\(_2\)PO\(_4\), 0.7 MgSO\(_4\), 2.1 CaCl\(_2\), 25 NaHCO\(_3\), 0.1 EDTA, 10 dextrose, 5.5 pyruvate, and 0.015 bovine serum albumin (all from Sigma). Perfusate osmolarity was 280 ± 2 mosM. Papaverine at 5 mg/l was also used in all solutions to ensure vasodilation of the preparation. Osmotic test solutions were identical to the control except for the presence of one additional solute that served as the osmotic agent during the experiment. Test solute concentrations added to the perfusate concentrations given above contained (in mM), unless otherwise noted, 30 urea, 20 NaCl, 30 glucose, 30 sucrose, 30

![Fig. 1. Weight transient responses to osmolarity steps (experiment 121202). Timing for step changes in osmolarity is indicated at top. The discontinuity in weight at the beginning of the albumin transient is an artifact arising from the increased perfusate viscosity, which causes an increased aortic volume due to an elevated aortic pressure. Before \( t = 0 \) there was an equilibration period in which weight rose to a steady level.](http://ajpheart.physiology.org/)
raffinose, 5 inulin, and 0.5 albumin. Solution osmolarity was validated with a freezing-point osmometer for the small solutes (Osmette A, Precision Systems). For the albumin test solution the concentration, C, was measured by protein refractometry (accurate to about ±0.2 g/l) and the osmolarity was calculated by the formula \( \pi = 0.345C + 0.002657C^2 + 2.26 \times 10^{-5}C^3 \) from McDonald and Levick (34). The increases in osmolarity due to the additions of the test solute were (in the same order) 30, 38, 30, 30, 30, 5, and 0.5 mosM. All in osmolarity due to the additions of the test solute were (in the albumin solution. The volume of the buffer was at least nine times that of solution and was changed at least three times during dialysis.

The albumin was purified by at least 72 h of dialysis at 4°C with a membrane with a molecular cut-off mass of 12,000–14,000 Da (Spectra/Por, Spectrum Laboratories) to remove small vasoactive compounds and control the ionic composition of the solution. The dialysis buffer was a physiological salt solution containing the same concentrations of NaCl, KCl, KH2PO4, MgSO4, and CaCl2 as the standard Ringer solution and was changed at least three times during dialysis. The volume of the buffer was at least nine times that of the albumin solution. The final concentration of albumin in the dialyzed solution was determined by refractometry; it was typically ~100 g/l, with a total osmolarity ~2 mosM higher than that of the buffer. This solution was then diluted to the experimental concentration of 34 g/l on the day of the experiment. Inulin was similarly purified by dialysis against deionized water using a membrane with a molecular cut-off mass of 2,000 Da (Spectra/Por, Spectrum Laboratories).

**Perfusion system.** A perfusion system (Fig. 2) was developed to allow rapid switching between control and test solutions. Solutions were circulated continually through membrane oxygenators (Medtronic Minimax Plus) with a 95% \( \text{O}_2 \)-5% \( \text{CO}_2 \) gas mixture and a 10-μm in-line filter. A constant-flow perfusion pump (Gilson Minipuls II) was used to send fluid through a heat exchanger, consisting of a stainless steel tubing coil submerged in a temperature-controlled water bath and a temperature-controlled windkessel on its way to the heart. Two identical perfusion lines were kept running continuously. A switching valve located ~0.1 ml before the heart ensured a sharp change between the lines.

**Isolated heart preparation.** The protocols for animal use were developed in accordance with National Institutes of Health (NIH) guidelines and were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Washington (IACUC protocol no. 2027-09). Adult female New Zealand White rabbits (2–3 kg) were sedated with acepromazine (1 mg/kg sc) 20–30 min before surgical anesthesia was induced by intramuscular ketamine (40 mg/kg) and xylazine (5 mg/kg). After tracheotomy and start of mechanical ventilation, the chest was opened with a midline incision through the sternum and a ligature was placed around the aorta. The rabbit was then given 300 U of heparin through the marginal ear vein. After 2 min, the aorta was occluded with the ligature to prevent air from entering the coronary arteries. The heart was removed immediately and temporarily immersed in iced Ringer solution until contractions ceased. It was then perfused retrogradely through the aorta while suspended from a T-shaped cannula (Fig. 2).

The isolated heart was trimmed of excess fat and other tissue. To drain the left ventricular (LV) cavity of leakage across the aortic valve a cannula was inserted through the thin apical myocardium (introducing it via a pulmonary vein). The right ventricle (RV) was drained similarly (introducing the cannula via the pulmonary artery). Coronary flow was taken to be the RV drain flow and was checked by calculation of perfusion pump flow \( \rightarrow \) LV drain flow. Preparations in which flow from the LV drain comprised >5% of the total flow were discarded because they likely had a damaged aortic valve. The heart was paced with a stimulator (Harvard Apparatus) at a constant rate of ~150 beats/min, and perfusion rate was set at ~20 ml/min (2–4 ml/min \( \cdot \) g\(^{-1} \)).

After isolation, the heart was equilibrated for at least 30 min. During this time the heart gained 1–2 g because of the

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**Fig. 2.** Isolated perfused heart system with continuous measurement of weight. Left: retrograde aortic flow. Coronary flow is the sum of pulmonary arterial flow and right ventricular (RV) drainage. The left ventricular (LV) vent flow was small and was considered to be leakage across the aortic valve. Right: a dual-perfusion system allows for rapid switching between different perfusates over the course of an experiment. The oxygenation circuit is shown for only 1 of the perfusates.

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low colloid osmotic pressure of the perfusate but reached a steady baseline weight by the end of the equilibration period. At the end of the equilibration period, a series of switches was made between the two perfusion lines, both of which contained the control solution at the same flow. By adjusting height and the gauge of fine needles at the end of the return line, we equalized the pressures and resistances of the two perfusion lines so that switching from one to the other caused a pressure jump of <1 mmHg. One perfusion line was then changed to the test solution, and the experimental protocol was begun.

Data recording. Heart weight $W(t)$ and aortic perfusion pressure were recorded from a force transducer (Transducer Techniques) and a pressure transducer (Statham). The transducer outputs were amplified with a custom-built amplifier, and the weight record was filtered by an analog resistor-capacitor filter with a time constant of 0.2 s to remove high-frequency noise. Signals were digitized and acquired by a Macintosh Power Mac 7100 running LabView 4 (National Instruments) at 250 Hz. Data points were reduced to one per second by taking the average of each 250 data points generated per weight and pressure signals were recorded continuously from the beginning of perfusion to the completion of the experiment. At the conclusion of all experiments, the heart was quickly removed from the experimental equipment, blotted, weighed, and then sectioned and dried at 100°C to a constant dry weight.

Analytical Techniques

The analysis of these experiments with sensitivity analysis and parameter optimization was based on a novel model of microcirculatory solute and water exchange. All analysis was performed on a LINUX workstation using the XSIM modeling environment. The XSIM simulation interface and the model described in the next section can be downloaded via http://nsr.bioeng.washington.edu/Software/DEMO, under “Blood-Tissue Exchange Models: Whole-organ models: osmotic”. This software package was developed by the National Simulation Resource for Circulatory Mass Transport and Exchange.

The model. The model used is described in detail in the companion paper (29) and is summarized in the APPENDIX. The standard parameters were adjusted to match the conditions of each experiment. Before the start of the experiment the model’s flow and aortic pressure were set to the values measured experimentally; venous outflow and lymph back pressure were at atmospheric pressure. We used the measured dry weight of a heart and the values of water content of vascular, interstitial, and cellular regions for in vivo rabbit hearts (17) to calculate initial values for plasma volume, $V_p$, interstitial space, $V_{int}$, and cell space, $V_{pc}$. To correct for edema we assumed that background weight gained during the equilibration period and later in the experiment was due exclusively to fluid accumulation in the interstitium; $V_{int}$ was increased so that model $W(t)$ matched the weight measured experimentally.

Sensitivity analysis. Sensitivity analysis provides insight into complex models and is an aid to experimental design. The sensitivity functions, defined as the instantaneous fractional change in a model output (in this case organ weight, $W$) induced per fractional change in a model parameter, $P$, were approximated by comparing model solutions produced with a 1% increase in the parameter values. Relative sensitivities ($\delta W/W(\delta P/P)$ or $\delta \ln W/\ln P$) rather than absolute sensitivities ($\partial W/\partial P$) were used to provide dimensionless sensitivity functions. Because the sensitivity functions are dependent on

the parameter values (8), sensitivity analysis was performed after an initial manual fit to a small number of preliminary data sets. The sensitivity curves shown in RESULTS were generated with the mean parameter values obtained from optimization against the full data set but do not differ significantly from the preliminary results used for experimental design.

Parameter estimation. Optimization of model fits to data was used to determine three model parameters: $1) L_{p,endo}$, the hydraulic conductivity through the transendothelial pathway, $2) A_{sp}/S$, the fractional small-pore area, and $3) r_{sp}$, the small-pore radius. The estimates of these parameters are not sensitive to the parameter values for the large-pore system, $A_{lp}/S$, which was set at $10^{-6}$, and $r_{lp}$, which was set at 24 nm. These values were determined from the observations of lymph-to-plasma concentration ratios in the heart (32, 38) and assumed to apply to our hearts. The complete weight record (d$W$/dt) including both the switch to the test solution and the return to control was used as the data for each optimization procedure. Optimization was performed with SENSOP (11), which uses sensitivity functions to optimize parameter sets and provide confidence ranges from the covariance matrix. Local minima in the sum of squares of differences between model solutions and data were not observed; this is to be expected when there are many data points per weight transient (6–30 min/transient at 1 sample/s is 360–1,800 samples per parameter optimization run) and few free parameters (a maximum of 3). The parameters for capillary and parenchymal cell volumes, $V_c$ and $V_{pc}$, were those from Ref. 19. The capillary surface area $S$ is 500 cm$^2$/g (6).

The three free parameters are interdependent to some extent and require use of the full extent of the weight transients and the full set of solutes. The larger solutes have the greatest influence on estimates of $r_{sp}$, because for small solutes the $\sigma_{sp}$ is close to zero. Consequently, it is from the overall d$W$/dt = $S J_V$ that an overall $L_{p}(L_{p,\text{total}})$ is calculated, thus defining $L_{p,\text{endo}}$ through the iterative parameter adjustments to account for the observed balances of solute and water fluxes that fit the rate of water flux and dissipation of solute gradients.

Calculation of derived parameters. The model parameters optimized to fit the data are explicitly related to the phenomenological coefficients of Kedem and Katchalsky (28), namely, solute permeabilities $P$ and reflection coefficients $\sigma$ through the solute effective radius $r_s$ and the pore radius $r_p$ (either $r_{sp}$ or $r_{lp}$) and the total pore areas. The equations governing this relationship are given in the APPENDIX (Eqs. A11–A13).

RESULTS

We obtained estimates of the parameters $L_{p,\text{endo}}$, $A_{sp}/S$, and $r_{sp}$ from a total of 111 high-quality osmotic weight transient data sets in 12 different Ringer-perfused rabbit hearts. Coronary flows during the experiment ranged from 1.5 to 4 mL·min$^{-1}$·g$^{-1}$; aortic pressures were typically between 20 and 30 mmHg in these vasodilated hearts. Hearts at the conclusion of the experiments weighed 7.5–10 g and were 82–87% water, compared with initial weights of 5.5–6.5 g estimated from the final dry weight and the known wet-to-dry ratio of freshly excised rabbit heart (17). Edema during the experiments was therefore substantial, but most of this fluid gain occurred during the initial 20- to 30-min equilibration period; the baseline weight was
relatively stable over the time of a single transient, but a gradual weight gain occurred throughout the duration of the complete experimental period, such as can be seen in Fig. 1.

**Parameter Estimates**

The means of the parameter estimates obtained from the complete set of osmotic weight transient data are presented in Table 1. The endothelial pathway for transcapillary water-only exchange accounts for ~28% of the total transcapillary hydraulic conductivity, the large-pore pathway accounts for 5% of the hydraulic conductivity, and the majority, 67%, is via the small-pore pathway. The small-pore hydraulic conductivity of $4.4 \times 10^{-8}$ cm·s⁻¹·mmHg⁻¹ is through a small fraction of the total capillary surface area, $A_{sp}/S = 2.2 \times 10^{-4}$, equivalent to a pore area per unit path length of 4.4 cm⁻¹. The small pore has an effective radius of ~6.9 nm, almost one-third of that of the large pore, assumed to be 24 nm in radius from prior model fits to lymph sampling data (32, 38). The coefficient of variation for individual parameter estimates was only ~1%, which is substantially smaller than the differences between parameter estimates from different data sets. Table 1 therefore treats each transient as providing an independent set of parameter estimates and gives the mean and the standard deviation of the set of parameter estimates.

Table 2 shows the parameter estimates obtained with each osmotic agent. Similar estimates were obtained regardless of the solute used, with the most significant disparity being the difference between $r_{sp}$ by inulin vs. albumin osmotic transients. Each individual transient could not be used to obtain estimates of all three free parameters, either because there was too much correlation between pairs of free parameters or because there was too little sensitivity of the weight response to the values of the parameter (see Discussion). When a given parameter could not be determined for a transient induced by a given osmotic agent (indicated by an asterisk in Table 2), the average estimate obtained from solutions with other osmotic agents was used.

These parameter estimates can be used to calculate solute $P$ and $\sigma$ for the solutes through each pathway (Table 3). Solute $P$ for small solutes (NaCl through raffinose) are dominated by diffusion through the small pore and are almost proportional to their free molecular diffusion coefficients in water, although there is the expected consistent decrease in the permeability-to-diffusion coefficient ratio ($P/D$) from ~3.8 to 3.0 cm⁻¹. Inulin and albumin, being much larger, are significantly hindered compared with the other solutes and have smaller $P/D$ and larger $J_{sp}$.

**Fitting the Model to Data on W(t)**

A representative fit to an osmotic weight transient experiment on an isolated rabbit heart is shown in Fig. 3. After a step increase in perfusate osmolarity with 20 mM NaCl, there is an initial rapid loss of water from the interstitium and cells to the plasma as osmotic equilibrium is restored. This rapid shift of water diminishes the osmotic pressure gradient in the test solute and also creates opposing concentration gradients in the resident solutes previously at equilibrium (osmotic buffering). Interstitial hydrostatic pressure falls as $V_{isf}$ decreases. This is followed by a slower phase where the solute enters the interstitium to partially dissipate its transcapillary concentration gradient; mechanical elastic and secondary osmotic forces act to partially restore interstitial volume. Water loss from cardiomyocytes is small, and the rise of perfusate osmolarity from 280 to 318 mosM would result in a steady-state loss of only 14% of cell water even if the

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**Table 1. Summary of capillary pathway parameter estimates**

<table>
<thead>
<tr>
<th>Pathway</th>
<th>$A_{p}/S$</th>
<th>$r_{sp}$ nm</th>
<th>$L_{p,endo}$ cm·s⁻¹·mmHg⁻¹</th>
<th>$L_{p,endo}/L_{p,total}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endothelial $k$ = 1</td>
<td>0.9998</td>
<td>6.9±0.16</td>
<td>$1.8 \times 10^{-8}$</td>
<td>0.28</td>
</tr>
<tr>
<td>Small pore $k$ = 2</td>
<td>$2.2 \times 10^{-4}$</td>
<td>6.9±0.16</td>
<td>$4.4 \times 10^{-8}$</td>
<td>0.67</td>
</tr>
<tr>
<td>Large pore $k$ = 3</td>
<td>$1 \times 10^{-6}$ (from Refs. 32 and 38)</td>
<td>6.9±0.16</td>
<td>$3.3 \times 10^{-9}$</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Values are means ± SD. $A_{p}/S$, fractional pore area; $r_{sp}$, pore radius; $L_{p}$, capillary hydraulic conductivity; $L_{p,endo}$, capillary hydraulic conductivity through transendothelial pathway; $L_{p,sp}$, small-pore capillary hydraulic conductivity; $L_{p,total}$, large-pore capillary hydraulic conductivity. $L_{p,total} = 6.5 \pm 1.5 \times 10^{-8}$. With mean capillary pressure ($p_{cap}$) = 5.0 mmHg, mean interstitial fluid pressure ($p_{inter}$) = 4.6 mmHg, and with lymphatic pressure ($p_{lymph}$) = 0, the capillary-to-lymph flux was 0.003 ml·g⁻¹·min⁻¹.

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**Table 2. Parameter estimates by solute**

<table>
<thead>
<tr>
<th>Osmotic Agent</th>
<th>No. of Transients</th>
<th>No. of Hearts</th>
<th>$A_{p}/S$</th>
<th>$r_{sp}$ nm</th>
<th>$L_{p,endo} \times 10^9$, cm·s⁻¹·mmHg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 mM NaCl</td>
<td>23</td>
<td>8</td>
<td>$2.19 \times 10^{-4}$</td>
<td>6.9*</td>
<td>1.51 ± 0.36</td>
</tr>
<tr>
<td>30 mM urea</td>
<td>15</td>
<td>5</td>
<td>$2.19 \times 10^{-4}$</td>
<td>6.9*</td>
<td>2.17 ± 0.97</td>
</tr>
<tr>
<td>30 mM glucose</td>
<td>11</td>
<td>4</td>
<td>$2.0 \times 10^{-4}$</td>
<td>6.9*</td>
<td>1.67 ± 0.43</td>
</tr>
<tr>
<td>30 mM sucrose</td>
<td>34</td>
<td>8</td>
<td>$2.0 \times 0.77 \times 10^{-4}$</td>
<td>6.9*</td>
<td>1.75 ± 0.52</td>
</tr>
<tr>
<td>30 mM raffinose</td>
<td>15</td>
<td>6</td>
<td>$2.4 \times 0.4 \times 10^{-4}$</td>
<td>6.9*</td>
<td>2.27 ± 0.48</td>
</tr>
<tr>
<td>5 mM inulin</td>
<td>5</td>
<td>3</td>
<td>$1.8 \times 0.3 \times 10^{-4}$</td>
<td>5.20 ± 0.26</td>
<td>1.45 ± 0.49</td>
</tr>
<tr>
<td>0.5 mM albumin</td>
<td>8</td>
<td>7</td>
<td>$2.7 \times 0.94 \times 10^{-4}$</td>
<td>8.24 ± 1.54</td>
<td>1.8*</td>
</tr>
</tbody>
</table>

Values are means ± SD. *Not estimated by this solute; simulations used aggregate values.
cell was a flaccid bag and slightly less if there was resistance to deformation. A steady-state interstitial-to-plasma concentration ratio is achieved when the rate of solute entry into the interstitium is equal to the rate of removal by lymph.

The time course of weight change is substantially different for larger solutes like albumin (Fig. 4). Because only 0.5 mM albumin was used, the initial rate of weight loss is less than for NaCl, despite albumin’s higher \( \sigma \). However, the duration of the weight transient is longer because transcapillary concentration gradients persist as the test solute penetrates the interstitium only very slowly. Osmotic buffering effects are minimal because the low \( J_w \) causes only a negligible transcapillary gradient in the resident solutes. Because of the low \( J_w \), the low \( \sigma \) for the small solutes, and the very low \( P \) for albumin, the \( J_w \) is of water and solute, so the \( \Delta P_{\text{osm}} \) persists for a long time and causes a water shift that is large, decreasing \( V_{\text{inf}} \).

### Sensitivity Analysis

Sensitivity analysis indicated that analysis of transients over a range of molecular weights from NaCl to albumin was needed to provide estimates of the small-pore system parameters \( A_{\text{sp}}/S \) and \( r_{\text{sp}} \) and the \( L_{p,\text{endo}} \) of the transendothelial pathway. Data spanning a wide range of molecular sizes are crucial to getting reasonable estimates, as there is no single solute size at which all three parameters have substantial and distinctly different sensitivity functions (Fig. 5).

The values of the large-pore parameters cannot be resolved by osmotic transient data because the sensitivity functions \( \partial \ln W/\partial \ln r_{\text{lp}} \) and \( \partial \ln W/\partial \ln A_{\text{lp}} \) with NaCl, sucrose, inulin, and albumin as the osmotic agent are all nearly zero over the time course of an osmotic transient experiment; therefore, these parameters are estimated from lymph-to-plasma concentration ratios for a set of solutes (32, 38).

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### Table 3. Solute phenomenological transport parameters

<table>
<thead>
<tr>
<th>Solute</th>
<th>( D_{w}^{c} ), cm(^2)/s</th>
<th>( r_{\text{sp}} ), Å</th>
<th>( \sigma_{\text{sp}} )</th>
<th>( P_{\text{sp}} ), cm/s</th>
<th>( \sigma_{\text{lp}} )</th>
<th>( P_{\text{lp}} ), cm/s</th>
<th>( (P_{\text{sp}} + P_{\text{lp}})/D ) cm(^{-1} )</th>
<th>( \sigma_{d} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>( 1.72 \times 10^{-5} )</td>
<td>2.5</td>
<td>0.0038</td>
<td>( 6.4 \times 10^{-5} )</td>
<td>0.0003</td>
<td>( 3.3 \times 10^{-7} )</td>
<td>3.72</td>
<td>0.28</td>
</tr>
<tr>
<td>Urea</td>
<td>( 1.50 \times 10^{-5} )</td>
<td>2.2</td>
<td>0.0029</td>
<td>( 5.7 \times 10^{-5} )</td>
<td>0.0002</td>
<td>( 2.9 \times 10^{-7} )</td>
<td>3.80</td>
<td>0.28</td>
</tr>
<tr>
<td>Glucose</td>
<td>( 7.79 \times 10^{-6} )</td>
<td>4.4</td>
<td>0.0132</td>
<td>( 2.6 \times 10^{-5} )</td>
<td>0.0005</td>
<td>( 1.5 \times 10^{-7} )</td>
<td>3.33</td>
<td>0.29</td>
</tr>
<tr>
<td>Sucrose</td>
<td>( 6.03 \times 10^{-6} )</td>
<td>5.2</td>
<td>0.0117</td>
<td>( 1.9 \times 10^{-5} )</td>
<td>0.0011</td>
<td>( 1.1 \times 10^{-7} )</td>
<td>3.15</td>
<td>0.29</td>
</tr>
<tr>
<td>Raffinose</td>
<td>( 5.02 \times 10^{-6} )</td>
<td>6.0</td>
<td>0.0228</td>
<td>( 1.5 \times 10^{-5} )</td>
<td>0.0014</td>
<td>( 9.2 \times 10^{-8} )</td>
<td>2.99</td>
<td>0.29</td>
</tr>
<tr>
<td>Inulin</td>
<td>( 1.85 \times 10^{-6} )</td>
<td>16.6</td>
<td>0.17</td>
<td>( 2.5 \times 10^{-6} )</td>
<td>0.0111</td>
<td>( 2.8 \times 10^{-8} )</td>
<td>1.35</td>
<td>0.39</td>
</tr>
<tr>
<td>Albumin</td>
<td>( 7.87 \times 10^{-7} )</td>
<td>36</td>
<td>0.62</td>
<td>( 1.4 \times 10^{-6} )</td>
<td>0.052</td>
<td>( 8.8 \times 10^{-8} )</td>
<td>1.78</td>
<td>0.70</td>
</tr>
</tbody>
</table>

\( D_{w}^{c} \) diffusion coefficients in water at 37°C. Equations for small-pore reflection coefficient (\( \sigma_{\text{sp}} \)), large-pore reflection coefficient (\( \sigma_{\text{lp}} \)), small-pore permeability (\( P_{\text{sp}} \)), large-pore permeability (\( P_{\text{lp}} \)), and osmotic reflection coefficient as a whole (\( \sigma_{d} \)) are Eqs. A12, A13, and A16 in the APPENDIX.
Osmotic Transients at Variable Flows

We found that flow had no effect on the parameter estimates produced by our analysis. Osmotic transients with 30 mM sucrose as the osmotic agent produced weight responses with an increasing slope as flow increased from 1.75 to 3.9 ml·min⁻¹·g⁻¹ (Fig. 6). This trend was consistently observed over 22 transients in two different hearts. The parameters estimated from these data sets did not have a variability higher than that observed in the standard study and showed no apparent trend as a function of flow.

DISCUSSION

The use of an anatomically detailed model of the coronary microcirculation has enabled us to design osmotic weight transient protocols that are effective in revealing the physiological parameters. The osmotic transient methods provide a means of distinguishing the exchange process than has previously been obtained in the heart.

Design of Experiment

Sensitivity analysis provides a tool to develop strategies for parameter optimization. When applied to our osmotic transient protocol, sensitivity analysis suggested a means to estimate the parameters $L_{p,endo}$, $r_{sp}$, and $A_{sp}/S$. The parameters $A_{sp}/S$ and $r_{sp}$ can be determined from albumin transients because the shapes of $\partial \ln W/\partial A_{sp}$ and $\partial \ln W/\partial r_{sp}$ differ greatly and $\partial \ln W/\partial L_{p,endo}$ and the fluxes across endothelial cells are small. $L_{p,endo}$ must then be determined from NaCl transients, holding $A_{sp}/S$ fixed. Given that the model is an appropriate descriptor, mid-sized solutes provide redundant information, and by using their known molecular sizes the fits to their transients should only require minor adjustments of parameter estimates.

This strategy makes sense given our understanding of the microcirculatory exchange model. Small hydrophilic solutes (mol wt < 1,000) only induce a significant $J_v$, through the transendothelial pathway, where it can be assumed that they have a $\sigma$ equal to 1 because aquaporin channels and cell membranes exclude these solutes. Although $J_v$ is proportional to $L_{p,endo}$, the osmotic driving force for fluid exchange is dissipated as the test solute permeates through the pores into the interstitium, a flux that is predominantly diffusive and proportional to $A_{sp}/S$. As a first approximation, the magnitude of the weight transient is governed by the ratio of these processes, or $L_{p,endo}$ to $A_{sp}/S$. As solutes become larger, sensitivity to $L_{p,endo}$ per unit osmolarity change stays the same; experimentally, because smaller osmotic step changes are used for larger solutes, the absolute magnitude of the maximum weight is usually smaller. For any specific $A_{sp}/S$, increasing solute size, and therefore increasing $\sigma$, reduces the rate of solute permeation into the interstitium and increases water flux out of the interstitium. For solutes larger than NaCl the sensitivity to $A_{sp}/S$ is initially negative (increasing small-pore area results in an increased water efflux). Increasing pore size, $r_{sp}$, leads to lower $\sigma_{sp}$, increased water flux, and reduced solute permeation. The sensitivity to $r_{sp}$ is greater the larger the solute.
Sensitivity analysis shows that the parameters governing transport through the large pores cannot be determined from the experimental data: even the largest solutes have a $\sigma$ near 0 for the large pores. Thus varying the osmolarity with any solute fails to affect $J_v$ or to influence the pattern of weight change. Although permeation of large solutes through the large-pore path is a significant fraction of the total diffusive permeability, the tail of the weight transient response is also independent of the parameterization of the large-pore pathway because their area is so small that flux of large solutes into the interstitium is too slow to affect the osmotic driving force, even late in the osmotic transient. Weight loss, $-\frac{\partial W}{\partial t}$, goes to zero when interstitial hydrostatic pressure $p_{\text{isf}}$ decreases and interstitial osmotic pressure $\pi_{\text{isf}}$ (exerted by the permanent protein components of the interstitial matrix) rises to balance the increased osmotic pressure in the capillaries, that is, $\Delta p - \Delta \pi$ goes to zero. This result is dependent on the assumption that the large-pore pathway is in fact in the neighborhood of $A_{tp}/S = 10^{-6}$ and $r_{lp} = 24$ nm or larger. Our model simulations show that a putative large-pore pathway would not make a measurable (>5%) contribution to the total osmotically induced water flux by albumin unless $r_{lp}$ were made smaller and there were more pores. Transients would be affected if $A_{tp}/S$ were as large as $5 \times 10^{-6}$ and $r_{lp}$ were as small as 12 nm, but these values are well outside of the range compatible with the lymph-to-plasma concentration ratios of large plasma proteins (32, 38).

**Interpretation of the Weight Transient Curve**

Grabowski and Bassingthwaighte (19) and Vargas and Johnson (49, 50) used the initial slope of the osmotic transient weight response, $\frac{dW}{dt} = J_v = \sigma L_{p,\text{endo}} S R T C$, where $R$ is the gas constant and $T$ is absolute temperature, to estimate the solute reflection coefficient. Because they assumed that coupled transport of water and solute occurred through a single pathway, they estimated relatively narrow pore dimensions by hydrodynamic theory. In an actual heterogeneous capillary, this measurement provides $\sigma_d$, the osmotic reflection coefficient for the membrane as a whole. By itself, the apparent $\sigma_d$ for a heterogeneous membrane is insufficient to provide a complete description of exchange kinetics. Realizing this, Pappenheimer proposed in 1969 (35) that ~50% of transcapillary water exchange occurs through a water-only pathway

---

Fig. 5. Sensitivity functions $\frac{\partial \ln W}{\partial \ln (\text{parameter value})}$, where $W$ is weight, for the 3 fitting parameters fractional small-pore area $A_{sp}/(S \Delta r)$, small-pore radius $r_{sp}$, and capillary hydraulic conductivity through the transendothelial pathway $L_{p,\text{endo}}$ for osmotic transients induced by 20 mM NaCl ($\Delta \pi = 38$ mosM), sucrose (B), inulin (C), and albumin (D). The weight response to cases in A and D are given in Figs. 3 and 4, respectively.
to explain early data on hydraulic conductivity and solute permeability in skeletal muscle measured by his isogravimetric technique (36) and the indicator-dilution work of Alvarez and Yudilevich (3). More recently, Watson (53) and Wolf (54–57) have proposed three pathway pore models for solute exchange in mammalian skeletal muscle, with 41% of steady-state flow through a water-only pathway, 17% through a large-pore pathway of radius 28.5 nm, and 42% through a small-pore pathway of radius 4.57 nm. The high reflection coefficients obtained from osmotic transient experiments can then be understood as an averaging between a transendothelial pathway that excludes all solutes (giving them a $\sigma$ of 1) and relatively unrestricted aqueous pathways with $\sigma$ close to 0 for at least the smaller hydrophilic solutes.

Here we use a model that explicitly accounts for the fluxes across each transcapillary pathway to directly estimate the parameters governing exchange through each. This approach allows us to extract more information from an osmotic weight transient curve than the simple Vargas and Johnson analysis. On the other hand, we have not taken into account many of the details of interstitial transport, e.g., differential protein velocities due to convection combined with molecular exclusion (see Ref. 33) and therefore might not represent the transit times of proteins from blood to lymph accurately. In a system with two or more pathways, the distribution of fluxes changes during the course of an osmotic transient experiment (Fig. 7), as appreciated by Rippe and Haraldsson (40). A step increase in capillary osmotic pressure induces water flux only through pathways that have a high $\sigma$ for the test solute. For small solutes like NaCl, the only pathway with a nonnegligible $\sigma$ is the transcellular pathway. Regardless of its fraction of total hydraulic conductivity, an increase in capillary NaCl concentration draws water out of the tissues predominantly across the endothelial cells and out of parenchymal cells, reduces $p_{\text{w,tissue}}$, and diminishes lymph flow, $J_{W,L}$, as shown in Fig. 7. Consequently, the apparent $\sigma$ of the capillary wall at early times in an osmotic transient is larger than later, because the fraction of $J_\sigma$ through the transendothelial cellular pathway is greater. In contrast, albumin can induce osmotically driven fluid movements through the small-pore system as well as across the endothelial cells. In contrast, changes in hydrostatic pressures, commonly used in lymph sampling studies, produce the same change in driving force for all pathways, and thus $\sigma_{\text{apparent}}$ approaches a constant value at high $J_\sigma$, when solute permeation, $J_\sigma < J_\sigma \times C_\sigma$, where $J_\sigma$ is solute flux and $J_\sigma \times C_\sigma$ is unhindered, expected solute flux.

We investigated the significance of explicitly modeling fluxes through each pathway individually by fitting model results generated by the three-path model with a single-path model that lumped all transcapillary exchange into a single effective $P$ and $\sigma$. The osmotic transient results presented in Fig. 3 are typical of small solutes and can be closely matched by using a parametrically reduced form of the model with a single lumped pathway for transcapillary exchange. The estimated NaCl permeability by the reduced model is $1 \times 10^{-8} \text{ cm/s}$, 50% higher than the multipath model estimate ($6.4 \times 10^{-9} \text{ cm/s}$). Also, $\sigma$ of the single-path model is 0.15, assuming that the $L_p$ of this single “effective” pathway is equal to the sums of the $L_p$ values of the three-path description. The higher apparent solute permeability predicted by the single-path model at least partially explains the historic discrepancy between indicator dilution and osmotic transient measurements of small-solute permeability. The actual permeability estimates obtained by Vargas and Johnson are higher than modern estimates, even accounting for this error, presumably because they used no albumin in their perfusate. A single-path reflection coefficient of 0.15 is incompatible with the observed lymph-to-plasma concentration ratio of unity for small solutes at even the highest filtration rates. It also implies a channel width of ~3 nm, incompatible with multiple-indicator dilution and electron microscopic observations.

Parameter Values

Our estimates of the general parameters governing exchange in the microvasculature of the heart are compatible with previous results. Our $L_p,\text{total}$ of $6.5 \times 10^{-8} \text{ cm/s} \cdot \text{mmHg}^{-1}$ (Table 1) is two-thirds of the Vargas and Johnson (49) estimate of $1.0 \times 10^{-7} \text{ cm/s} \cdot \text{mmHg}^{-1}$ in isolated, Ringer-perfused rabbit heart. The most likely reason for our lower estimate...
is that Vargas and Johnson used a completely protein-free Ringer solution, which artificially increased transcapillary hydraulic conductivity. A novel result is our estimation that 28% of the $L_p$ is contributed by the transendothelial pathway. This value is greater than the 5–10% estimated in frog mesentery (15) but less than the 40% predicted by Wolf (54, 55) in skeletal muscle. Our estimates of $d$ for small solutes are just over 0.28, a consequence of a reflection coefficient of 1 in the transcellular pathway and a reflection coefficient close to zero through the large and small pores. For albumin, our estimate of $d$ = 0.7 is higher than $d = 0.59$ estimated from filtration-rate independent $C_l/C_r$ measurements (38), in line with theoretical expectations (13, 40), but it is lower than the 0.80–0.87 $d$ obtained in other organs (56). Because permeabilities are known to be artificially elevated in the Ringer-perfused heart, it is possible that the reflection coefficient is slightly higher than our value in vivo.

The solute permeabilities obtained in this study are also consistent with estimates obtained from indicator dilution studies in Ringer-perfused rabbit and guinea pig heart preparations (44). Solute permeability is proportional to $A_{sp}/(S/\Delta r)$, where $A_{sp}$ is the cleft or pore surface area, $S$ is the capillary surface area, and $\Delta r$ is the length of the pathway from capillary lumen to interstitium. These terms are kinetically inseparable. In anatomic studies $S$ is ~500 cm$^2$/g (6), and in the geometry of our model it is 487 cm$^2$/g (29). From electron microscopy the distance $\Delta r$ is ~0.5 μm; thus an estimate of $A_{sp}/(S \Delta r)$ of 4.4 cm$^{-1}$ is equivalent to $A_{sp}/S$ of 0.022% and $A_{sp}$ of 0.11 cm$^2$/g.

For translating from the parameters of our model the traditional permeability-surface area product $PS$ (in ml·g$^{-1}$·min$^{-1}$) for glucose would be
For glucose, multiple-indicator dilution methods in Ringer-perfused hearts (rabbits, except where specified otherwise) gave $PS$ equal to $2.3 \pm 0.7$ (5), $1.2 \pm 0.16$ (31), and $2.3 \pm 0.3$ (in guinea pig; Ref. 30). For sucrose, indicator dilution estimates were $2.3 \pm 0.9$ (18), $2.0 \pm 0.42$ (44), and $1.9 \pm 0.6$ (in guinea pig; Ref. 43). Rather high values of $5.1 \pm 1.4$ ml·g$^{-1}$·min$^{-1}$ (10) were obtained in rat hearts with albumin-free perfusate and are therefore not directly comparable to our studies reported here. Permeabilities are lower normally in vivo. In blood-perfused dog hearts the $PS$ values are lower: for glucose, $PS_c = 0.3 \pm 0.1$ ml·g$^{-1}$·min$^{-1}$ (3, 59); for sucrose, $PS = 0.24 \pm 0.07$ (5), $0.22 \pm 0.1$ (59), and $0.26 \pm 0.02$ (22) ml·g$^{-1}$·min$^{-1}$.

Our estimates of $A_{lp}/S$ are compatible with those of Guller et al. (21); for NaCl they used an intraproe diffusion coefficient $D = 5 \times 10^{-6}$ cm$^2$/s, taken from studies of Na diffusion in extracellular space (42, 46) on the basis that the diffusion in the cleft between endothelial cells is reduced by the presence of glycoa- lyx. Their values of $D$ within the cleft are probably more nearly correct than assuming one equivalent to that in water, as we did for Table 3. Estimating the total length of interendothelial cleft per unit capillary surface area as Guller et al. (21) did would suggest a total $A_{lp}/S$ of $2.25 \times 10^{-3}$, given a 9-nm width. In accordance with Guller et al. (21), because our functional estimate of $A_{lp}/S$ was only $2 \times 10^{-4}$, we would conclude that <10% of the cleft is open and that diffusion through the other 90% is blocked by interendothelial gap junctions (2, 9).

**Flow Effects, An Artifact of Compartmental Analysis**

Investigators using the osmotic transient approach observed that flow had an effect on the weight response. Our results confirmed the observation that the initial rate of weight loss during an osmotic response increases with increasing flow. Consequently, when compartmental analysis techniques are used to obtain reflection coefficients, $\sigma_d$ appears to increase with increasing flow rates (57). This is because the Vargas and Johnson (49) estimate of reflection coefficient is based on the prediction that the initial volume flux is proportional to a step change in concentration along the whole length of the capillary. Because in actuality the concentration step travels along the capillary with the fluid velocity and is being dissipated as it travels, the initial water fluxes are smaller in reality than the compartmental model assumes, with the result that the compartmental model underestimates the apparent $\sigma_d$.

Vargas and Johnson (49, 50) handled the flow-limi- nation problem by performing experiments at increas-
model fits these observations over the whole range of flows without altering the parameters governing transcapillary exchange.

**Errors and Limitations**

**Low oncotic pressure preparations.** The isolated, Ringer-perfused heart is a convenient preparation for osmotic transient studies but differs from the in vivo blood-perfused heart. Ringer-perfused hearts beat less strongly than blood-perfused hearts and so develop more edema because of poorer squeezing of the interstitial fluid (ISF) and because of the lowered oncotic pressure of the perfusate. By the end of an experiment, hearts typically had water contents of 82–85%, as measured by wet-to-dry weight ratio, compared with in vivo values of 78 ± 1% (17, 58). Because total perfusate osmolarity is kept at normal levels, it seems likely that much of this change is due to an increased interstitial volume. In our analysis, we have assumed that all weight gain during the baseline equilibration period is caused by expansion of the ISF volume, almost a doubling of interstitial volume.

A problem with using low-protein perfusates is that capillary permeability tends to rise (19, 24, 25), so the parameters estimated for the Ringer-perfused heart are not necessarily representative of the in vivo values. Whereas we used a background level of 1 g/l albumin in all solutions to help maintain normal permeability properties, it is possible that other serum proteins are also necessary to maintain completely normal permeabilities. There is possibly some degradation of the capillary glycocalyx, which contributes some of the resistance to exchange. As interpreted by our pore theory model, such degradation would be observed by increases in both pore radius and pore area. On the basis of comparisons of multiple-indicator dilution experiments in different heart preparations, we expect roughly a doubling of the pore area and an increase in effective radius from 5 to 7 nm when going from blood-to Ringer-perfused preparations (29). Therefore, in the in vivo condition the transendothelial fraction of $L_p$ is probably closer to 40–50% rather than the 28% we found in these experiments (Table 2).

**Pressure changes during experiment.** Drops in arterial pressure are known to occur during an osmotic transient with a step increase in concentration at constant flow (19, 47, 48). If these pressure changes propagated to the capillaries they would partially offset the increase in perfusate osmolarity, leading to a smaller than expected change in the Starling forces for a measured rate of fluid exchange. However, in a constant-flow preparation, capillary pressures will be reduced only if capillary and postcapillary venular resistances are selectively reduced.

Coronary arterial smooth muscle is known to dilate in response to osmolarity changes (4, 51). To test the hypothesis that the pressure changes are indeed caused by arteriolar coronary dilation, we fully dilated the arterial vessels with papaverine, a smooth muscle relaxant, causing a reduction in perfusion pressure (data not shown). This intervention also kept capillary pressures low and partially offset the initial weight gain on the low-oncotic pressure perfusate. In the presence of papaverine, the pressure transient following an osmotic change was diminished compared with control and typically <2 mmHg. This number represents an upper bound on the variation in hydrostatic pressure that could occur at the capillary level during an experiment.

**Source of fluid loss.** Gravimetric measurements cannot distinguish whether the fluid lost from an organ originates from the cellular or interstitial spaces. Regardless of its original origin, the model predicts that this fluid is always hypotonic compared with interstitial fluid because at least a portion of the flux is pure water coming through the transendothelial pathway. The smaller the molecular size of the osmotic agent, the more hypotonic is the volume flux because it is localized more exclusively to the transcellular pathway. This prediction is in agreement with the observations of Effros (16). Our model predicts that about two-thirds of the fluid loss during a small-solute transient in the heart originates in the parenchymal cells. Wagensteen et al. (52) used morphometric measurements in lungs to show that most of the water extracted during a shift in perfusate osmolarity originated from the intracapillary volumes. Morphometric or indicator dilution studies in heart to determine cellular and interstitial fluid volumes before and after an osmotic transient could provide additional validation of model predictions.

**Potential influence of Donnan forces on intracellular volumes.** Stein (45) gives the Post-Jolly equations for Donnan distribution across the cell membrane. The general concept is that if there is a cation that leaks passively into the cells it must be pumped out, otherwise the cell expands. The summarizing equation for cell volume is $V = (A_i/[Na]_e) \times (1 + k_{\text{leak}}/k_{\text{pump}})$, where $A_i$ is moles of intracellular protein, $[Na]_e$ is extracellular sodium concentration, and $k_{\text{leak}}$ and $k_{\text{pump}}$ are first-order rate constants (Eq. B7.1.6 in Ref. 45).

The consequence of this is that if $k_{\text{leak}}$, $k_{\text{pump}}$, and $[Na]_e$ do not change during an osmotic transient, the volume $V$ is not affected. This is to say that $V$ is controlled by the osmotic concentrations and not by Donnan effects. To examine this in more detail, one would see that a 30 mM sucrose step increase would cause cell shrinkage and increase intracellular Na content by ~10%, decreasing the leak flux by 10% and increasing the pump flux by 10%, creating a situation for reducing the intracellular Na content. But neither $k_{\text{leak}}$ nor $k_{\text{pump}}$ needs to change for this to happen. [Adaptive cell behavior is commonly found, however; for example, $k_{\text{leak}}$ changes (45)]. The time frame for adaptation by changes in leak and pump rates is not known for the heart. Lymphocytes adapt to a new steady state in ~20 min, although they never return to baseline (20). If cardiac cell adaptation is at the same rate, this would not affect the interpretation of the responses to small solute molecules but would influ-
ence our thinking about albumin-induced transients. More research is needed here.

**Errors in parameter estimates.** Much of the variation in parameter estimates in this study likely arose in the real variability of capillary permeability between hearts and within the same heart over the course of a series of experiments. The coefficients of variation (standard deviation of parameter estimates divided by the mean parameter estimates) for $L_{\text{p,endo}}$, $A_{\text{sp}}S$, and $r_{\text{sp}}$ were 33%, 32%, and 23%, respectively. In contrast, the confidence limits on the free parameter values determined by optimization were typically ~1% for fitting of our model to the individual experimental data sets. This is an underestimate of the true uncertainty in the parameter estimates because having only three free parameters in the optimization does not account for variation in other model parameters in estimating the uncertainty in the parameter estimates. The variation in parameter estimates varied by nearly as much over the course of a given heart experiment as they did in all 12 hearts. This was so even when the same osmotic agent was used at different times during the experiment. However, transients very close to each other in time produced quite similar parameter estimates. It is likely that the permeability of our preparations increased over the 3–4 h of each experiment because of the effects of isolation and prolonged exposure to Ringer solution. The overall error in the study is comparable to that achieved by other methods for measuring capillary permeability properties in whole organs. The osmotic transient method has the advantage of providing more complete information on transport across the capillary wall than either the multiple-indicator dilution or lymph sampling methods.

In conclusion, we have gained insight into the processes of water and solute across the capillary and cell membranes through extensive, carefully controlled experiments using the osmotic weight transient method in isolated, perfused hearts and quantitative analysis via a comprehensive and detailed model of the underlying physiology. By using this more precise model, we have extracted more information from the weight transient record than was previously possible. Our results suggest that the small-pore system is well represented by a population of pores with radius of 6.9 ± 1.7 nm and a fractional pore area $A_{\text{sp}}/S$ of 0.022 ± 0.007%. The size and density of the large-pore pathway cannot be determined by osmotic transient methods, but lymph sampling data suggest that it is likely ~24 nm in radius with an $A_{\text{sp}}/S$ of ~0.0001%. There is a significant pathway for solute-free water exchange in myocardial microvessels, accounting for about a quarter of the transcapillary hydraulic conductivity. This measurement is too large to result from the permeability of water through pure lipid bilayers, so aquaporin water channels presumably play a role as water traverses both luminal and abluminal endothelial plasmalemma and myocyte sarcolemma. The analysis of osmotic transient data we have presented is consistent with our application of the same model to indicator dilution and steady-state lymph sampling data in the companion paper (29). Now for the first time all the various types of observations are brought into compatibility by a properly comprehensive model.

**APPENDIX**

The model used is described in detail in the companion paper (29). Briefly, it consists of an axially distributed blood-tissue exchange region in which fluid and solutes exchange between vascular, interstitial, and parenchymal cell volumes. The following equations describe the coupled transcapillary exchange of fluid ($J_{\text{VC}}$) and solutes ($J_{k}$), lymphatic drainage of interstitial fluid ($F_{\text{L}}$), and water exchange across the parenchymal cell membrane ($J_{\text{V,pc}}$). These fluxes determine changes in the fluid volumes of interstitium ($V_{\text{f,isf}}$) and parenchymal cells ($V_{\text{pc}}$), perfuse velocities ($u$) in a constant-volume capillary, and solute quantities of $N_{s}$ different solutes in all three regions ($n_{c,j}$, $n_{isf,j}$, and $n_{pc,j}$).

\[
\frac{\partial u}{\partial x} = -SJ_{\text{VC}}/V_{c} \quad \text{(A1)}
\]

\[
\frac{\partial V_{\text{f,isf}}}{\partial t} = S_{c}J_{\text{VC}} + S_{pc}J_{\text{V,pc}} - F_{\text{L}} \quad \text{(A2)}
\]

\[
\frac{\partial n_{c,j}}{\partial t} = -\frac{\partial}{\partial x} (u \cdot n_{c,j}) - S_{c}J_{c,j} \quad \text{for } j = 1 \to N_{c} \quad \text{(A4)}
\]

\[
\frac{\partial n_{isf,j}}{\partial t} = S_{c}J_{c,j} - F_{c}C_{isf,j} \quad \text{for } j = 1 \to N_{c} \quad \text{(A5)}
\]

\[
\frac{\partial n_{pc,j}}{\partial t} = 0 \quad \text{for } j = 1 \to N_{c} \quad \text{(A6)}
\]

Three separate pathways exist for the coupled exchange of water and solute across the capillary: one pathway for solute-free water exchange and small-pore and large-pore pathways for coupled fluid and solute exchange. The fluid flux through each transcapillary pathway is given by

\[
J_{\text{VC}} = L_{\text{pc}}\left(p_{c} - p_{\text{ad}} \right) - \sum_{j} \left[ \sigma_{j,k} \Pi_{c,j} - \Pi_{\text{isf},j} \right] + \Pi_{\text{M}} \quad \text{(A7)}
\]

where $L_{\text{pc}}$ is the hydraulic conductivity through the $k$th pathway, $\sigma_{j,k}$ is the reflection coefficient of the $j$th solute in the $k$th pathway, $\Pi_{c,j}$, and $\Pi_{\text{M}}$ are the osmotic pressures of the $j$th solute in capillary and interstitium, respectively, and $\Pi_{\text{M}}$ is the osmotic pressure exerted by interstitial matrix proteins. Total $J_{c}$ is the sum of the $J_{c}$ values for the individual pathways, and the rate of weight change is $J_{\text{vc}} = \sum J_{c}$ for $k = 1 \to 3$. dW(t)/dt = $J_{\text{vc}}$.

Similarly, the water flux across the parenchymal cell membrane from cells to interstitium is given by

\[
J_{\text{V,pc}} = L_{\text{p,pc}}\left( p_{pc} - p_{\text{ad}} \right) - \left( \Pi_{pc} - \Pi_{\text{M}} - \sum_{j} \Pi_{\text{isf},j} \right) \quad \text{(A8)}
\]

The total solute flux of solute $j$ from capillary to interstitium, $J_{\text{isf},j}$, is given by the sum of diffusive and convective transport for each path

\[
J_{\text{isf},j} = \sum_{k} \left[ J_{\text{VC}}(1 - \sigma_{j,k})C_{c,j} + P_{j,k}(C_{c,j} - C_{\text{isf},j}) \left( \frac{P_{e,j,k}}{P_{e,j,k} - 1} \right) \right] \quad \text{(A9)}
\]

$P_{e,j,k}$ is the Péclet number for the $j$th solute traveling through the $k$th pathway, defined by

\[
\frac{d}{dx} \left( u \cdot n_{c,j} \right) \quad \text{(A4)}
\]

\[
\frac{dV_{f,isf}}{dt} = S_{c}J_{c,j} - F_{c}C_{isf,j} \quad \text{for } j = 1 \to N_{c} \quad \text{(A5)}
\]

\[
\frac{dn_{pc,j}}{dt} = 0 \quad \text{for } j = 1 \to N_{c} \quad \text{(A6)}
\]
Osmotic Weight Transient Analysis

\[ P_{e,j,k} = \frac{J_{\alpha}(1 - \sigma_{j,k})}{P_{\alpha}} \]  

(A10)

The pore equations of Curry (12) were used to determine \( L_p, P \), and \( \sigma \) through the large- and small-pore pathways

\[ L_p = \left( \frac{A_p}{S,\Delta r} \right) \frac{r^2_p}{8\eta} \]  

(A11)

\[ P = \left( \frac{A_p}{S,\Delta r} \right) (1 - \alpha)F(\alpha)D \]  

(A12)

where \( \alpha \) is the ratio of solute radius to pore radius, \( r_p \) is the equivalent pore radius in nanometers; the area of the \( k \)th pathway, \( A_p = N_p \cdot \Pi r^2_p \) = pore surface area; \( \Delta r \) is the length of the pore from capillary lumen to ISF; \( D \) is the free diffusion coefficient of the solute in the pore; \( F(\alpha) \) is a factor (0 < \( F < 1 \)) describing hindrance to diffusion, given by Curry’s Eq. 5.17 (13) as taken from Faxen’s 1959 paper

\[ F(\alpha) = 1 - 2.10444\alpha + 2.08877\alpha^2 - 0.94813\alpha^3 \]

\[ - 1.372\alpha^6 + 3.87\alpha^8 - 4.19\alpha^9 \]  

(A14)

and \( G(\alpha) \) accounts for the difference in solute and water velocities; 0.5 < \( G < 1 \), by Curry’s Eq. 5.51 (14)

\[ G(\alpha) = \frac{1 - 2\alpha^2/3 - 0.20217\alpha^5}{1 - 0.75851\alpha^5} \]  

(A15)

which is accurate for \( \alpha < 0.6 \) and overestimates \( G \) a little for higher \( \alpha \) values (lower \( G \) values). A pore radius is not defined for the water-only pathway across the endothelial cell; \( L_{p,endo} \) is estimated directly from the weight transients and, neglecting the change that occurs in the small volume of the endothelial cells, represents conductance across the luminal and abluminal surfaces in series.

The apparent osmotic reflection coefficient for the membrane, \( \sigma_{m} \), can be calculated from \( \sigma \) for each of the individual pathways from

\[ \sigma_{d} = \sum \frac{L_{p,d}}{L_{p,total}} \]  

(A16)

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DISCLOSURES

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