Effect of intraperitoneal pressures on tissue water of the abdominal muscle

EL RASHEID ZAKARIA, J OANNE LOFTHOUSE, AND MICHAEL F. FLESSNER
Nephrology Unit, Department of Medicine, University of Rochester Medical Center, Rochester, New York 14642

Zakaria, El Rasheid, Joanne Lofthouse, and Michael F. Flessner. Effect of intraperitoneal pressures on tissue water of the abdominal muscle. Am J Physiol Renal Physiol 278: F875–F885, 2000.—A major factor that affects solute and water transport through tissue is the state of tissue hydration. The amount of interstitial water directly affects the transport coefficients for both diffusion and convection. To investigate the effect of simultaneous exposure of tissue to hydrostatic and osmotic pressures on the state of tissue hydration and the pattern of distribution of tissue water, we investigated the effect of simultaneous exposure of tissue to hydrostatic and osmotic pressures on the state of tissue hydration. The amount of interstitial water directly affects and water transport through tissue is the state of tissue hydration. The amount of interstitial water directly affects and to 1.5 \( \text{u} \) (510 mosmol/kg) solution at intraperitoneal pressures \( \text{P}_{\text{ip}} \) between 0 and 6 mmHg, and we infused isotopic markers intravenously and determined their equilibrium distribution volumes \( V_D \) in the anterior abdominal muscle (AAM) by quantitative autoradiography. Total tissue water volume \( (V_{\text{TW}}) \) was determined from dry-to-wet weight ratios. \( V_T \) of \( ^{14}\text{C} \) urea, equals the sum of the extracellular water volume \( (V_{\text{EC}}) \) and intracellular water volume \( (V_{\text{IC}}) \). If \( V_T \) = interstitial water volume and \( V_V \) = vascular water volume \( (V_D \text{of } ^{13}\text{I}-\text{labeled IgG}) \), then \( \theta_{V_{\text{EC}}} = \theta_{V_{\text{IC}}} + \theta_{V_{\text{V}}} \). AAM hydrostatic pressure profiles were measured by a micropipette/servo-null system and demonstrated that elevation of \( \text{P}_{\text{ip}} \) above 3 mmHg significantly \( (P < 0.05) \) increases mean tissue pressure \( (P_T) \) to the same level regardless of intraperitoneal osmolality. The increase in \( P_T \) resulted in a nonlinear tissue expansion primarily in the interstitium regardless of osmolality. From 0 to 6 mmHg, \( \theta_{V_{\text{IC}}} \) (in ml/g dry tissue) increased from 0.59 ± 0.02 to 1.7 ± 0.05 and to 1.5 ± 0.05 after isotonic and hypertonic dialysis, respectively, whereas \( \theta_{V_{\text{IC}}} \) increased from 2.8 ± 0.08 to 3.0 ± 0.1 after isotonic dialysis and decreased to 2.6 ± 0.1 after hypertonic dialysis. After dialysis at 6 mmHg with isotonic or hypertonic solutions, \( \theta_{V_{\text{IC}}} \) increased from 0.034 ± 0.001 to 0.049 ± 0.001 and 0.042 ± 0.002, respectively. \( \theta_{V_{\text{IC}}} \) during hypertonic dialysis at \( \text{P}_{\text{ip}} \) between 0 and 6 mmHg increased in a nonlinear fashion \( (F = 26.3, P < 0.001) \), whereas \( \theta_{V_{\text{IC}}} \) invariably decreased \( (F = 11.1, P < 0.001) \) and \( \theta_{V_{\text{IC}}} \) doubled from its control value at low \( \text{P}_{\text{ip}} \). In conclusion, elevation of intraperitoneal hydrostatic pressure causes tissue expansion, primarily in interstitium, irrespective of osmolality of the bathing solution. Tissue hydrostatic pressure is therefore the primary determinant of tissue properties with respect to hydration, which in turn affects diffusive and convective transport.

The simultaneous stresses of hydrostatic and osmotic pressures on tissue occur in peritoneal dialysis, a technique that relies on solute and water exchange between a hypertonic solution in the peritoneal cavity and the blood circulating in the surrounding tissues. Typically 2–3 liters of a hyperosmolal dialysis solution containing glucose as an osmotic agent are repeatedly instilled into and drained from the peritoneal cavity to remove excess solutes and body water. Because of the relatively large fill volume, the intraperitoneal hydrostatic pressure \( (P_T) \) in peritoneal dialysis patients increases to between 2 and 10 mmHg \((10, 18)\), whereas the initial osmolality of the fluid depends on the concentration of dextrose in the solution and varies between 330 and 510 mosmol/kg. Despite the widespread use of peritoneal dialysis, the details of the mechanisms underlying the complex process of fluid and mass transport between the blood and dialysate are still to be defined.

The general equation (Darcy's Law, see Ref. 12) that governs fluid flow through tissue is

\[
Q = -KA \frac{dP_T}{dx}
\]

where \( Q \) equals the flow through an area of tissue A, \( K \) equals tissue hydraulic conductivity, and \( dP_T/dx \) is the hydrostatic pressure gradient in the tissue. We have previously demonstrated that when intraperitoneal pressure \( (\text{P}_{\text{ip}}) \) is raised with instillation of fluid into the cavity, \( P_T \) in the anterior abdominal wall muscle increases, and that the \( P_T \) profiles were nearly identical with either a hypertonic solution (460 mosmol/kg) or an isotonic solution (290 mosmol/kg) in the cavity \((3)\). In separate studies \((7–9)\), we demonstrated that the flow rate of a solution containing a protein marker from the cavity into the abdominal wall was time dependent and proportional to \( \text{P}_{\text{ip}} \), and that the magnitudes of the flow for either isotonic or hypertonic solutions were the same at a given hydrostatic pressure in the peritoneal cavity. An intriguing but complicating feature of the hypertonic case is that the net water movement is from the tissue toward the cavity, the direction that is opposite to the hydrostatic pressure-driven flow from the cavity \((9)\).

In experiments with isotonic solutions, we have shown that the dependency of the flow on \( \text{P}_{\text{ip}} \) is due to both a marked increase in the interstitial hydraulic conductivity \( (K) \) and a moderate increase in the interstitial hydrostatic pressure gradient \( (dP_T/dx) \), when \( \text{P}_{\text{ip}} \)

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
HYDROSTATIC AND OSMOTIC PRESSURE EFFECTS ON TISSUE

exceeds a threshold pressure of 1.5 mmHg (23). Theoretically, \( K \) is a complex function of the tissue structure and the interstitial water volume, \( \theta_{\text{II}} \) (12). In a subsequent study (24), we reported that the increase in \( K \) with \( P_{\text{F}} \) is due to the combined effects of expansion of the interstitium, dilution of the interstitial macromolecules from the influx of water, and a washout of mobile interstitial hyaluronan in the direction of flow. These prior results have indicated that the dynamics of the volume change in whole tissue and interstitium are important determinants of tissue hydration and the resistance to hydraulic fluid flow across tissue. Our previous study (24) of the expansion of the subperitoneal tissue space was carried out with isotonic solution. Although clinical dialysis routinely employs hypertonic solution, the effect of combined hydrostatic and osmotic pressure of the peritoneal solution on the surrounding tissue space is unknown. Our hypothesis is that the expansion of the interstitium as well as the total tissue water space will be altered by the hyperosmotic solution.

To test this hypothesis, we determined the in vivo effect of hydrostatic and osmotic pressures on the tissue water of the anterior abdominal muscle. The experiments were designed to measure the components of tissue (ml/g of dry or wet weight tissue) making up the total tissue water. These are as follows: the extracellular fluid volume \( \theta_{\text{EC}} \), the vascular volume \( \theta_{\text{V}} \), the intracellular volume \( \theta_{\text{IC}} \), and the interstitial volume \( \theta_{\text{II}} = \theta_{\text{EC}} - \theta_{\text{V}} \). Our model tissue is the rat anterior abdominal muscle (AAM), when it is exposed to isotonic or hypertonic solution in the peritoneal cavity, which is maintained at hydrostatic pressures between 0 and 6 mmHg.

METHODS

Animals

All experiments were performed in 250–350 g female Sprague-Dawley rats (Charles River Laboratories). Animals had free access to water and standard rat chow until the morning of the experiment. At least three animals were used for each pressure level investigated. All procedures were approved by the University of Rochester Committee on Animal Resources.

Materials

Tracers. Immunoglobulin G (anti-rabbit IgG, no. IM-134; Amersham Life Science, Arlington, IL) was used as a marker of the local vascular volume \( \theta_{\text{V}} \). Prior to the experiment, the isotope was checked for degradation and for free \(^{125}\text{I}\) by precipitation with trichloroacetic acid (TCA). If free \(^{125}\text{I}\) was greater than 1%, then the solution was purified further by mixing it with saline and concentrating the mixture with a Centricon 30 microconcentrator (Millipore, Bedford, MA) by centrifugation (IEC Centra CL2). Dilution and concentration were repeated until the free \(^{125}\text{I}\) was <1% by TCA precipitation. \(^{14}\text{C}\) Mannitol was purchased from Moravek Biochemicals (Brea, CA). The volume of distribution and half-life of the product in the rat have previously been determined to be 0.174 \( \pm \) 0.006 l/kg and 13 min, respectively (4). With these values, an infusion rate of labeled mannitol was chosen to maintain a constant plasma concentration during the course of the experiment.

\(^{3}\text{H}_{2}\text{O}\) and \(^{14}\text{C}\) Jurea were purchased from Moravek Biochemicals and used as supplied.

Dialysis solutions. The dialysis solutions employed in the present experiments were made from an isotonic Krebs-Ringer bicarbonate solution (containing, in mol/l, 0.12 NaCl, 0.01 KCl, 0.002 calcium chloride disolvate, 0.025 NaHCO\(_3\), 0.0028 KH\(_2\)PO\(_4\), and 1.18 ml of 1 M MgSO\(_4\)·7H\(_2\)O). All solutions were filtered with a 0.45-µm pore size membrane (Nalgene) and stored at 4°C. The hypertonic solution was made by adding mannitol to the base solution to a concentration of 4%; the initial osmolality of the hypertonic solution was 510 \( \pm \) 5 mosmol/kg. Bovine serum albumin (5%) was added to all solutions before each experiment.

Surgery

Anesthesia was induced by an intramuscular injection of pentobarbital sodium (60 mg/kg) to the hind leg and maintained with subsequent intravenous injections. Surgery was initiated on loss of the blink reflex. A tracheostomy was performed to reduce airway resistance. Two arterial lines were established using PE-50 catheters. The left carotid artery was cannulated to allow for continuous blood pressure measurements on a pressure measurement system (model PE-10z Statham pressure transducer; Window Graf, Gould Valley Instruments, OH), and a tail artery catheter was used for blood sampling. A venous catheter was secured into the left external jugular vein for continuous infusion of \(^{14}\text{C}\) mannitol from an infusion pump (model 22; Harvard Apparatus, Holliston, MA). The animal's rectal temperature was continuously monitored and maintained between 35.5 and 38.5 with a servo-controlled warming blanket (Harvard Apparatus) and an overhead heating lamp. The peritoneal cavity was exposed through a midline abdominal incision \( \sim \) 1.5 cm, and the hollow viscera (duodenum to rectum) were removed using the technique as described in our previous publication (26). The slitlike abdominal incision was closed using a continuous suture after careful inspection to ensure there was no bleeding. This maneuver was necessary to ensure that fluids in the cavity have access to the entire abdominal wall. With the aid of a trocar, a multihole catheter was placed through the abdominal wall into the peritoneal cavity and secured with a purse stitch. A three-way valve was connected to the multihole catheter to administer and sample the dialysate and to continually monitor the free 125I. A glass capillary manometer. A urethral catheter was inserted for collection of urine during the experiment.

Measurements

Radioactive tracer detection. \(^{14}\text{C}\) - and \(^{3}\text{H}\)-labeled tracers were quantified with a scintillation counter (model LS6000IC; Beckman, Fullerton, CA). \(^{125}\text{I}\) labeled tracers were quantified with a counter (Gamma 4000; Beckman, Irvine, CA).

Interstitial hydrostatic pressure. The hydrostatic pressure profile within the AAM was measured by the technique of Wilg and colleagues (22) as later modified by Flessner (3) to allow for in vivo measurements of the interstitial pressure profile in the rat anterior abdominal wall. Details of the procedure are found in our previous publication (3).

Quantitative autoradiography. Quantitative autoradiography (QAR) was used to determine the local concentration of each tracer in the tissue at the time of animal death. The general assumption for the determination of a particular space within the tissue was that the tracer was equilibrated between the plasma and the volume of distribution of the tracer within the tissue. The volume of tissue, \( \theta_{\text{EC}} \), equals the ratio of the tissue concentration to the plasma concentration,
Briefly, tissue tracer concentrations were determined from the thin tissue sections from the frozen carcass at the end of each experiment (see Dialysis procedure in Experimental Protocols, below). The sections were placed with standards (tissues with known isotopic concentration) against X-ray film (Biomax MR; Kodak, Rochester, NY) to produce autoradiograms. After developing the films, the tissue slides were stained with hematoxylin and eosin. Each slide was examined by light microscopy to determine the mesothelial layer and the skin side. This procedure is important in tissue samples where tracer concentration profiles are to be determined. The films were analyzed with a computerized densitometer (model MCID; Imaging Research, St. Catherines, Ontario, Canada), which measures optical density (OD) vs. position in the tissue. The isotopic standards are used to construct a calibration curve (concentration vs. OD) to convert the unknown OD values from the tissue samples to concentration. By superimposing the tissue histology over the autoradiogram, the location of the reading was carefully determined, and a concentration vs. position curve was obtained (concentration profile data) or mean concentration in a large area of the tissue. These concentrations divided by the plasma concentration provided an estimate of the volume that was marked by the specific tracer. The term “local” refers to a particular location within the tissue or that has been obtained from QAR. The use of this macro-QAR technique is detailed in our previous publications (5, 24).

Extracellular volume. The extracellular volume, \( V_{EC} \), is defined as a unit of volume within the AAM tissue that is not occupied by cells or solid material. \( V_{EC} \) was determined by an intravenous infusion of \([14C]\)mannitol at a rate to compensate for renal excretion and to maintain a constant concentration of the labeled mannitol. The tracer equilibrates with the extracellular space, and its tissue concentration divided by the plasma concentration provides the estimate of \( V_{EC} \). After surgical preparation, 5 \( \mu \)Ci of \([14C]\)mannitol was given as an intravenous injection followed by a continuous infusion at a rate of 0.25–0.5 \( \mu \)Ci/min for 90 min. Thirty minutes after commencing the infusion, the dialysis fluid was injected into the peritoneal cavity in an amount sufficient to reach the desired \( P_{IP} \). Attempts were made to obtain at least one pressure profile measurement between the tissue and the cavity. In addition, the hypertonic solution was connected to the three-way valve attached to the intraperitoneal catheter. The reservoir was maintained at the exact level above the right atrium to produce the desired \( P_{IP} \), which was determined every 15 min with a water manometer. Attempts were made to obtain at least one pressure profile measurement in the AAM during the 1-h dwell time. At the end of the experiment the following steps were taken in rapid succession as follows: the animal was euthanized by an anesthetic overdose followed by decapitation. The fluid was drained from the cavity, and the animal was rapidly frozen using chlorodifluoromethane (Dust-off; Falcon Safety Products, Branchburg, N.J.) precooled to −75°C. The abdominal wall was carefully cut from the carcass with an autopsy saw. Four small portions of muscle (50–100 mg each) were taken from the abdominal wall. Each piece was thawed, gently blotted to remove any residual fluid, and placed in a previously weighed vial to determine its weight. The tissue was then solubilized and counted for either \([125I]\)-IgG or \([14C]\)mannitol. From the abdominal wall, thin sections (20 \( \mu \)m) were taken horizontally with a cryomicrotome (model OTF; Bright-Hacker, Fairfield, N.J.) and dried on a slide warmer. Sections were used for single-label \([125I] \) or \([14C] \) QAR and for histology after staining with hematoxylin and eosin.

The initial QAR profiles, which were produced during hypertonic dialysis with the steady intravenous infusion of \([14C]\)mannitol, appeared to be sloping toward the cavity. In the above protocol, there was no \([14C]\)mannitol in the peritoneal cavity, which set up a diffusive gradient between the tissue and the cavity. In addition, the hypertonic solution caused convection of fluid and solute from the tissue into the cavity. After mathematical analysis, it was surmised that the combined convective and diffusive fluxes from the tissue to the cavity likely distorted the shape of the mannitol concentration curve. Therefore, the equilibrium distribution volumes \( V_D \) of \([14C]\)mannitol in the AAM at various \( P_{IP} \) were determined after loading the tissue with the tracer from the plasma as well as from the peritoneal side. This design attempt to eliminate any diffusion gradient between the tissue and the cavity. However, the loading of the tissue from the peritoneal side has the potential to raise the concentration in the tissue and could result in an overestimation of the equilibrium \( V_D \) of \([14C]\)mannitol or \([14C]\)urea, especially at the peritoneal edge. Two \( P_{IP} \) pressure levels were tested in this experiment.

**Extracellular Volume.**

The extracellular volume, \( V_{EC} \), is defined as a unit of volume within the AAM tissue that is not occupied by cells or solid material. \( V_{EC} \) was determined by an intravenous infusion of \([14C]\)mannitol at a rate to compensate for renal excretion and to maintain a constant concentration of the labeled mannitol. The tracer equilibrates with the extracellular space, and its tissue concentration divided by the plasma concentration provides the estimate of \( V_{EC} \). After surgical preparation, 5 \( \mu \)Ci of \([14C]\)mannitol was given as an intravenous injection followed by a continuous infusion at a rate of 0.25–0.5 \( \mu \)Ci/min for 90 min. Thirty minutes after commencing the infusion, the dialysis fluid was injected into the peritoneal cavity in an amount sufficient to reach the desired \( P_{IP} \). Attempts were made to obtain at least one pressure profile measurement between the tissue and the cavity. In addition, the hypertonic solution was connected to the three-way valve attached to the intraperitoneal catheter. The reservoir was maintained at the exact level above the right atrium to produce the desired \( P_{IP} \), which was determined every 15 min with a water manometer. Attempts were made to obtain at least one pressure profile measurement in the AAM during the 1-h dwell time. At the end of the experiment the following steps were taken in rapid succession as follows: the animal was euthanized by an anesthetic overdose followed by decapitation. The fluid was drained from the cavity, and the animal was rapidly frozen using chlorodifluoromethane (Dust-off; Falcon Safety Products, Branchburg, N.J.) precooled to −75°C. The abdominal wall was carefully cut from the carcass with an autopsy saw. Four small portions of muscle (50–100 mg each) were taken from the abdominal wall. Each piece was thawed, gently blotted to remove any residual fluid, and placed in a previously weighed vial to determine its weight. The tissue was then solubilized and counted for either \([125I]\)-IgG or \([14C]\)mannitol. From the abdominal wall, thin sections (20 \( \mu \)m) were taken horizontally with a cryomicrotome (model OTF; Bright-Hacker, Fairfield, N.J.) and dried on a slide warmer. Sections were used for single-label \([125I] \) or \([14C] \) QAR and for histology after staining with hematoxylin and eosin.

The initial QAR profiles, which were produced during hypertonic dialysis with the steady intravenous infusion of \([14C]\)mannitol, appeared to be sloping toward the cavity. In the above protocol, there was no \([14C]\)mannitol in the peritoneal cavity, which set up a diffusive gradient between the tissue and the cavity. In addition, the hypertonic solution caused convection of fluid and solute from the tissue into the cavity. After mathematical analysis, it was surmised that the combined convective and diffusive fluxes from the tissue to the cavity likely distorted the shape of the mannitol concentration curve. Therefore, the equilibrium distribution volumes \( V_D \) of \([14C]\)mannitol in the AAM at various \( P_{IP} \) were determined after loading the tissue with the tracer from the plasma as well as from the peritoneal side. This design attempt to eliminate any diffusion gradient between the tissue and the cavity. However, the loading of the tissue from the peritoneal side has the potential to raise the concentration in the tissue and could result in an overestimation of the equilibrium \( V_D \) of \([14C]\)mannitol or \([14C]\)urea, especially at the peritoneal edge. Two \( P_{IP} \) pressure levels were tested in this experiment.
series: 3 mmHg or 6 mmHg (n = 3 each). After surgical preparation (see Surgery, above), the renal pedicles were bilaterally ligated. Twenty-five microucroies of [14C]mannitol were injected via a short venous catheter and allowed to equilibrate for 30 min. At 30 min, the dialysis solution, prepared as 150 ml of 4% mannitol in 5% bovine serum albumin in Krebs ringer and containing 40 µCi of [14C]mannitol, was injected into the peritoneal cavity in an amount sufficient to raise \( P_{ip} \) to either 3 or 6 mmHg above heart level. The rest of the fluid was used to fill the reservoir, which is maintained at the desired pressure level. Peritoneal fluid and blood were sampled at 15-min intervals, for tracer concentration and osmolality. The animal was killed and rapidly frozen after a 60-min dwell time. Samples of AAM were collected for QAR (see Quantitative autoradiography, above) and for dry-to-wet weight ratios. Comparison of the tissue-averaged concentrations (over the initial 1,000 µm of tissue adjacent to the cavity) from these bidirectional mannitol experiments with those of the experiments in which tracers mannitol had been infused intravenously demonstrated no significant differences. However, because of the distorted slope of the unidirectional studies, only the bidirectional [14C]mannitol concentration profiles are presented in the RESULTS.

Assessment of the equilibrium \( V_{o} \), the rate of [14C]mannitol or [14C]urea in AAM as measurements of \( t_{1/2}^{EC} \) or \( t_{1/2}^{U} \), respectively, is based on the assumption that the tissue tracer concentration is equal to that in the plasma at the time of tissue sampling. Implicit within this assumption is that 60 min of tracer equilibration time is sufficient to reach a steady state of diffusion equilibrium in the AAM. In hypertonic dialysis, the osmolality of the dialysis fluid decreases with time, due to dilution from the osmotic flux into the cavity and to loss of the osmotic agent by diffusion into the tissue.

Bidirectional [14C]mannitol transport studies. To test the effects of dwell time and the decrease in osmolality on the estimation of \( V_{o} \) of [14C]mannitol in the AAM, we designed a series of experiments in which the dwell time was set at 120 min with the dialysis fluid exchanged at 60 min, to keep osmolality within 90% of its initial value. A total of three rats (280 ± 21 g) were used in this series. The animals were surgically prepared (see Surgery, above) and were rendered anephric by bilateral ligation of the renal pedicle; this eliminated the need to continuously infuse the tracer to make up for renal clearance. Fifteen microucroies of [14C]mannitol were given as an intravenous bolus injection. For each animal, the dialysis solution was prepared as 150 ml of 4% mannitol, 5% bovine serum albumin in Krebs ringer solution containing 40 µCi of [14C]mannitol. Initial osmolality of the solution was (511 ± 1.2 mosm/kg). The dialysis solution was instilled into the peritoneal cavity in an amount sufficient to raise \( P_{ip} \) to ~3 mmHg. Approximately 20 ml of the dialysis solution was added to the reservoir connected to the peritoneal cavity. The rest of the fluid was kept in a water bath at 37°C to be used for the second exchange. At 60 min, the peritoneal fluid was aspirated in a 60-ml syringe within 1 min. The rest of the dialysis solution from the water bath was injected into the peritoneal cavity to raise \( P_{ip} \) to ~3 mmHg. The reservoir was reconnected. The fresh solution was allowed to dwell for another 60 min. Blood and peritoneal fluid were sampled 15 min after initiation of the experiment and every 30 min thereafter for a total dwell of 120 min. At 120 min, final blood and peritoneal fluid samples were obtained; the animal was then euthanized, and the abdominal muscle was harvested (see Dialysis procedure, above) and prepared for QAR. If \( t_{1/2}^{EC} \) obtained from these 120-min dwell studies equaled \( t_{1/2}^{EC} \) obtained from 60-min dwell times, then our assumption that 60 min equilibration for a small-molecular-weight tracer to reach a steady state of diffusion equilibrium in the AAM will be justified.

Bidirectional [14C]urea transport studies. The purpose of these studies was the determination of \( V_{o} \), a surrogate for the volume of tissue that was made up of water. Since the technique of QAR requires dehydration of the frozen tissue slice prior to placement against the X-ray film, [3H]O cannot be used as a marker for tissue water content. To ensure that the tracer urea is equivalent to tritiated water in terms of volume of distribution, six animals were surgically prepared (see Surgery, above). A 5% bovine serum albumin in Krebs-Ringer solution was instilled into the peritoneal cavity to produce \( P_{ip} \) of 1.5 mmHg, and 150 µCi of either [3H]O or [14C]urea was given as an intravenous bolus injection. The total bolus dose was carefully determined from the initial concentration and the weight of the syringe before and after the injection. Blood and peritoneal fluid were sampled every 15 min for a total equilibration time of 180 min. Final peritoneal, blood sample, and the total urine volume were collected, and their specific activities were determined in a beta counter. The volume of distribution was calculated as the given dose of [3H]O or [14C]urea divided by the tracer concentration at time 0, which was found by extrapolating the plasma concentration vs. time curve to \( t = 0 \) on a log-log plot. The plasma curve was fitted to \( C(t)/C_{0} = e^{-kt} \), where \( C(t) \) is the plasma tracer concentration at time \( t \), \( C_{0} \) is the tracer concentration at time 0, and \( k \) is the decay constant. In these control experiments we determined \( V_{o} \) of both [3H]O and [14C]urea in rats (227 ± 5 g rat, mean ± SE, \( n = 3 \)) and [14C]urea in rats (527 ± 5 g rat, mean ± SE, \( n = 3 \)), as well as the decay constants. The \( V_{o} \) was determined to total body water (TBW) by [3H]O averaged 658 ± 14 ml/kg rat (\( n = 3 \)), which is comparable to 626 ± 8 ml/kg rat as obtained using [14C]urea as a marker, whereas the half-lives (\( T_{1/2} \)) were 545 ± 21 min and 317 ± 47 min, respectively. Since \( k = 0.693/T_{1/2} \), the corresponding values for \( k \) were calculated to be 1.27 × 10^{-3} min^{-1} and 2.19 × 10^{-3} min^{-1}. From these parameters, the bolus injection and infusion rate were calculated to attain a concentration of labeled urea in the plasma equal to that in the peritoneal cavity.

The design and the experimental procedures are identical to those in Dialysis procedure (above). The experiments were performed in six groups of animals of 1.5, 3, 4.4, and 6 mmHg. For each pressure level, three animals were used. Twenty-five microucroies of [14C]urea was given as an intravenous bolus injection via a short catheter. The given dose was carefully determined from the weight of the syringe before and after injection and from the total counts recovered from the syringe and needle. For each animal, 200 ml of hypertonic dialysis solution (4% mannitol in 5% BSA in Krebs-Ringer) containing 25 µCi of [14C]urea was prepared. The dialysis solution was injected into the peritoneal cavity in an amount sufficient to raise \( P_{ip} \) to the desired level. This radioactivity in the cavity would oppose any tracer diffusion into the cavity from the tissue. Approximately 20 ml of the solution was added to the reservoir, which was connected to the peritoneal cavity. The rest of the fluid was kept in a water bath at 37°C. Peritoneal fluid was allowed to dwell for 60 min. At 60 min, the peritoneal fluid was rapidly aspirated in a 60-ml syringe as completely as possible. Because the cavity was closed, no attempt was made to completely recover the fluid from the peritoneal cavity, and therefore some residual fluid remained. A fresh solution from the water bath was injected into the peritoneal cavity to raise \( P_{ip} \) to the desired pressure. The fresh solution was allowed to dwell for another 60 min. Blood and peritoneal fluid were sampled at 15 min, then every 30 min for a total of 120 min equilibration and dwell time. At the end of the experiment, final peritoneal fluid and blood samples were aspirated in a 60-ml syringe within 1 min.
were obtained for radioactivity and osmolality measurements, and the animal was euthanized. Tissue samples from the abdominal wall were harvested and processed for single-label ([14C]urea) QAR and for dry-to-wet weight determination as noted in Dialysis procedure.

Statistics

All data are presented as means ± SE unless stated otherwise. One-way ANOVA was used to analyze the effect of a single factor (i.e., P_{ip}) on measured θ_{TW}, θ_{EC}, θ_{IC}, and θ_{IV}. Two-way ANOVA was used to check for possible variation in θ_{TW}, θ_{EC}, θ_{IC}, and θ_{IV} resulting from dialysis fluid osmolality and P_{ip}. A Bonferroni t-test was also used to assess differences in means between the groups. All calculations were performed with PRIZM V 1.03 (San Diego, CA). A statistic was considered to be significant if the probability of a type 1 error was P < 0.05.

RESULTS

Hydrostatic Pressure Profiles

Figure 1 displays the measured hydrostatic pressure profiles (means ± SE) for P_{ip} elevations during hypertonic dialysis or isotonic dialysis (isotonic data from Ref. 24 was plotted for comparison). The hypertonic profiles were similar to our previous measurements at the corresponding P_{ip} using isotonic dialysis solution in the peritoneal cavity (3, 24). A one-way ANOVA demonstrated that elevation of P_{ip} > 3 mmHg significantly (F = 4.15, P < 0.05) increased the mean tissue pressure as calculated from the distance-averaged hydrostatic pressure (P_{T} within the first 600 µm from peritoneal edge) regardless of the osmolality of the dialysis solution. A multiple comparison Bonferroni t-test at each P_{ip} demonstrated that the numerical difference between the calculated P_{T} for the two solutions was not statistically significant (all Bonferroni P > 0.05). The mean P_{T} values are listed in Table 1.

Pressure-Volume Curves

The change in the interstitial fluid volume (θ_{IV}) in the AAM as a function of dialysis fluid osmolality and mean interstitial pressure P_{T} is shown in Fig. 2. We have plotted both the results from hypertonic dialysis in this study with those obtained from our previous study with isotonic dialysis solution (from Ref. 24). Measurements in tissue were obtained within the first 1,000 µm from the peritoneal edge. As seen in Fig. 2, regardless of the dialysis fluid osmolality, the shape of the pressure volume curve did not change, i.e., the response of θ_{IV} to changes in the interstitial pressure is nonlinear with a nearly linear rise in θ_{IV} between P_{T} of 0.7 and 3 mmHg and then a decrease in the slope of the curve above 3–4 mmHg. In both curves, θ_{IV} doubles from its control value between P_{T} of 0.7 and 4 mmHg, irrespective of dialysis fluid osmolality. The vascular volumes (θ_{IV}, not shown in Fig. 2) in the AAM assessed at P_{ip} elevations to 1.5, 3, and 6 mmHg were constant at 0.01 ± 0.003 ml/g wet tissue, regardless of the P_{ip} or the osmolality of the dialysis solution.

Extracellular Volume Profiles

Figure 3 displays the results designed to measure θ_{EC} profiles ([14C]mannitol concentration normalized to the plasma concentration) in the AAM as a function of distance from peritoneal edge. All profiles were determined after tissue loading with the tracer from the blood as well as from the peritoneal side during hypertonic dialysis. Dwells were performed at P_{ip} = 3 mmHg for either 60 or 120 min as indicated. There was no significant difference between the two 3-mmHg curves. The calculated θ_{EC} from the distance-averaged tissue tracer concentration was 0.32 ± 0.003 and 0.33 ± 0.004 ml/g tissue after dialysis at P_{ip} = 3 mmHg and dwell times of 60 min and 120 min, respectively. Results from 60-min dwell experiments at P_{ip} = 6 mmHg are also shown to demonstrate the effect of higher pressure on θ_{EC}. The higher pressure causes the profile to be displaced upwards with an overall distance-averaged tracer concentration of 0.39 ± 0.005 ml/g tissue. These results suggest that tissue [14C]mannitol concentration profiles are not affected by increasing the equilibration time. However, with raising P_{ip} to 6 mmHg, the concentration profile within the tissue increases its value at every position in the tissue. The decrease in θ_{EC} near the peritoneal surface is likely due to the mismatch in concentration within the peritoneal cavity and the plasma which results from the marked water flow which is free of solute (and therefore tracer) into the cavity during the early period of hypertonic dialysis.

Total Tissue Water and Its Distribution During Hypertonic Dialysis

Figure 4 displays the pattern of distribution of total tissue water (θ_{TW}) in the AAM after 2 h of either isotonic or hypertonic dialysis at P_{ip} of 6 mmHg, compared with data from AAM not exposed to dialysis (control, P_{ip} = 0 mmHg). θ_{TW} was determined from dry and wet weights,
and all data are expressed as means ± SE in milliliters per gram dry tissue weight. \( \theta_{TW} \) in control rats was 3.45 ± 0.09 but significantly increased (F = 30.63, P < 0.001) after dialysis at \( P_{Pip} = 6 \) mmHg, regardless of dialysis solution osmolality, to 4.71 ± 0.14 for isotonic dialysis and to 4.22 ± 0.15 for hypertonic dialysis. This tissue expansion was primarily in the interstitium \( (\theta_{TI}) \), which increased its volume fraction from control condi-

Fig. 2. Interstitial fluid volume \( (\theta_{I}) \) in the abdominal wall after isotonic dialysis (open symbols, data replotted from Ref. 24), or hypertonic dialysis (solid symbols) as plotted vs. mean interstitial pressure \( (P_{I}) \). Regardless of the osmolality of the peritoneal fluid, \( \theta_{I} \) changes in a nonlinear fashion at graded increases in \( P_{Pip} \), which results in increases in \( P_{I} \). There was no significant difference in the distance-averaged \( P_{I} \) between the two solutions, as calculated from the first 1,000 µm from the peritoneal edge.

Fig. 3. Extracellular water volume \( (\theta_{EC}) \) profiles in the anterior abdominal muscle (AAM) as a function of distance \( x \) from peritoneal edge. All profiles were determined after tissue loading with the tracer \( ([14C] \text{mannitol}) \) from the blood as well as from the peritoneal side during hypertonic dialysis. Solid circles and open circles, respectively, are profiles after 60-min and 120-min dwell visits. Both dwellers were performed at \( P_{Pip} = 3 \) mmHg. There was no significant difference between the two 3-mmHg curves. Solid squares are profiles after 60-min dwell visits at \( P_{Pip} = 6 \) mmHg. Data are means ± SE. Higher pressure results in \( \theta_{EC} \) to be displaced slightly upward.

Table 1. Data compiled from hypertonic dialysis experiments performed at \( P_{Pip} \) between 1.5 and 6 mmHg

<table>
<thead>
<tr>
<th>( P_{Pip} )</th>
<th>0 mmHg</th>
<th>1.5 mmHg</th>
<th>3 mmHg</th>
<th>4.4 mmHg</th>
<th>6 mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBW, ml/kg rat ( (V_{B}^{\text{W}}) )</td>
<td>248 ± 20</td>
<td>280 ± 21</td>
<td>244 ± 12</td>
<td>234 ± 8</td>
<td></td>
</tr>
<tr>
<td>Urea ( T_{1/2} ), min</td>
<td>600 ± 9</td>
<td>563 ± 14</td>
<td>568 ± 56</td>
<td>504 ± 42</td>
<td></td>
</tr>
<tr>
<td>Initial osmolality, mosmol/kg</td>
<td>1.668 ± 155</td>
<td>1.062 ± 221</td>
<td>1.631 ± 397</td>
<td>1.378 ± 377</td>
<td></td>
</tr>
<tr>
<td>Mean ( P_{TW} ), mmHg</td>
<td>513 ± 0.7</td>
<td>505 ± 3</td>
<td>513 ± 2</td>
<td>508 ± 3</td>
<td></td>
</tr>
<tr>
<td>( \theta_{I} ), ml/g dry tissue</td>
<td>3.29 ± 0.05</td>
<td>3.59 ± 0.16</td>
<td>3.93 ± 0.22†</td>
<td>4.43 ± 0.33†</td>
<td>4.22 ± 0.15‡</td>
</tr>
<tr>
<td>( \theta_{I} ), ml/g wet tissue</td>
<td>0.74 ± 0.01</td>
<td>0.78 ± 0.01</td>
<td>0.83 ± 0.01*</td>
<td>0.86 ± 0.03*</td>
<td>0.85 ± 0.01*</td>
</tr>
<tr>
<td>( \theta_{EC} ), ml/g dry tissue</td>
<td>2.70 ± 0.04</td>
<td>2.81 ± 0.12</td>
<td>2.66 ± 0.15</td>
<td>2.84 ± 0.21</td>
<td>2.43 ± 0.21</td>
</tr>
<tr>
<td>( \theta_{EC} ), ml/g wet tissue</td>
<td>0.55 ± 0.01</td>
<td>0.57 ± 0.01</td>
<td>0.51 ± 0.01*</td>
<td>0.52 ± 0.02†</td>
<td>0.50 ± 0.02*</td>
</tr>
<tr>
<td>( \theta_{I} ), ml/g dry tissue</td>
<td>0.56 ± 0.01</td>
<td>0.74 ± 0.03</td>
<td>1.22 ± 0.07*</td>
<td>1.55 ± 0.12*</td>
<td>1.39 ± 0.12‡</td>
</tr>
<tr>
<td>( \theta_{I} ), ml/g wet tissue</td>
<td>0.17 ± 0.002</td>
<td>0.20 ± 0.003</td>
<td>0.31 ± 0.01*</td>
<td>0.33 ± 0.01*</td>
<td>0.34 ± 0.01*</td>
</tr>
<tr>
<td>( \theta_{I} ), ml/g dry tissue</td>
<td>0.03 ± 0.001</td>
<td>0.04 ± 0.002</td>
<td>0.04 ± 0.004</td>
<td>0.04 ± 0.003</td>
<td>0.04 ± 0.003</td>
</tr>
<tr>
<td>( \theta_{I} ), ml/g wet tissue</td>
<td>0.01 ± 0.00</td>
<td>0.01 ± 0.00</td>
<td>0.01 ± 0.00</td>
<td>0.01 ± 0.00</td>
<td>0.01 ± 0.00</td>
</tr>
</tbody>
</table>

Values are means ± SE. Intraperitoneal pressure \( (P_{Pip}) \) was typically maintained within ±0.2 mmHg of the desired pressure. Partitioning of total tissue water in anterior abdominal muscle as calculated from dry-to-wet weight ratio or from the equilibrium distribution volumes of [14C]urea in hypertonic dialysis experiments at \( P_{Pip} \) between 0 mmHg and 6 mmHg. Values in parentheses were determined with [14C]urea. TBW, total body water; \( T_{1/2} \), half time. See text for description of \( * \) values. *P < 0.001, †P < 0.01, and ‡P < 0.05 compared with controls \( (P_{Pip} = 0 \) mmHg).
Bonferroni test showed no significant difference in interstitial expansion ($\theta_{i}$) ($F = 1.47, P > 0.05$) or the local vascular volume ($\theta_{iv}$) ($F = 0.013, P > 0.05$) between hypertonic and isotonic dialysis.

As seen in Table 1, total body water normalized to kilogram body weight tends to be numerically lower with gradual elevation of Pip, but the difference among the group means was not significant ($n = 12, F = 1.42, P > 0.1$). $\theta_{i}$ and $\theta_{iv}$ were determined from a separate set of experiments, whereas total tissue water fraction was either determined from dry-to-wet weight ratio from AAM tissue ($\theta_{tw}$) or obtained from the normalized tissue $[^{14}C]$urea concentration ($\theta_{urea}$). Elevation of Pip from 1.5 to 6 mmHg caused $\theta_{urea}$ to increase from $0.78 \pm 0.01$ to $0.85 \pm 0.01$ ml/g wet tissue ($F = 29.7, P < 0.001$). This increase in $\theta_{urea}$ is primarily elicited in $\theta_{i}$, which accounted for 20% of $\theta_{urea}$ at $P_{ip} = 1.5$ mmHg, but significantly increased ($F = 135.4, P < 0.001$) to 40% of $\theta_{urea}$ after Pip elevation to 6 mmHg. $\theta_{iv}$ was not affected by this pressure change. However, $\theta_{ic}$ was significantly reduced ($F = 21.2, P < 0.001$) from 0.57 ml/g wet tissue (73% of $\theta_{urea}$) at $P_{ip} = 1.5$ mmHg, to 0.50 (59% of $\theta_{urea}$) upon elevation of Pip to 6 mmHg.

Bonferroni test showed no significant difference in interstitial expansion ($\theta_{i}$) ($F = 1.47, P > 0.05$) or the local vascular volume ($\theta_{iv}$) ($F = 0.013, P > 0.05$) between hypertonic and isotonic dialysis.

As seen in Table 1, total body water normalized to kilogram body weight tends to be numerically lower with gradual elevation of Pip, but the difference among the group means was not significant ($n = 12, F = 1.42, P > 0.1$). $\theta_{i}$ and $\theta_{iv}$ were determined from a separate set of experiments, whereas total tissue water fraction was either determined from dry-to-wet weight ratio from AAM tissue ($\theta_{tw}$) or obtained from the normalized tissue $[^{14}C]$urea concentration ($\theta_{urea}$). Elevation of Pip from 1.5 to 6 mmHg caused $\theta_{urea}$ to increase from $0.78 \pm 0.01$ to $0.85 \pm 0.01$ ml/g wet tissue ($F = 29.7, P < 0.001$). This increase in $\theta_{urea}$ is primarily elicited in $\theta_{i}$, which accounted for 20% of $\theta_{urea}$ at $P_{ip} = 1.5$ mmHg, but significantly increased ($F = 135.4, P < 0.001$) to 40% of $\theta_{urea}$ after Pip elevation to 6 mmHg. $\theta_{iv}$ was not affected by this pressure change. However, $\theta_{ic}$ was significantly reduced ($F = 21.2, P < 0.001$) from 0.57 ml/g wet tissue (73% of $\theta_{urea}$) at $P_{ip} = 1.5$ mmHg, to 0.50 (59% of $\theta_{urea}$) upon elevation of Pip to 6 mmHg.

**Figure 5.** A: dialysis fluid osmolality as a function of dwell time. For each Pip level investigated, dialysate osmolality is normalized to the initial dialysis solution osmolality before instillation. Rise in dialysate osmolality at 60 min is due to replacement of the dialysate with a fresh solution. This technique allowed for keeping the mean dialysate osmolality greater than 90% of its initial value. B: dialysate $[^{14}C]$urea concentration normalized to plasma concentration for each Pip level investigated is plotted as a function of dwell time. This ratio is close to unity during the entire dwell despite the osmotic flux into the cavity and incomplete drainage of dialysate at 60 min. C: equilibrium distribution volume of $[^{14}C]$urea ($\theta_{urea} = \theta_{EC} + \theta_{IC}$) profiles in the AAM at each Pip level investigated. There was a good correlation between $\theta_{tw}$ as determined from dry-to-wet weight ratios and the distance-averaged $\theta_{urea}$. The normalized concentration of mannitol is plotted in C to illustrate possible osmotic effects on the tissue water closest to the peritoneum ($C(x)/C(0)$, where $C(x)$ and $C(0)$ are tissue concentration and concentration of mannitol at $x = 0$, respectively). Symbols correspond to Pip in mmHg: 0, open diamonds; 1.5, open circles; 3, solid circles; 4.4, open squares; and 6, solid squares.

**Tissue Profiles of $\theta_{urea}$**

Figure 5A displays the averaged dialysis fluid osmolality as normalized to initial osmolality and plotted vs. dwell time for each Pip experiment ($n = 3$ each). Sixty minutes after initiation of the dwell, dialysis fluid osmolality dropped to ~80% of its initial value before instillation. This is due to the osmotic fluid flux into the cavity and initial diffusion of mannitol out of the cavity into surrounding tissue. The rise in dialysis fluid osmolality after 60 min is due to the replacement of the dialysis solution with fresh solution. For each set of Pip investigated, the normalized average dialysis fluid osmolality during the 120-min dwell was slightly above 90% of the initial value. Figure 5B shows the dialysate to plasma concentration ratios of $[^{14}C]$urea as plotted.
vs. dwell time for each set of $P_{ip}$ experiments. The variability of the data is attributed to a low number of observations in each point ($n = 3$), to incomplete drainage of peritoneal fluid after the first 60 min cycle, and to the accompanying osmotic water flux into the cavity. Figure 5C displays the measured tissue $\theta_{urea}$ (ml/g wet tissue, from $[^{14}C]$urea) profiles in the AAM plotted vs. distance from the peritoneal edge. Normalized tissue tracer concentration provided an estimate of the $\theta_{urea}$ (ml/g wet tissue) in the AAM for each of the $P_{ip}$ investigated. With the exception of the first 200 µm from the peritoneal edge, the profiles are rather flat for all the $P_{ip}$ investigated. The lower tissue tracer ($[^{14}C]$urea) concentration near the peritoneal edge is likely due to the fact that the mannitol concentration, and, therefore, osmolality is highest at the edge of the peritoneum. The normalized tissue concentration profile of tracer mannitol that diffuses from the peritoneal cavity is plotted to illustrate this (data from Ref. 6), and it is assumed that the transport of the tracer mannitol from the cavity mirrors that of the unlabeled mannitol. The greatest effects of the hypertonic solution would presumably be on the intracellular space near the peritoneum.

In Fig. 6, the fractions making up the total tissue water ($\theta_{urea}$) in the AAM are shown for control nondialyzed rats and for animals that underwent hypertonic dialysis at $P_{ip}$ elevations between 1.5 and 6 mmHg. A significant increase in $\theta_{urea}$ is only observed in animals dialyzed at $P_{ip} > 1.5$ mmHg. This is consistent with our finding that 1.5 mmHg is a threshold for a hydrostatic-driven convection during peritoneal dialysis in rats. The increase in $\theta_{urea}$ is primarily in the interstitium, $\theta_{i}$, which doubled its volume upon elevation of $P_{ip} \geq 3$ mmHg. There was no significant change in $\theta_{iv}$; however, $\theta_{iv}$ invariably decreased in all animals dialyzed at $P_{ip} > 1.5$ mmHg.

**DISCUSSION**

The present study investigated the changes in total tissue water and its distribution in AAM upon simultaneous exposure to changes in hydrostatic and osmotic pressures. As demonstrated in the present study (see Fig. 6) and in our previous publications (9, 24), an intra-peritoneal threshold pressure must be reached for changes in tissue water to occur. The threshold pressure in the rat peritoneal cavity is ~1.5 mmHg, which results in an average tissue pressure ($P_T$) of 1.2 mmHg in the abdominal wall. Below this threshold pressure, there is neither a significant change in tissue water content nor a significant change in the pattern of distribution of tissue water. Above the threshold pressure, a hydrostatic pressure profile is set up across the abdominal wall, as seen in Fig. 1, which illustrates the profiles for both the hypertonic dialysis of this study and the isotonic solution of our previous study (24). As stated in the results, the hydrostatic pressure profiles varied with the $P_{ip}$ and had no dependency on the tonicity of the solution in the peritoneal cavity; this observation matches our previous pressure profile measurements in the anterior abdominal wall (3). Since the slope of the tissue pressure profile ($dP_T/dx$) defines the driving force for hydrostatic pressure-driven convection from the cavity into the interstitial space of the anterior abdominal wall muscle during dialysis (see Eq. 1), it is not surprising that a significant increase in tissue water is observed regardless of osmolality of peritoneal fluid. This tissue expansion is primarily elicited in the interstitium ($\theta_{i}$) (see Fig. 4), which doubles from its control value irrespective of dialysis fluid osmolality when $P_{ip}$ is raised from 1.7 to 4 mmHg (Fig. 2). Observations of tissue hydration depend to a great extent on the tissue model employed, and a comparison to those used in other physiological studies will be made below. In addition, these observations allow us to predict the specific alterations to the transport coefficients for diffusion and convection through the tissue space when it is expanded by fluid in the cavity during peritoneal dialysis.

The Model Tissue and the Assumptions

The model tissue employed in this study is rat AAM simultaneously exposed to changes in hydrostatic and osmotic pressures. The model allows for measurement of tissue hydrostatic pressure and tracer concentration profiles for any $P_{ip}$ and peritoneal fluid osmolality. The total tissue water and its distribution in AAM were measured indirectly using dry-to-wet weight ratios or radioactive tracers. Since there is no specific marker for the interstitium, it must be determined from the difference between extracellular fluid volume ($\theta_{ec}$) and intravascular fluid volume ($\theta_{iv}$). $[^{14}C]$mannitol is assumed to mark the total $\theta_{ec}$ in the AAM in 90-min equilibration time. To test the validity of this assumption, we performed control experiments in which the tracer equili-
bration and dwell time were increased to 120 min and in which the osmolality of the dialysis solution was maintained within ~90% of its initial value. The design of these control experiments allowed for equilibration of the interstitium from nearly constant and equal tracer concentrations in the plasma and dialysis solution. Since there was no significant increase in the overall $\theta_{EC}$ after increasing the equilibration time, we feel confident that the 90-min equilibration time used in the present study is sufficient for $[^{14}C]$mannitol in the plasma to equilibrate throughout the extracellular space. This is supported by previous studies by Larsson et al. (14) who found that the $V_D$ of $^{51}$Cr-EDTA (mol mass = 341 Da) in rat skeletal muscle does not change after infusion times of 60, 90, or 120 min. Wiig and Reed (20) reported that the extracellular spaces measured with $^{51}$Cr-EDTA in anephric cats were similar 2 h and 6–7 h after tracer injection. Our mathematical model (5) with parameters for mannitol (4) predicts that a steady-state tracer concentration is approached in tissue within 10–20 min. The 90-min equilibration time used in the majority of the present studies should therefore be more than sufficient to approach an equilibrium.

Studies of the Interstitium by Others

Levick and McDonald (13) have demonstrated simultaneous flow into and flow out from the rabbit knee after increases in both intra-articular pressure and albumin concentration in the infusate, suggesting an internal circulation of fluid. However, the rabbit knee synovium is only 20 µm thick, with little “tissue” surrounding the blood capillaries. It therefore resembles a capillary surrounded by a minimum of interstitial space. The rat AAM is ~2,000 µm thick with the nearest blood capillaries typically 40 µm from the peritoneum. Thus the mechanisms operating in the rabbit synovial model may be different from those of our model system.

Wiig and Reed (20) dialed cats with 20% glucose in saline solutions in the peritoneal cavity and measured $\theta_{EC}$, $P_T$, and $\theta_{TW}$ in gracilis and sartorius muscles, which are anatomically separate from the tissue adjacent to the cavity. The high glucose concentration in the peritoneal cavity causes a rapid and large flux of fluid into the peritoneal cavity. The immediate effect of this large flux is an increase in the effective transcapillary oncotic pressure in favor of capillary absorption. This resulted in the decrease in all volumetric parameters in tissues distant from the peritoneal cavity (20, 21). Because of a greater absolute reduction of $\theta_{TW}$ than in $\theta_{IC}$, the authors concluded that $\theta_{EC}$ was also reduced after hypertonic dialysis. The authors extended their experiments in dogs with the same measurements in gracilis and sartorius muscles (21). $\theta_{IC}$ was reduced by 43% with reduction in $\theta_{TW}$ greater than the fall in $\theta_{IC}$. In both species, a remarkable reduction in $P_T$ was observed after hypertonic dialysis: from −0.8 ± 0.9 to −4.0 ± 1.1 mmHg in cats and from −0.1 ± 0.8 to −4.2 ± 1.7 mmHg in dogs. However, blood glucose had increased 5- to 10-fold from a control value of 4–5.5 mmol/l, despite insulin injection.

The results and conclusions from these studies in cats and dogs cannot be compared with our studies because of the different experimental designs. First, by varying the volume and composition of the intraperitoneal solution, we have exposed the AAM directly and simultaneously to increases in osmolality and hydrostatic pressure, which results in effects on $\theta_{IC}$ and $\theta_{TW}$. In the cat and dog studies, $\theta_{IC}$ was manipulated by dehydration alone. Second, in none of the animal’s dialyzed at $P_{IP} > 0$ mmHg with the hypertonic solution were negative pressures in the tissue recorded, whereas all $P_T$ values were negative in the cat and dog studies. Third, the net change in $\theta_{TW}$ in this study involves more complex mechanisms of fluid shift consisting of an osmotic flux into the cavity with simultaneous hydrostatic-driven fluid flux into the AAM in situations where $P_T$ reaches a threshold of ~1.2 mmHg. In contrast, in the cat and dog studies, there was likely little hydrostatic-driven fluid flow, and the use of a 20% glucose-based solution caused depletion of the plasma volume by 30% and increased the effective colloid osmotic pressure by 76% compared with baseline values. This may in part explain the lower $\theta_{IC}$ obtained after hypertonic dialysis (20, 21). In summary, our results are derived from manipulation of the interstitial side of the Starling forces, whereas previous studies were based on changes in the plasma pressures.

Pressure-Volume Curve

The pressure-volume curve assessed in this study (Fig. 2) revealed the classic nonlinear form reported in literature (16, 19, 21, 24), in which the tissue pressure ($P_T$) must reach a pressure threshold to elicit a change in $\theta_{IC}$. This threshold pressure varies according to tissue type. In the AAM the interstitial threshold pressure is ~1.2 mmHg, compared with 3.5–6 mmHg in cat intestine (1) and ~6.6 mmHg in synovium lining the rabbit knee (11, 12, 15). It is important to note that although the fluid loss rate from the peritoneal cavity is nearly a linear function at $P_{IP} > 1.5$ mmHg regardless of the osmolality of peritoneal fluid (9), the accompanying tissue expansion which is primarily in the interstitium is a nonlinear function of $P_{IP}$ (see Fig. 2).

Effects of Hypertonic Dialysis on Local Water Content Profiles

Under normal physiological conditions in the intact rat, the total water content in the rat abdominal muscle accounts for ~75% of the total tissue volume. $\theta_{IC}$ is maintained constant during a steady state in which fluid filtration toward the interstitium caused by the slight imbalance in the transcapillary Starling forces is balanced by an equal amount of fluid drained by interstitial lymphatics. Experimental perturbation of tissue pressure results in complex changes in fluid shifts. In a recent work (24) using isotonic dialysis, we have shown that raising $P_{IP}$ between 1.2 and 8 mmHg significantly increases tissue water content of the ab-
dominal wall muscle interstitium. This occurs primarily in \( \theta_{ip} \) because \( P_{ip} \) sets up a hydrostatic pressure difference across the anterior abdominal wall and results in a tissue pressure gradient (Fig. 1) that drives water flow from the cavity into the muscle interstitium (9) and doubles its volume between \( P_{ip} \) of 1.5 mmHg and 4 mmHg (24). Hypertonic dialysis with a small solute such as mannitol or sucrose adds further complexity to the fluid dynamics of the tissue space by imposing a hyperosmolar solution at the peritoneum, which results in a decreasing profile of the osmotic agent in the tissue. The concentration profile has been determined for labeled mannitol (5, 6) and is illustrated in Fig. 5C. Since the hyperosmolarity depends on the concentration of the agent used (for an ideal solution, \( \pi = CRT \), where \( C \) is concentration, \( R \) is the gas constant, and \( T \) is absolute temperature), the osmolar profile would be expected to parallel this concentration profile. Figure 5 demonstrates that most of the hypertonicity due to mannitol is lost in the 500–600 \( \mu \)m adjacent to the peritoneum; the cells and microvessels of this region of the tissue are subject to this osmolar force during dialysis.

The tissue profile for \( \theta_{urea} \), a surrogate for total tissue water, illustrates some of the effects of hypertonicity in the tissue space closest to the peritoneum. All of the curves decrease in magnitude in the 200 \( \mu \)m closest to the peritoneum, with the lowest value measured at the peritoneum where the osmolality is highest. Part of this decrease in local concentration of urea may be due to the dilution of the tracer in the cavity caused by the influx of solute-free water due to the osmotically driven flow. Evidence for the dilution is provided in Fig. 5B with values of the ratio of dialysate urea concentration to that in plasma concentration \((D/P)_{urea}\) less than 1 for some of the \( P_{ip} \) values. When the urea tracer concentration in the cavity drops below the plasma concentration, urea from the tissue will diffuse from the tissue toward the dialysis fluid; this might cause a slight decrease in the local concentration of urea in the vicinity of the peritoneum. However, a clear pattern is present in all curves of Fig. 5C, including those curves with \((D/P)_{urea}\) equal to one. The decrease in total tissue water appears to coincide with the region of highest hypertonicity (see mannitol concentration profile in Fig. 5).

Although we have no independent measure of the intracellular space adjacent to the peritoneum, we can surmise from the relationship \( \theta_{IC} = \theta_{urea} - \theta_{EC} \) and from the relatively flat profiles of \( \theta_{EC} \) (Fig. 3; also see figure 2 of Ref. 24) that the hyperosmolarity that is present in the vicinity of the peritoneum results in the decrease of the intracellular space. The fluid that transports from \( \theta_{IC} \) may contribute to the local tissue expansion or to the osmotically induced flow from the tissue to the cavity.

Hypertonicity in the peritoneal cavity produces a quantitatively lower \( \theta_{TW} \) at \( P_{ip} \) > 1.5 mmHg than does isotonic dialysis due to a decrease in \( \theta_{IC} \) (see Figs. 4 and 6 and Table 1). As discussed in the introduction, we have previously observed that hypertonic dialysis produces an osmotically driven volume flux from tissue into the cavity (2, 6, 9) and a hydrostatic pressure-driven flux into surrounding tissue and that these events appear to occur at the same time (7–9). Whereas the underlying mechanisms of these complex flow phenomena are not completely clear at this time, \( \theta_{TW} \) (or \( \theta_{IC} \)) measured during hypertonic dialysis results from this combination of osmotic and hydrostatic forces and the complex flow into and out of the tissue space.

Effects of Hydrostatic and Osmotic Pressures on Transport During Peritoneal Dialysis

The general expansion of the tissue space surrounding the peritoneal cavity increases the rates of diffusion of solutes such as urea and creatinine through the tissue. The effective diffusion coefficient in tissue \((D_{eff})\) equals the product of the diffusivity of the solute within the tissue space \((D_u)\) and the volume of the tissue available to the solute \((\theta_u)\): \( D_{eff} = D_u\theta_u \) (17). For small solutes, \( \theta_u \) equals \( \theta_{EC} \). If the concentration gradient \((dC/dx)\) is assumed to be unchanged with tissue expansion, then the diffusion coefficient and therefore the rate of diffusion will increase in proportion to the tissue expansion. As shown in Fig. 6, \( \theta_{ip} = (\theta_{EC} - \theta_{IC}) \) increases with \( P_{ip} \) > 1.5 mmHg and reaches a value of twice baseline at a \( P_{ip} \) of 3 mmHg. Since this relatively low pressure is well within the lower range of \( P_{ip} \) values that have been measured in patients (10, 18), the interstitium surrounding the peritoneal cavity is likely in a constant state of expansion, and rates of small solute diffusion (mol mass ≤5,000 Da) are increased relative to those that would be observed without a large volume in the cavity.

Expansion of the extracellular volume in the tissue surrounding the peritoneal cavity increases the rate of convection. This follows from the dependability of the hydraulic conductivity of the tissue space on the interstitial volume. One theoretical expression that is used to relate the hydraulic conductivity of a porous bed to its structural properties is the Carmen-Kozeny equation (12): \( K = \theta_{ip}^2/S^3 \), where \( G \) is Kozeny factor (a dimensionless proportionality factor), and \( S \) is the wetted surface area within the porous bed. As shown in Figs. 2 and 6 and in Ref. 24, \( \theta_{ip} \) doubles once \( P_{ip} \) increases from 1.5 to 4.4 mmHg; this expansion would theoretically increase \( K \) by a factor of 8 \((K_{ip}/K_0 = 2^3)\) if \( S \) is constant. However, the expansion of the interstitium may increase the wetted surface area of interstitial matrix molecules, such as hyaluronan (12), and the prediction of in vivo hydraulic conductivity cannot be easily made independently from experiment. We have previously demonstrated that \( K \) in Eq. 1 varies directly as the \( P_{ip} \) is raised above 1.5 mmHg and increases by a factor of 5 between \( P_{ip} \) of 1.5 and 8 mmHg (23). An increase in tissue hydraulic conductivity will increase the rate of fluid flow through the tissue. Thus the raised \( P_{ip} \) expands the tissue, decreases its resistance to flow, and results in higher rates of convection through the tissue than if it were in a nonexpanded state. However, the direction of net flow will depend on the complex relationship between hydrostatic and osmotic pressure forces within the tissue space. Although the theory of
hydrostatic pressure-driven flow is well established (Eq. 1), the effects of osmotic gradients are still controversial.

In conclusion, instillation of a dialysis solution in the peritoneal cavity results in a rise in the intraperitoneal hydrostatic pressure, \( P_h \), that is maximally exerted across the AAM causing elevation of the local interstitial pressure, \( P_i \). It is not until \( P_i \) reaches a threshold pressure of \( \sim 1.2 \text{ mmHg} \) that a significant tissue expansion is observed. This tissue expansion is nonlinear, occurs primarily in the interstitium (\( \theta_i \)), and is unaffected by the osmolarity of the bathing solution. Since the interstitial space expands to the same degree under isotonic or hypertonic conditions, the primary determinant of the resistance to passive transport through the extracellular space in tissue surrounding the peritoneal cavity is the hydrostatic pressure exerted by the fluid in the cavity and the corresponding changes in the adjacent tissue space. The relatively small pressures seen in the cavity (2–20 mmHg) during dialysis are more than sufficient to cause interstitial expansion. Thus transport through the subperitoneal interstitium under clinical dialytic conditions occurs at an accelerated rate compared with movement through normal, nonexpanded tissue.

This work was supported by grants from the Whitaker Foundation, National Institute of Diabetes and Digestive and Kidney Diseases Grant R29-DK-48479, and by a grant-in-aid from the American Heart Association.

Address for reprint requests and other correspondence: M. F. Flessner, Box 675, 601 Elmwood Ave., Univ. of Rochester Medical Center, Rochester, NY 14642 (E-mail: Michael_Flessner@URMC.Rochester.edu).

Received 23 April 1999; accepted in final form 16 December 1999.

REFERENCES